STUDIES ON THE EPIDEMIOLOGY AND PATHOPHYSIOLOGY OF OVINE GASTRO-INTESTINAL HELMINTHIASIS.

A thesis submitted for the degree of Doctor of Philosophy in the Faculty of Veterinary Medicine, University of Glasgow.

by

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September, 1982.

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ACKNOWLEDGEMENT

From the depth of my heart I would like to record my thanks and indebtedness to my Supervisor, Professor J. Armour, and wish to express my deepest gratitude for his guidance and valuable advice, support, patience and understanding throughout the past three years, without which, this work would not have been achieved.

My gratitude is also due to Professor G.M. Urquhart, who allowed me to study in his department and for his advice, encouragement and help throughout the study.

I wish to thank most sincerely Dr. P. Holmes for his stimulating and fruitful discussion in the pathophysiological studies described in this thesis.

I greatfully acknowledge the skilfull technical help both in the field and laboratory work given by Mr. K. Bairden, whose never failing assistance was greatly appreciated.

I would like to extend my thanks to Dr. J. J. Parkins in the Animal Husbandry department for his help.

I should also show appreciation to Mr. S. Brown, Mr. C. Hartley, Miss J. Henderson, Mr. P. Tiebosch and Mrs. C. Connelly for their enthusiastic cooperation.

My thanks to the technicians in the Department of Physiology for their assistance in the Radioisotopic procedure in this thesis and to those in the Department of Animal Husbandry for their help.

Appreciation is also due to Mr. A. Finnie, A. May, and G. Cameron in the Department of Photography for the illustrations which appear in this thesis.

My thanks also to Mr. Murphy for his able assistance in the post-mortem.

I wish to thank Mrs. K. MacNeill and Mrs. E. Blake for typing the thesis.

I would like to thank the members of the Department of Veterinary Parasitology and others, too numerous to mention, for their assistance and sympathy which helped me through the past three years.

I am most greatful to the Iraqi Ministry of Higher Education and Research for their financial support which allowed me to complete the study.

Finally, I wish to record my debt and love to my family for their moral and financial support throughout the study.

SUMMARY.

This thesis is divided into two sections. In the first the literature pertaining to the epidemiology of ovine parasitic gastro-enteritis is reviewed and then, over a 2 year period, the epidemiology of this disease is studied in sheep from a flock which was housed each winter. In particular, the epidemiological effect of anthelmintic treatment of the adult sheep prior to turn-out in the spring and the interaction of the protozoal disease coccidiosis with the intestinal trichostrongyle N. battus are studied.

The impact of anthelmintic treatment administered during the housing period on the subsequent epidemiology of trichostrongyles varied according to the drug used. Where the drug levamisole was used the efficiency was sub-optimal, the ewes contaminated the pasture from the first day of grazing and the epidemiology of the disease did not vary from that in untreated flocks. In contrast, when the highly effective broad spectrum drug fenbendazole was used the faeces of the ewes remained clear of nematode eggs for several weeks and the level of pasture infection was considerably reduced.

By housing the sheep during the winter there was some indication in the first year that a build up of Eimeria spp oocysts occurred and both ewes and lambs shed many oocysts within 2 weeks of grazing. However, in the second year the number of oocysts shed were much reduced which suggested that the first year response

was due to favourable climatic conditions rather than the ingestion of massive numbers of oocysts indoors.

Although severe nematodiriasis occurred due to N. battus infection in both years, the disease was more severe in the second year when coccidiosis was apparently less prevalent and there was no indication that any positive interaction occurred between the two diseases.

Two other interesting observations emerged from the study. The first was that larvated N. battus eggs hatched in the autumn of the year when the eggs were deposited and gave rise to increased pasture larval populations on the herbage and worm burdens in the lambs. At the time these experiments were carried out this had not been recorded previously and eggs were thought to require a full winter exposure before hatching in the following spring. However, contemporaneous studies have recorded a similar phenomena and this may affect the current control measures based on a year to year hatching pattern. The second was, that the pasture larval sampling technique proved to be a much more sensitive indicator of pasture levels of infective larvae than did helminth-naive tracer lambs. This sensitivity of the pasture technique was particularly noticeable when pasture levels were high in 1980 but was also apparent throughout 1981.

In the second part of the thesis the pathophysiological aspects of gastro-intestinal trichostrongylosis are reviewed

and some of these effects are then studied in two experiments. In the first experiment radioisotopic techniques were used to assess the losses of plasma protein into the gastro-intestinal tract of naturally parasitised lambs. The leak of plasma protein was measured on three occasions using $^{51}CrCl_3$ while total faecal output was measured using chromic oxide. Losses of $^{51}CrCl_3$ - labelled plasma protein were significantly higher in lambs grazing heavily contaminated pasture than in those grazing lightly infected ground in two of the sampling occasions. The increased losses were associated with high trichostrongyle faecal egg counts hypoalbuminaemia and elevated plasma pepsinogen levels. This is the first time that a plasma protein leak has been positively demonstrated in grazing sheep.

In the second experiment the effects of an experimental challenge with larvae principally of the abomasal nematode O. circumcincta plus a few of the intestinal species, T. vitrinus and Ch. ovina were measured in ewes which had previously been exposed to infections with these parasites and were judged to be immune. Their immune status was shown by the presence of low worm burdens in both challenged and unchallenged ewes. However, despite those low worm burdens the larval challenge was associated with marked pathophysiological disturbances and in particular, elevated on plasma protein loss into the gastro-intestinal tract. The concurrent timing of these changes suggested that they had a common etiology possibly associated with hypersensitivity

reaction in the alimentary mucosa of immune sheep. The economic importance of this novel result is that impaired production of immune sheep may occur when they are under challenge and emphasises the need to control infections in ewes as well as in lambs.

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SECTION 1

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FACTORS INFLUENCING THE EPIDEMIOLOGY OF OVINE PARASITIC GASTRO-ENTERITIS.

- A LITERATURE REVIEW

INTRODUCTION

Clinical parasitic gastro-enteritis (PGE) in sheep is a disease syndrome which is usually seen in young animals during their first grazing season. The nematode species mainly responsible for PGE in temperate climates are : from the abomasum, Haemonchus contortus, Ostertagia species (O. circumcincta and O. trifurcata), Trichostrongylus axei ; from the small intestine, Trichostrongylus species (T. vitrinus, T. colubriformis), Cooperia curticei, Nematodirus species (N. battus, N. filicollis), Bunostomum trigonocephalum, Strongyloides papillosus ; from the large intestine, Oesophagostomum venolosum, Chabertia ovina, Trichuris ovis. Of these the Ostertagia spp, Trichostrongylus spp and N. battus are the most important in Great Britain.

Life Cycle.

The life cycle of these species are generally similar, consisting of a free living phase followed by a period of parasitic development within the host. The life cycle may be summarised as follows : -

Fertilised eggs, laid by the female parasites, pass to the pasture in the faeces and under suitable conditions of temperature and moisture (optimal conditions are a temperature of 22-26^{o}C and a relative humidity of over 85%) they develop to first stage larvae (L1) which feed and moult to become second stages (L2). Both L1 and L2 larval stages are motile feeding stages which rely mainly on

bacteria as a food source. The final stage of free living development results in a generation of third stage non-feeding larvae (L3) ; these are infective stages and are characterised by the retention of the second larval cuticle in the form of a sheath hence they are unable to feed and must survive on food material acquired in the early stages. The infective stages then migrate from the faeces to the immediately surrounding herbage. Infection occurs by ingestion of the ensheathed infective larvae (L3) and is followed by two parasitic moults through the fourth and fifth larval stages (L4, L5) to the mature adult. In many species the period between ingestion and the appearance of mature egg laying is 21 days, although in some such as Oesophagostomum and Chabertia spp it may be extended to several weeks (63 days). Under certain circumstances it may be considerably longer as development can be arrested at the 3rd, 4th and 5th stage, depending on the species, for several months ; this phenomenon is known as inhibited or arrested larval development (Connan, 1968b ; Reid and Armour, 1972 ; Michel, 1974), or hypobiosis (Gordon, 1970).

An exception to the above life cycles are the Nematodirus spp which differ in the important respect that the egg does not hatch until the L3 is present (Gibson, 1958 ; Thomas, 1959b ; Thomas and Stevens, 1960).

The factors which are important in the epidemiology of the gastro-intestinal parasites of sheep may be conveniently grouped under two main headings.

1. Factors which affect the contamination of the environment with parasite eggs or larvae.

2. The development of these eggs to the infective stage and the survival, availability and longevity of these larval stages.

FACTORS AFFECTING CONTAMINATION.

The level of contamination of the environmemnt is influenced by several factors including the biotic potential of the parasite, stock management, host immune status and the degree of hypobiosis.

a) Biotic potential.

Biotic potential may be defined as the capacity of an organism for biological success, and is usually related to reproductive ability eg. a parasite which can lay great numbers of eggs or undergo many generations within a short period of time. A wide spectrum of biotic potential exists in nematodes ; some such as H. contortus produce many thousands of eggs (Allonby & Urquhart, 1973). Others such as Trichostrongylus spp produce only a few hundred.

Obviously, heavy contamination of the environment occurs with parasites of high biotic potential and disease problems due to these parasites are built up rapidly.

b) Stock management.

Density of stocking can also influence the level of pasture contamination in nematode infection. It has the greatest influence during the period when climatic conditions are optimal for development of the contaminating eggs or larvae to the infective stage. Stocking density will also be of the utmost importance if the grazing stock are composed of fully susceptible animals such as weaned lambs, or the ratio of susceptible to immune stock per unit area is wide, as in sheep flocks with a high percentage of twins and triplets.

c) Immune status of the host.

Much of the seasonal variation and the rythms of parasitism of the gastro-intestinal nematodes in sheep are mediated by the immunity of the host. Thus young sheep are incapable of developing an adequate resistance to most nematodes, Urquhart, Jarrett and Mulligan, (1962). An exception to this rule is N. battus, (Gibson, 1959 ; Brunsdon, 1960) where the lambs are highly immune by 6 months of age. Although age immunity 'per se' does not operate against most gastro-intestinal nematodes of sheep, it may influence the course of infection, reduce its pathogenic effect and possibly increase the ability to acquire immunity.

Even when sheep have acquired a high degree of immunity to gastro-intestinal nematodes this can wane for a variety

of reasons and influence the contamination of pasture. The most important reduction in immunity is the so called 'spring rise' in nematode faecal egg counts, particularly in pregnant ewes. This was first described by Zawadowsky and Zvjagvintzev (1933) in Russia but only achieved global recognition following the studies by Scottish workers, commencing with those of Morgan and Sloan (1947) who accorded the term 'spring rise' to an increase in nematode faecal egg count (epg) observed during the spring.

After another decade Crofton(1958b) demonstrated that increased epg's also occurred in lactating ewes from autumn lambing flocks. He suggested that the increase was associated with parturition and lactation rather than season and proposed the term 'post parturient rise' in preference to spring rise in nematode faecal egg counts. Since then the association of lactation with an increased susceptibility to nematode infections has been clearly demonstrated by many workers including Brunsdon (1964a) Dunsmore (1965), and Connan (1968a). Also O'Sullivan and Donald (1970) found that if lambs were removed from ewes within 12 hours of birth then the ewes did not develop an increased susceptibility to nematodes.

Salisbury and Arundel (1970), observed that the rise in nematode faecal egg counts commenced prior to parturition and continued until about 6 weeks post-lambing and proposed the term peri-parturient rise (PPR) which is sometimes now

referred to as the peri-parturient relaxation in immunity (PPRI).

Several of the host factors thought to be responsible for this immunological impairment around parturition have been studied by Connan (1972) and Kelly and Dineen (1973). These workers demonstrated an association, either direct or indirect, with circulating levels of prolactin closely following the PPR in the nematode faecal egg counts. Thus prolactin increases from about five weeks prior to parturition, reaches a peak in early lactation, and then steadily declines until the lambs are weaned.

The parasitological factors that contribute to the PPR are resumption in development of larvae previously arrested in their development, the increased fecundity of existing adult female worms, the development to maturity of new infections and possibly the failure to eliminate existing infections.

The source of the PPR varies in different geographical regions. In arid or semi- arid zones where survival of infective stages (L3) is negligible during the dry season, the development of arrested larvae makes the major contribution. Some of these larvae will develop because of the relaxation in immunity, while others will develop due to the influence of seasonal factors as discussed later under hypobiosis. In temperate zones where larvae

on pasture survive the winter in reasonable numbers, the acquisition of fresh infection and the maintenance of the resulting gravid populations also make significant contribution to the PPR.

Evidence of the part played by an increased fecundity of existing female worms is more circumstantial ; thus the nematode faecal egg counts of ewes are higher if they are subject to excessive stress such as extremes of weather and poor nutrition, Morgan, Parnell and Rayski (1951). This is possibly due to an increased level of cortisone which is known to increase during periods of stress, particularly as the experimental administration of cortisone to sheep and cattle with nematode infections results in an elevated nematode faecal egg count (Armour 1967). Also, recent studies by Jansen (1977) have shown that nematode egg counts are higher in ewes with twins than in ewes with a single lamb. Since ewes with twins are under more stress and have to produce more milk, this could be responsible for higher levels of plasma corticosteroids which in turn influence the worms to produce more eggs. It is not clear if this is a direct effect on the worms or whether cortisone influences the immunity of the host. Jansen also showed that ewes lambing for a second time produce a lower epg than ewes lambing for the first time at the same age, either as a result of a higher basic immunity in the second lambing or higher stress factors in the first lambing (Jansen 1978 ;

1981).

In most areas of the world parturition of grazing animals is synchronised to occur with the climate favourable to pasture growth and also, unfortunately, suitable for the development and survival of the free living stages of most helminths. Clearly the epidemiological significance of the PPR is that it ensures a contamination of the pasture and further propagation of the parasite.

Host immunity can also limit the level of contamination by modifying the development of new infections or expelling existing burdens. Apart from rejection of fresh infections, the development of gravid worms may be constrained by immuno-arrested larval development, stunting of adult worms and reduced egg production by these worms (Urquhart, et al, 1962). The expulsion of existing adults may occur due to immunological (Stewart, 1953), or non-immunological reasons (Allonby and Urquhart, 1973). It is interesting that in contradistinction to the PPR and hypobiosis, the above phenomena tend to occur when infective larval stages are readily available and environmental conditions are favourable to translation, they are also particularly common in helminths of high biotic potential such as H. contortus (Allonby and Urquhart, 1973) and Ascaris suum (Jorgensen, Nansen, Neilsen, Eriksen and Andersen, 1975) and propbably reflect a

regulatory control by host/parasite to prevent over population by the latter.

d) Arrested larval development.

Larvae may become arrested in development within the host as a manifestation of acquired immunity (Urquhart, et al, 1962). For example in sheep O. columbianum (Gordon, 1949), Nematodirus spp (N. spathiger) (Donald, Dineen, Turner and Wagland 1964), H. contortus and O. circumcincta, (Connan, 1968b ; 1969 ; 1971).

However, there is increasing recognition that larvae may also arrest in development as a result of prior experience of certain climatic or seasonal influences, a phenomenon referred to as hypobiosis by Gordon (1970). The significance of hypobiosis on the epidemiology of ovine gastro-enteritis has been studied by several workers and has been reported in relation to various host parasite systems. Positive evidence for an association between seasonal climate and hypobiosis came from the epidemiological studies by Reid and Armour (1972) in South West Scotland, on the gastro intestinal nematodes of sheep. These workers introduced groups of susceptible (Tracer) lambs to graze on a permanent sheep pasture at monthly intervals from mid July to mid April and each group was then housed for 7 days prior to slaughter. The proportion of arrested larvae of all genera present except intestinal Trichostrongylus spp, increased markedly during

late autumn to reach a maximum of 84% and 100% (Ostertagia spp), and 73% and 100% (T. axei) in December and January respectively. The maximum numbers of worms of all genera were present between mid September and mid October. The increase in the proportion of arrested larvae occurred independently of either age or sex of the host, or size of the worm burdens. It was suggested that the primary factor involved in the arrestment of the larvae was an environmental conditioning of the larvae possibly producing endocrine or metabolic changes in the larvae.

A similar seasonal pattern has been described for various nematode species of sheep by other workers eg. in H. contortus (Connan,1968a ; Blitz and Gibbs, 1971 ; Waller and Thomas, 1975 ; Thomas, 1979). In Ostertagia spp, (Connan, 1969 ; Ayalew and Gibbs, 1973). For Trichostrongylus spp, (Eysker, 1978 ; 1980 ; Waller, Donald and Dobson, 1981). In N. filicollis (Michel,1974), C. curticei (Sommerville,1960), Ch. ovina, Connan (1974) and Ch. ovina and Oe. venulosum, Eysker (1980).

In sub-tropic zones with winter rainfall, arrested Ostertagia species larvae also accumulate in sheep, although this occurs at different seasons eg. in Victoria, Australia, it occurs in spring (Anderson, 1972), whereas in New Zealand, Brunsdon (1972), has recorded that the highest level of arrested larvae occurs during late autumn

and winter. The accumulation of significant populations of arrested larvae in the host usually coincides with the onset of environmental conditions which are adverse to the free living development of the nematode and the severity of the latter appears to influence the proportion of a nematode population which becomes arrested or hypobiotic. For example in Canada and Nigeria, where cold and dryness, respectively, are particularly severe at certain times of the year, the proportions of arrested larvae are high ; in Britain with a relatively mild winter, the proportions are moderate, and in Kenya, where two wet seasons occur with comparatively short arid periods intervening, the proportion is low.

Opinion on the aetiology of hypobiosis has polarised in two directions. First, that the seasonal effect is via the host either through the endocrine system or immunological response or secondly a direct effect on the metabolism of the free living stages. Experimental support for the latter theory was provided by Blitz and Gibbs (1972) and McKenna (1973) who observed that exposure of infective larvae to autumnal conditions induced inhibition in H. contortus ; they concluded that cold influenced the onset of arrestment and that a decreasing photoperiod also had some influence. The effect of photoperiod has been confirmed by Waller and Thomas (1975) and Thomas (1979). Gibbs (1973) also mentioned that decreasing day length increased the

proportion of hypobiosis whereas Cremers and Eysker (1975) were not able to demonstrate an effect of decreasing day length. In Cambridge, Connan (1975) observed that a high level of arrestment of H. contortus occurred when the larvae were cultured for 12 days at 25°C in the dark and therefore, suggested that conditioning of H. contortus for hypobiosis can be made during the pre-parasitic stage. In later experiments he showed that a high moisture content in the culture resulted in higher levels of arrested development and that this effect was less when culture periods were reduced to 6 days (Connan, 1979). Connan also showed that Ostertagia spp can be induced to become hypobiotic by exposure to a decreasing photoperiod and low temperature.

The results of studies in the Netherlands on arrested development of O. circumcincta and H. contortus by Eysker (1979 ; 1981) showed that when L3 of these species were given to sheep, after having been cultured in faeces for 7 days at 25°C, only a small proportion of the larvae became arrested and when O. circumcincta was cultured at 16°C no arrested stages were subsequently found. This effect of storage at 4°C is known to be effective in producing arrested development in other trichostrongyloids (Armour, 1970 ; McKenna, 1973 ; Armour and Bruce, 1974 ; Michel, 1974) and is thought to be similar to the field situation in late summer and

autumn as described by Reid and Armour (1972). In some instances the maturation of arrested larvae can occur spontaneously or synchronously eg. O. ostertagi in cattle, Armour and Bruce (1974). In O. circumcincta Reid and Armour (1973) and in H. contortus arrested larvae seem to mature synchronously over a period of several months (Blitz and Gibbs, 1972 ; Connan, 1975). The maturation of hypobiotic larvae has an important epidemiological function in that it results in an increased contamination of the environment, usually at the time when conditions are ripe for free living development. The nematode has, therefore, achieved its aim of ensuring another generation and this is the real importance of the phenomenon although in the process clinical disease may ensue.

FACTORS AFFECTING THE DEVELOPMENT, SURVIVAL, AVAILABILITY AND LONGEVITY OF FREE-LIVING STAGES.

Factors affecting the development, survival, availability and longevity of free living stages of helminths, and where appropriate their intermediate hosts, have been referred to as translation, Michel and Parfitt (1956). The factors which affect translation are mainly environmental, especially seasonal climatic changes and certain management practices ; inter current infections may also influence.

a) General environmental factors.

Several environmental factors which affect the development and survival of free living stages of parasitic nematodes

under field conditions have been studied by many authors (Gordon, 1948 ; Kates, 1950 ; 1965 ; Wallace, 1961 ; Levine, 1963 ; Ollerenshaw and Smith, 1969 ; Andersen, Levine and Boatman, 1970 ; Levine and Andersen, 1973 ; Donald, 1973 ; Kenneth and Todd, 1976 ; Michel, 1976 ; Southcott, Major and Barger, 1976 ; Callinan, 1978a ; 1978b ; 1979 ; Young, Anderson, Overend, Tweedie and Preston, 1980 ; Gibson and Everett, 1981 ; 1982). All of these authors stated that the principal limiting factors on egg hatching and larval development are temperature and moisture.

Under laboratory conditions, Silverman and Campbell (1959) studied development of the eggs and larvae of H. contortus and showed that at 7.2°C development to the infective stage did not occur ; at 11°C development from egg to infective stage (L3) took 15 days and that the development became more rapid as the temperature rose above 11°C, the(L3) being reached in 9 days at 14.4°C and in 5 days at 21.7°C.

Crofton (1965) worked out the effect of temperature on the minimum time taken for eggs of a number of species to hatch and has shown that the lowest temperature at which any hatching occurs is 4°C for O. circumcincta, 6°C for Ch. ovina, 8-9°C for T axei and T. vitrinus, 9°C for H. contortus, 15°C for B. trigonocephalum and 16°C for C. curticei. Furman (1944a) working with O. circumcincta, and using a controlled humidity and

temperature box, has found that non embryonated eggs kept in 5ml of water were killed in 1-2 days by a temperature of 45°C, but embryonated eggs resisted a temperature up to 37°C ; in ice at 6°C they survived for 10-22 days. Pre-infective larvae (L1, L2) were more sensitive than eggs to the effect of environmental temperature ; freezing or high temperatures killed them quickly while those in shallow water at 5°C could survive for 1-2 months.

Recent studies by Andersen, Wang and Levine (1966) and Donald (1968) have confirmed that the climatic factors exert a greater effect on the pre-infective larval stages than on the infective stage (L3) while Pandey (1972) showed that the latter withstood low temperatures better than excessively high ones.

Rainfall is also important for the development and survival of larvae on herbage, since dessication rapidly kills both eggs and larval stages, Rose (1963). The distribution of eggs or larval stages between, host feaces, herbage, root mat and soil, is dependent on temperature and moisture (Crofton, 1963). The faecal pat offers a protective environment for the survival of the egg or larval stages, and the degree to which this occurs varies with the host. Thus cattle faeces are more highly protective to the free living stages since they remain in the original form for a considerable time compared with sheep pellets, although Gordon (1967) has

pointed out that the faeces of sheep on improved pasture can occur in a form similar to cattle pats, rather than as pellets.

Other factors affect development and survival within the faeces and the distribution of the infective stages on the herbage, eg. the number of faecal deposits per unit area of pasture which is related to the size, age and structure of the flock of sheep and total area of pasture (Crofton,1958b) and the number of parasite eggs per faecal mass. The rate of development of egg to L3 and the rate of migration all depend primarily on the microclimate present in the herbage (Donald, 1968). This in turn depends on the soil type which may influence the growth and species composition of herbage, and these in turn can influence the formation of the layer of mat between the soil and the herbage. The mat is abundant in older pasture and holds a permanent store of moisture in which the relative humidity remains high even after weeks of drought, the presence of this moisture and pockets of air trapped in the mat, limits the rate of temperature change, and these factors favour the development and survival of larvae in the root mat.

Recently, Gibson and Everett, (1981, 1982) have shown that soil is a good medium for development of free living stages of N. battus because the eggs are exposed to a more even temperature and more constant moisture

than eggs in faecal pellets which are exposed to drying winds and the sun. Whether L3 of other species remain in soil as reported for O. ostertagi in cattle by, Al Saqur, Bairden, Armour and Gettinby (1982) is not known, though Andersen, Levine and Boatman (1970) found soil an insignificant source of L3. Parkin (1976) also suggested that free water is necessary for the hatching of the eggs of N. battus, and under favourable conditions larvae migrate from soil on to the herbage.

b) Migration of larvae.

Before the infective larvae are likely to be ingested by the host they must leave the faeces and migrate on to the herbage. Temperature and moisture are again the important regulating factors in this migration. Crofton (1948), found that migration ceased at temperatures below 13°C and where larvae migrated at higher temperatures an insignificant amount of migration occurred during hot, dry weather due to lack of water. Knapp (1964) reported that low relative humidity inhibits larval migration. Some observations on trichostrongyloid infective stages suggest that they migrate only a small distance from the faecal deposit in which they develop, (Furman, 1944 b ; Rose, 1963, 1964 ; Sturrock, 1965). In Russia, Voznyi (1978) studies the vertical migration of Ch.ovina larvae which introduced from faeces placed at the soil level of plants grown in small pots. He showed that after 24 hours 18% of the larvae had migrated up the stems, several

centimetres apart, as high as 16cm. He also found that moisture favoured migration. At 80-90% humidity 1.2 to 3.5% of the larvae migrated and at 25-30% only 0.06-0.2% larvae migrated. Higher temperatures were also important for larval migration, significantly more migrations occurring in high temperatures than in low temperatures.

The results of recent studies by Williams, Skinner and Kenneth (1980) on the lateral migration of H. contortus larvae on pasture showed that over 90% of infective larvae were within 10cm. of the faeces, and that the number decreased logarithmically as the distance from the faeces increased, and also they confirmed that moisture and temperature are the most important weather factors associated with lateral larval migration of H. contortus.

Other biological and mechanical factors which effect the migration and distribution of larvae, are heavy rain, dry grass, earth worms, several species of Arthopods, vertebrates such as rabbits, ground squirrels and birds, spores of the fungus Pilobolus and Psychodid flies (Robinson, 1962, Christie, 1963, Jacobs, Todd, Dunn and Walker, 1968 ; Grønvold, 1979 ; Williams, et al, 1980 ; Oakley, 1981).

c) Longevity of infective stages.

The survival of infective stage larvae varies according to the nematode species and the climatic condition for

a period of at least one year. Early field observations by Ransom (1916) showed that a paddock which had been left vacant for seven months still contained sheep trichostrongyloid infective larvae. Raffenuspeiger (1931) has shown that horse strongyle larvae on a pasture in Montana, U.S.A. remained alive in faeces throughout two winter and one summer season, while Griffiths (1937) found that larvae of O. circumcincta, N. filicollis and T. colubriformis retained their infectivity throughout a Canadian winter. Furthermore, studies in England by Taylor (1938) also found that a high proportion of the infective stages of sheep nematodes (Ostertagia and Trichostrongylus) can retain their infectivity for a few weeks after they had been placed on grass grown in boxes in the open field during winter. Crofton,(1948) studied hill pastures in England which were grazed by sheep and lambs from June - mid April and recovered many infective larvae from June throughout October, very few during November and practically none during the rest of the winter and spring. Parallel studies in the U.S.A. by Kates (1950) on sheep nematodes, and in England by Rose (1965) have shown that many sheep nematodes could survive on the pasture throughout the winter, thus confirming the findings of Crofton (1949 ; 1952). Using faeces containing a mixture of nematode eggs and spread on small grass plots from January to December, Gibson and Everett (1967 ; 1972 ; 1976) observed that infective larvae of T. colubriformis could be recovered from the herbage through the year except

for a period during the winter when eggs failed to develop to L3 (November to February). They also showed that O. circumcincta eggs survived during the winter months and completed their development to L3 when the temperature rose above the threshold of 27°C. The same authors found that no infective larvae were recovered from eggs of H. contortus during the year except for July to September, and it is concluded that the climatic conditions in Southern England are not ideal for development and survival of the pre-parasitic stages of H. contortus. Similar investigations were carried out by Boag and Thomas (1970) in the North of England and the results were broadly similar to those obtained by Gibson and Everett in the South, though larval survival appeared to be slightly shorter. In Canada, where the winters are prolonged and cold, survival of large numbers of trichostrongyloid L3 on the pasture is good (Smith and Archibald, 1969) and Slocombe (1974) and a similar pattern has been observed by Tharaldsen (1976) in Norway, and Taylor, Cawthorne, Kenny and Regan (1973) in Northern Ireland. It is interesting that in the above countries where the winters are long and severe and the grazing season short, survival is so good. It may be related to the extensive snow cover providing a favourable microclimate.

In Australia, Donald (1968) and Anderson (1972) have confirmed that the infective stages of sheep nematodes survive well during the mild winter in that country, but

are susceptible to the high temperature and dry conditions which occur in spring and early summer.

d) Seasonal fluctuations of strongyloid infective L3 in sheep pasture.

The relationship between the above factors ie. the various sources of contamination, larval availability and the build up of infections causing outbreaks of clinical parasitism in sheep have been studied in different countries, with a wide range of farm type and climate. In early studies in Britain the relationship was assessed by : -

1) Introducing susceptible animals on to known infected pasture and counting, at regular intervals, the numbers of infective larvae per unit weight of herbage samples.

2) By experimentally infecting animals and grazing them at specific times on parasite free pasture, and estimating the pasture populations of larvae from samples of herbage.

These two techniques were used to study pasture populations of sheep gastro intestinal nematode larvae Crofton (1949 ; 1952) and more specifically Nematodirus species larvae (Thomas and Stevens 1960 ; Gibson 1959 ; 1963). In further studies in Britain and Australasia

pasture sampling techniques were used to study the seasonal fluctuation of larvae on the herbage grazed by permanent sheep flocks (Boag and Thomas, 1971 ; Donald and Waller, 1973 ; Vlasoff, 1973) and other workers used this technique with the introduction of worm free lambs (tracers or indicators) which were grazed for short intervals, slaughtered and their worm burdens used to assess the pasture infectivity (Reid and Armour, 1972 ; Donald, Morley, Waller, Axelsen and Donnelly, 1978 ; Waller, Dobson, Donald and Thomas, 1981). The direct pasture sampling method gave an estimate of the average concentration of L3 on the herbage of a defined area of pasture at a specific time while the worm counts from tracer animals provided an estimate of larval intake by the animals over the period during which it had been grazed on the pasture. The tracer method can also yield information on the fate of ingested larvae, in particular the presence or otherwise of arrested larvae which is not available by the pasture sampling technique. The results of these studies clearly showed that there was a marked seasonal variation in the numbers of L3 present during the year. It was also apparent that only a small number of generations occurred each year, the actual number being primarily climate dependent eg. in cooler areas only one or two generations of parasite occur annually in the grazing season (Heath and Michel, 1969 ; Boag and Thomas, 1971 ; Thomas and Boag, 1972 ;

Thomas, 1974a,b ; Boag and Thomas, 1977) while in warmer climates such as Northern Nigeria more than four generations of trichostrongyloid develop in the rainy season Eysker and Ogunsusi (1980). The fluctuations of larvae on the herbage are controlled by the ability of the eggs and larvae of different species to develop under different climatic conditions and these fluctuations determine the seasonal succession of species observed in the field.

Numerous surveys have been conducted in various parts of the world to determine the seasonal incidence of the various species of sheep nematode. In Britain, Morgan and Sloan (1947) Morgan, Parnell and Rayski (1950 ; 1951) Wilson, Morgan, Parnell and Rayski (1953) studied the seasonal fluctuations of faecal egg counts and the worm burdens of hill sheep in Scotland, and showed that both the worm faecal count and the worm burdens began to rise in March, reached a peak in May and then declined in August and persisted at a low level during winter. The same authors noted that the most prevelant species found in hill sheep was Ostertagia spp and that these were principally responsible for the general trend. This dominance of Ostertagia spp agrees with reports from other areas of Britain, Crofton (1963) Michel (1969) Boag and Thomas (1971) Gibson and Everett (1972) Thomas (1973) Reid and Armour (1972, 1975) and Waller and Thomas (1978). H. contortus is less important in Scottish sheep than

Ostertagia spp, but in parts of England large numbers of H. contortus adults derived from arrested larvae ingested in the previous summer are found during the spring (Connan, 1968b ; Waller and Thomas, 1975). Trichostrongylus spp apparently increase during the early winter and for some weeks from March onwards ; Ch.ovina burdens have been shown to increase in late winter and early spring and were probably derived from larvae whose development had been arrested previously (Ross, Dow and Purcell, 1969 ; Connan, 1974).

With N. battus the maximum number usually occurred in late May but this varied by 3-4 weeks according to the particular climate in any one region and in any one year (Gibson, 1959 ; Thomas and Stevens, 1960 ; Boag and Thomas, 1975).

It has also been shown that the level and sources of infection varied according to pasture management. Thus when ewes and lambs were grazed on 'clean pasture' ie. pasture not contaminated by sheep at least for one year, the ewes were the only source of infection, this occurring during the PPRI. The pasture was contaminated in April-May with many eggs which resulted in a peak of larval availability in June or July depending on the temperature pattern in the area. As a result, a build up of worm burdens and a high egg output occurred in lambs in late July/August, (Michel, 1969 ; Boag and Thomas, 1971, 1973 ;

Gibson and Everett, 1972, 1973), thus virtually the whole of the August worm infection in the lambs had come directly from the ewes. The August lamb egg output then gave rise to a further smaller wave of larval infections in September-October ; where this second wave of infection occurred outbreaks of clinical disease were common. However, when ewes and lambs were stocked on pasture grazed by sheep in the previous year the lambs acquired infection from two sources. Firstly, over-wintered infective larvae on the pasture, (Rose, 1965 ; Gibson and Everett, 1967). Secondly, the eggs deposited by the ewe undergoing the post-lambing rise in nematode faecal egg counts. The over-wintered larvae available on the pasture as soon as the lambs began to graze, resulted in the establishment of worm infections in June. Occasionally, the numbers of larvae over-wintering were sufficient to cause a check in lamb growth rate at a time of fast growth (Boag and Thomas, 1971 ; Thomas and Boag, 1973 ; Gibson and Everett, 1973a ; Donald and Waller, 1973 ; Brunsdon, 1974 ; Armour and Urquhart, 1974 ; Michel, 1976).

Gibson and Everett, (1973a,b : 1975a,b ; 1976) conducted a series of field/plot trials in which they simulated the post- parturient rise contamination by O. circumcincta and also different levels of residual, over-wintering pasture infection. The results demonstrated that on pasture receiving only the post-

parturient ewe contamination, the build up of larval populations on the herbage and consequently the danger to lambs, would be free from early June onwards. On pasture where only over-wintered infestation was present larval build up did not occur until lamb autoinfection began in mid August. They also reported that the different patterns of larval availability resulting from the two sources, together with time of lambing, temperature and rainfall have a profound effect on faecal output and the development of worm burdens, and hence the performance of lambs.

Outbreaks of disease therefore, occur in two phases ; the first is seen in lambs during the grazing season and usually occurs from July onwards, the ewes being the principal source of L3 which produce disease (Michel, 1969 ; Boag and Thomas, 1971 ; Thomas and Boag, 1972 ; Gibson and Everett, 1973 b ; Boag and Thomas, 1977). The second which seems to be less common occurs later in August or September, the lambs being the principal source of infection. This pattern is remarkably similar to that of Type 1 ostertagiasis in calves (Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart (1965). Occasional outbreaks have also been reported in winter, particularly in housed sheep and resulting from maturation of arrested larvae as in bovine Type 11 ostertagiasis (Reid and Armour, 1973).

A different pattern of build up occurs with Nematodirus spp infections (Crofton and Thomas, 1951, 1954 ; Stamp, Dunn and Watt, 1955 ; Thomas and Steven, 1956 ; Baxter, 1957 ; Downey and Conway, 1963 ; Helle, 1969), since eggs passed by lambs in the late spring and early summer of one year do not hatch to become infective L3 stages until the following year, usually in May and June when they rapidly accumulate on pasture (Scarnell and Rawes, 1959 ; Gibson, 1963). Experimental studies by Gibson (1959, 1963) and Thomas and Steven (1960) suggested that N. filicollis follows a less constant pattern, since hatching occurs over a much more prolonged period, from autumn to spring, and as a result disease outbreaks could occur at any time. Studies by Boag and Thomas (1975) on pasture larval patterns of Nematodirus spp showed that N. filicollis larvae regularly first appear on the pasture in autumn and that high numbers of N. filicollis L3 accumulate during autumn and winter, culminating in a peak in spring. Nematodiriasis is a 'lamb to lamb' disease with only one annual cycle of infection and resistance to infection develops by the time the lambs are six months old (Gibson, 1959) so that the adult sheep play little part in the life cycle of the parasite. As a result, in Britain, outbreaks have occurred due to N. battus infection in May and June principally in lowland flocks (Thomas and Steven, 1960 ; Boag and Thomas, 1975) and also in hill lambs grazing on re-seeded pasture where heavy congregation of stock has occurred, (Gibson, 1973 ;

Armour and Urquhart, 1974 ; Reid, 1976). If susceptible lambs graze on contaminated pasture during the larval peak for several years the levels of infection will increase until it reaches epidemic proportions.

THE ROLE OF INTERCURRENT INFECTIONS IN HELMINTHIASIS.

There is some evidence that intercurrent infections with other organisms can also affect the pathogenesis of some helminthiasis. Reid (1976) has reported that an interaction between N. battus and Eimeria spp occurs in sheep. During outbreaks of nematodiriasis in 4-8 week old lambs he found large numbers of coccidial oocysts in the faeces of these lambs ; when the lambs were treated with anthelmintic to remove the N. battus worms, several animals failed to respond and diarrhoea continued. Treatment of the diarrhoeic lamb with an anti-coccidial, sulphadimidine, then resulted in the disappearance of diarrhoea. Since many lambs had large numbers of coccidial oocyst in the faeces without eveidence of Nematodirus infection but were clinically normal, Reid suggested that the interaction of the two species enhanced the pathogenic effects of the coccidia. This has been confirmed by Gregory, Joyner, Catchpole and Norton (1980b) who found that lesions of coccidiosis are closely associated with parasite development. However, in Norway (Helle, 1971) estimated that the presence or absence of nematodes did not seem to have any influence on the number of oocyst in the faeces of lambs and stated that the establishment of coccidia in lambs in the spring, occurred during the first days on pasture when few nematodes had been ingested. This, of-course, does not rule out Reid's

theory that the presence of both parasites results in greater pathogenicity.

Several interactions between parasites and bacteria have been reported, sometimes with enhanced clinical disease resulting. In the rat infection with the gut nematode Nippostrongylus brasiliensis and the protozoan Trypanosoma brucei, Urquhart, Murray, Jennings and Bate (1973) resulted in an impaired immune expulsion of the nematode in rats also infected with the protozoan compared to monospecific infections ; a similar observation was made with the mouse parasites Nematospiroides dubius and Trichuris muris by Jenkin and Benhke (1977). In cattle, enhanced pathogenicity has been reported when, Fasciola hepatica and Salmonella dublin were both present (Aitken, Jones and Hughes, 1976) while in pigs infections with the nematode Trichuris suis and the spirochete Treponema hyodysenteriae resulted in a particularly severe typhilitis, (Beer and Rutter, 1972). In some of these reports it is difficult to decide whether pathogenicity is enhanced or whether immuno-suppressing effect of one parasite on the other occurs.

With the exception of the Norwegian studies (Helle, 1971) all of the above epidemiological studies have been carried out in flocks which graze throughout the year and the impact of winter housing, a management procedure which is increasing in prevalence, has not been studied. The first part of this thesis deals with observations on the epidemiology of parasitic gastro-enteritis in a flock of sheep which lambed indoors in late March and then grazed until the following

November. The possible interaction between PGE and coccidiosis which might occur as a result of the congregation of sheep indoors favouring a build up of coccidial oocysts is also studied.

MATERIALS AND METHODS.

EXPERIMENTAL ANIMALS.

The ewes and lambs used in the experiments were of the Scottish Blackface breed. They came from two sources. The permanent flock was purchased from a local farmer prior to the onset of the experiments in which the custom was to house the flock during the winter. The tracers were purchased from a local farm when a week old and then reared under helminth free conditions. During the experiments the ewes and lambs were grazed on plots situated within the grounds of Glasgow University, which had been grazed regularly by sheep for a number of years. The area of each grazing plot was approximately 0.33 hectares and separation of adjacent plots was achieved by means of a double wire mesh fence with 1.5 metres between fences. In 1981, supplementary feeding consisting of $\frac{1}{2}$ to 1kg of concentrates daily was given to lambs after weaning ie. mid July-November.

CLINICAL EXAMINATION.

When the ewes and lambs were examined their condition was assessed on the basis of appearance, appetite and body weight. The latter was measured by using a weigh crate suitable for small ruminants (Avery Scales Ltd., Glasgow, Scotland).

BIOCHEMICAL, HAEMATOLOGICAL TECHNIQUES.

Blood samples for pepsinogen and packed cell volume percentage (PCV) estimation were taken directly from the jugular vein into

heparinised vacutainer tubes (Becton-Dickson Ltd., York House, Empire Way, England). After determination of the PCV by the Micro-haematocrit method (Hawksley and Son Ltd., London, England) the remainder of the samples were centrifuged at 2000 revolutions per minute (rpm) for 20 minutes. The plasma obtained was then processed to determine the level of pepsinogen present, the method used being that described by Edwards, Jepson and Wood (1960). In this technique the plasma is incubated with bovine serum albumin (BSA) at pH 2.0 for 24 hours and the phenolic amino acids (tyrosine) liberated estimated using the Folin-Ciocalteau reaction. Corrections are made for the normal (ie. non-incubated content of tyrosine substances) and also for the release of these substances from BSA when incubated alone. The detailed procedure used is given in Appendix 1.

Plasma pepsinogen values have been expressed as international units (i.u.) of tyrosine to the nearest 0.1 i.u.

PARASITOLOGICAL TECHNIQUES.

a) Faecal Analysis.

This was done using a modified McMaster flotation (Gordon and Whitlock, 1939) and where necessary a simple centrifugal flotation technique.

b) The Modified McMaster Method.

In this technique 3 g. of faeces were homogenised with 42ml of water and the resultant suspension passed through a 250 micron sieve (Endecotts Test Sieves Ltd., Morden, London). After thorough mixing of the filtrate, 15ml were withdrawn into each of two flat bottomed centrifuge tubes (capacity 15ml) and the latter centrifuged at 2000 rpm for two minutes. The supernatants from both tubes were then discarded and the remaining faecal mass broken up by rotary agitation. One tube was then filled to its former level with saturated salt solution and after inverting 6 times a volume of the suspension, sufficient to fill both chambers of the McMaster slide was quickly transferred by pipette to a McMaster slide. The number of eggs and oocysts under the etched areas of both chambers of the slide were counted and the result multiplied by 50 to give the number of eggs and oocysts per gram of faeces according to the following calculation :

3 g. of faeces in 42ml gives 1 g. in 15ml.

and

The volume under one etched chamber of the McMaster slide equals 0.15ml.

The No. of eggs and oocysts seen in one square

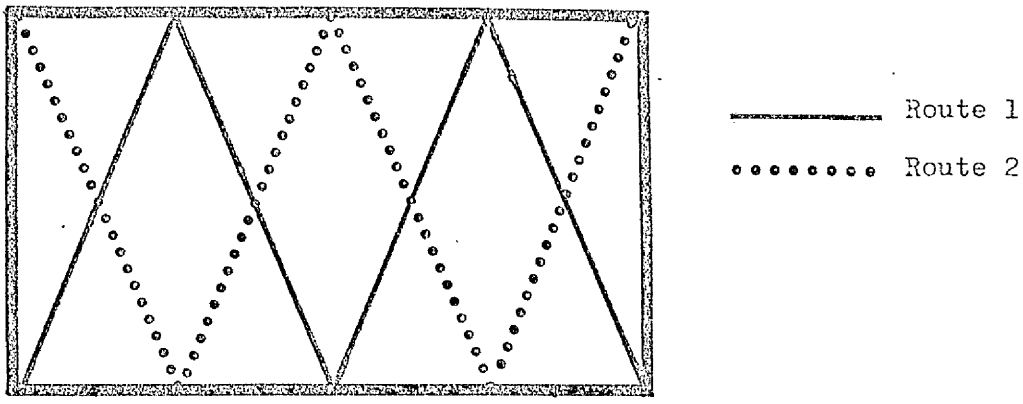
$\therefore$ x 100 (15 ÷ 0.15) = the No. of eggs/gram

or - The total No. of eggs and oocysts seen in both chambers x 50 (15 ÷ 0.3) = the No. of eggs/gram.

When eggs and oocysts were not detected in the McMaster slide the second centrifuge tube was filled with saturated salt solution, a coverglass placed on top and the tube centrifuged at 1500 rpm for one minute. By this process all trichostrongyle eggs and oocysts in the sub-sample were concentrated at the top of the tube and by carefully transferring the coverglass to a plain glass slide, could be counted. Eggs and oocysts recovered in this way were recorded as the actual number of eggs and oocysts per gram. Differentiation of 100 oocysts and the percentage species distribution was then determined.

c) Analysis of herbage for trichostrongyle L3.

Pasture samples were collected by traversing the experimental plot as shown in the following diagram :



Fifty evenly spaced stops were made along routes 1 and 2 and at each stop four plucks of grass (the amount that could be grasped between thumb and forefinger) were taken giving a total of 400 plucks per plot. The grass was then processed by a method similar to that used by Parfitt (1955) and was as follows :

After weighing, the grass was soaked in 20 litres of warm water plus 5ml .non-ionic detergent ("Lissapol", I.C.I. Ltd., U.K.). Twenty four hours later the herbage was transferred to a fresh 20 litres of warm water and rinsed overnight after which, as much water as possible, was removed by squeezing and the grass spread on trays. After drying the herbage was again weighed and this dry weight (to the nearest gram) was used in the final calculation thus allowing the number of larvae present to be expressed as L_3/kilogram dried herbage.

The grass washings were allowed to sediment for a minimum of six hours, the supernatant drawn off and the sediments bulked. After filtration through a double milk filter (Maxa Filters, A. McCaskie, Stirling, Scotland) the larvae retained were recovered using a Baerman apparatus, consisting of a glass funnel closed at the stem with a length of rubber tubing and clip and filled with warm water (Plate 1). A 150 micron aperture

Plate 1. Baermann apparatus for the extraction of nematode larvae from herbage and faeces.

sieve supported the milk filter, thus allowing the motile larvae to migrate to the warmer side of the temperature gradient. Twelve hours later, 200ml of fluid was withdrawn from the neck of the funnel and reduced by a process of sedimentation and centrifugation to a final volume of 10ml. The larvae in 1ml were differentiated and counted. The criteria for larval identification were those detailed in Technical Bulletin No. 18 (Ministry of Agriculture, Fisheries and Food) and used by Keith (1953) ie. body length, prolongation of the second larval sheath beyond the tail of the third stage larvae stages.

d) Preparation of larval inocula.

Faeces containing Trichostrongyle ova spp were collected from naturally infected ewes and lambs using a bag and harness system. These were subsequently mixed with Vermiculite, 100 g. aliquots placed in screwtop glass jars and incubated at 23°C for 14 days. The infective larvae were recovered by standard Baerman techniques as described previously.

Larval inocula was prepared by counting the number of larvae present in 40 x 0.025ml aliquots. A minimum total number of 400 larvae were counted and to facilitate examination the larval dilution was so arranged that the

number of L_3 per 0.025ml aliquot did not exceed 30. Once the number of L_3 present in 1ml was known the volume necessary to provide the required inoculum was pipetted out and made up to a volume of approximately 20ml prior to dosing the ewes. Throughout the whole counting procedure emphasis was placed on regular agitation of the suspension to prevent clumping.

e) Differential larval counting.

After faecal culture, a differential larval count of trichostrongyle spp was carried out. The infective larvae were recovered by standard Baerman techniques as described previously, in a final volume of 1ml achieved by sedimentation and centrifugation. One drop of iodine solution was added and at least 100 larvae identified using the criteria outlined in the MAFF Technical Bulletin No. 18.

f) Necropsy procedure.

Grazing animals were housed for at least seven days prior to post-mortem. A captive bolt pistol was used to kill the sheep which were then immediately bled out. After opening the abdomen, the pyloric sphincter was ligatured and the gastro-intestinal tract then removed

from the body cavity.

The abomasum, small intestine and large intestine were separated and the contents were washed into buckets and the volume made up to a standard 4 litres (except where an unusually large amount of material was present when a greater volume of water was required) and duplicate samples of 200ml withdrawn and formalised for subsequent examination. The abomasal mucosa scraped off and digested in three times its volume of pepsin/hydrochloric acid mixture for six hours at 42°C. The digested mixture was then made up to 2 litres and 200ml samples withdrawn as before. The parasites present in 10 x 4ml aliquots were counted identified and classified as adult male or female, developing fourth or fifth larval stages or early fourth stage larvae (EL_4) depending on bursal or vulvar development, the presence of a sheath projection and size respectively. The large intestine and its contents were washed through a 40 mesh sieve on which the worm will be retained.

METEOROLOGICAL OBSERVATIONS.

For the outdoor experiments wet and dry temperatures were recorded at ground level using a mechanically operated thermograph (Negretti and Zambra Ltd., Ayesbury, England) placed in a Stevenson screen. Weekly rainfall was measured using a Symons rain gauge, the results being expressed in millimetres.

A 2 YEAR STUDY ON PARASITIC GASTRO-ENTERITIS AND COCCIDIOSIS IN A SHEEP FLOCK HOUSED DURING WINTER.

1980 AND 1981

INTRODUCTION TO FIELD AND EXPERIMENTAL STUDIES.

From the preceding literature review it is clear that most epidemiological studies on ovine parasitic gastro-enteritis have been conducted in flocks which grazed throughout the year ie. where infections were continuous although the level of larval availability fluctuated considerably. No attempt has been made to examine the impact of winter housing of flocks, a management procedure which is becoming more common, on the epidemiological pattern of parasitic gastro-enteritis in lambs.

The possibility that other parasitic disease such as coccidiosis might increase in flocks which are congregated together during winter housing also requires evaluation ; particularly as the interactions between coccidiosis and nematodiriasis are thought to enhance the severity of the overall clinical effect of both diseases (Reid, 1976).

The field experiments reported in this section of the thesis were designed to evaluate both of the above possibilities in a flock from a farm where winter housing has been in vogue for 10 years. The study was carried out over 2 years and the results from each will be presented separately.

EXPERIMENTAL DESIGN 1980.

Eight Blackface ewes and their 12 lambs were purchased at the end of March, 1980, from a farm where the ewes are housed annually from December until early April. The ewes lambed in mid March so the lambs were approximately 2 weeks old at purchase. Prior to lambing all the ewes in the farm were treated with levamisole (ICI, Macclesfield, England) at recommended dose rates. On arrival at Garscube Estate, Glasgow University, on April 4th the ewes and lambs were introduced onto permanent sheep pasture known to be contaminated with overwintered ovine trichostrongyloid larvae.

The lambs were weaned in mid July and the ewes were then removed from the paddocks and slaughtered ; at this time, three lambs were also removed for slaughter. The remaining lambs, designated permanents (P), continued to graze on the same pasture until the end of October when they were also slaughtered. All the sheep were housed for 7 days prior to slaughter.

During each month from July through November groups of 3 worm free tracer lambs were grazed for a period of 2 weeks each ; at the end of 2 weeks the tracers were housed for 7 days, slaughtered and their worm burdens used to assess pasture infectivity. The idea of housing for 7 days was to allow the ingested larvae to develop beyond the 4 day stage at which arrestment of larvae sometimes occurs and so facilitates the identification of the latter.

All the permanent lambs received fendendazole (Panacur, Hoechst U.K. Ltd., Milton Keynes, Bucks), on two occasions, namely on 17th July and 9th September, a regime of treatment commonly practiced in the upland and hill farms of West Scotland. Individual lambs showing severe nematodiriasis in May were also treated with fenbendazole. The experimental design is summarised in Table 1.

OBSERVATIONS.

Climatic data.

The maximum and minimum temperatures were assessed weekly using a thermograph housed in a Stevenson screen. Weekly rainfall was also recorded using a Symon rain gauge.

Clinical.

The animals were clinically examined twice weekly at which time particular attention was paid to appetite and faecal consistency. At fortnightly intervals all the sheep were weighed.

Haematology and Biochemistry.

The sheep were bled fortnightly (ewes from April-July and lambs from June to the end of October) and the blood was collected from the jugular vein into tubes containing heparin. Packed cell volume percentage (PCV) estimations were determined by the

TABLE 1 Experimental Design 1980.

Animals	Management	Treatment with fenbendazole	Slaughter
Eight ewes	Set stocked from 4th April to 17th July, 1980.	-	21st July, 1980.
* Nine Lambs(P)	Set stocked from 4th April to 31st October, 1980.	17th July & 9th September	7th November, 1980.
Three Lambs (P)	Set stocked from 4th April to 17th July, 1980.	-	21st July, 1980.
5 x 3 Lambs(T)	3 lambs set-stocked for 2 week period July - November	-	From August to end of November, 1980

* one lamb No. (Y45) died in mid - August of severe parasitic gastro-enteritis.

microhaematocrit method and after centrifugation was removed for determination of plasma pepsinogen levels by the method of Edwards, et al, (1960).

Parasitological data.

Faeces

Faecal samples were collected twice weekly from ewes and lambs to detect the presence of trichostrongyle eggs and Eimeria species oocysts by the modified McMaster method (MAFF Technical Bulletin No. 18, 1971) and the results expressed as numbers of eggs and oocysts per gram (epg) and (opg). The faecal samples were also cultured twice weekly and the resultant larvae differentiated at genus level.

Herbage

Weekly herbage samples were examined for the presence of trichostrongyle infective larvae (L3) by the method of Parfitt (1955).

Post-mortem worm burdens

At necropsy the method of Ritchie, Anderson, Armour, Jarrett, Jennings and Urquhart (1966) was used to establish the worm burdens present.

RESULTS.

After assessing the climatic data the remaining results are considered under three main headings namely, coccidiosis, nematodiriasis and other trichostrongyle infections plus *Ch. ovina*. In the first two instances in order to avoid repetition, reference will be made only to clinical and parasitological data ; the section on other trichostrongyles will also include all the biochemical, haematological and post-mortem findings.

COCCIDIOSIS

Climatic data

The temperature and rainfall patterns are shown in Fig. 1. This pattern is fairly typical for the West of Scotland although in late August and September the rainfall was rather higher and the temperature rather lower than normal.

Clinical findings

A watery sometimes very severe diarrhoea characteristic of coccidiosis occurred in individual lambs after three weeks grazing and continued intermittently for another 3 weeks. No clinical evidence of diarrhoea could be detected in the ewes. During the diarrhoeic phase the faeces were subjected to routine bacteriological examination which proved to be negative.

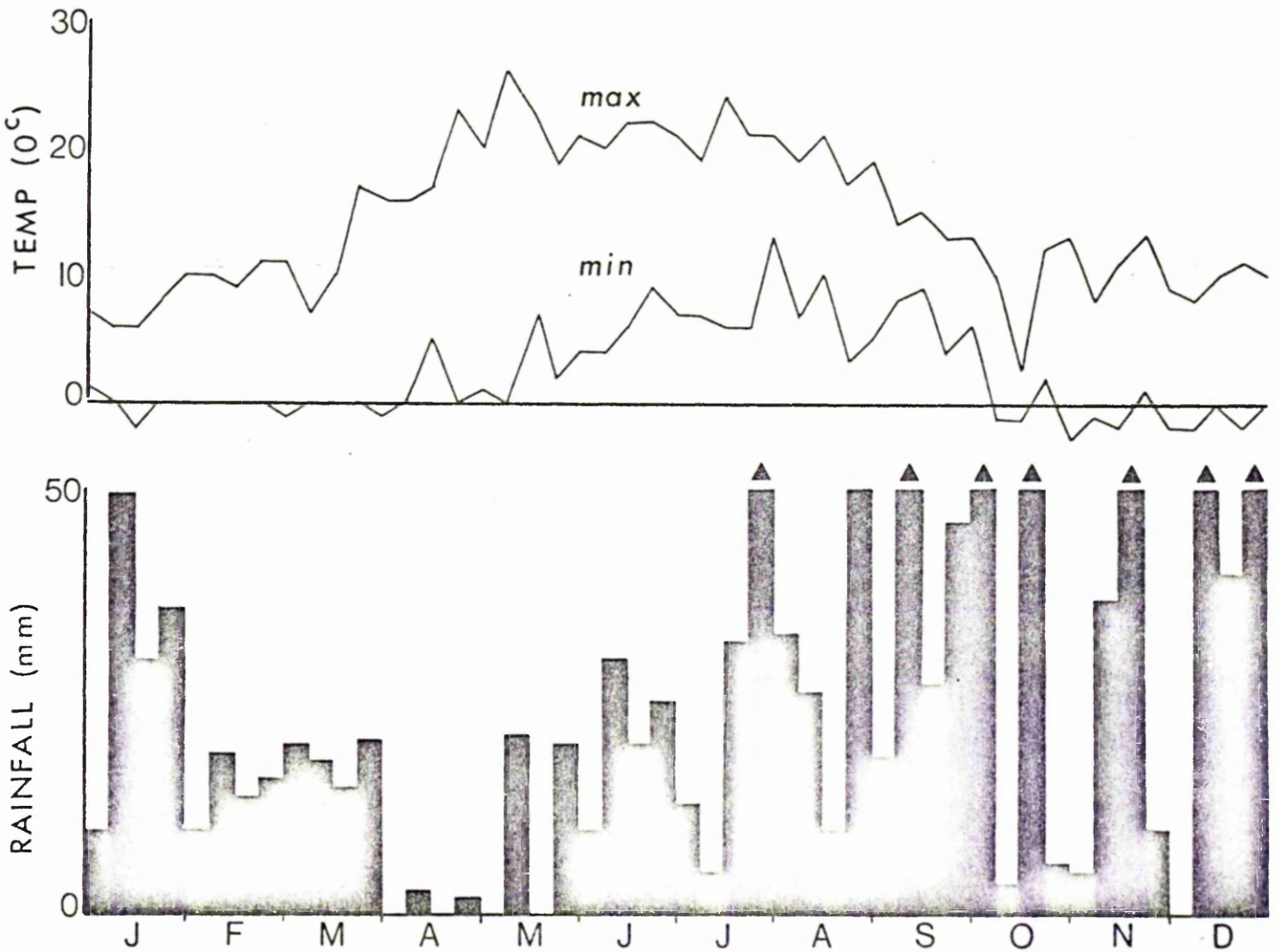


Figure 1. Weekly climatic data 1980.

Faecal oocyst counts

The mean oocyst counts for ewes and lambs are shown in Fig. 2. Positive oocyst counts were regularly recorded from ewes in this experiment and mean values ranged from 0-600 opg. Considerable variation occurred in the oocyst counts of individual ewes as can be seen from Table 2, and ranged from 0-4520. The numbers of oocysts shed in the faeces increased during the middle of May, then declined, to increase again at the beginning of July. The predominant species present were E. oviniodalis and E. crandallis.

The faecal samples from the lambs became positive for coccidial oocysts after only two weeks grazing ie. when they were 3-4 weeks old and rapidly attained a mean peak of 1.29 million with individual counts exceeding 4 million opg ; thereafter, the counts declined and fluctuated around 2 to 300,000 for 6 weeks then declined to very low levels after 10 weeks ie. at the end of June. Eleven different Eimeria species were present and the morphological characteristics of nine of these are shown in Plate 2. The mean numbers of Eimeria species in lambs ranged from 0-1,295,557 while the individual oocyst counts, shown in Appendix 2 ranged from 0-4,252,000. The commonest species were E. ovinoidalis and E. crandallis and the percentage of these species recorded during May were 48% and 35% respectively. The percentage of the different species is given in Table 3.

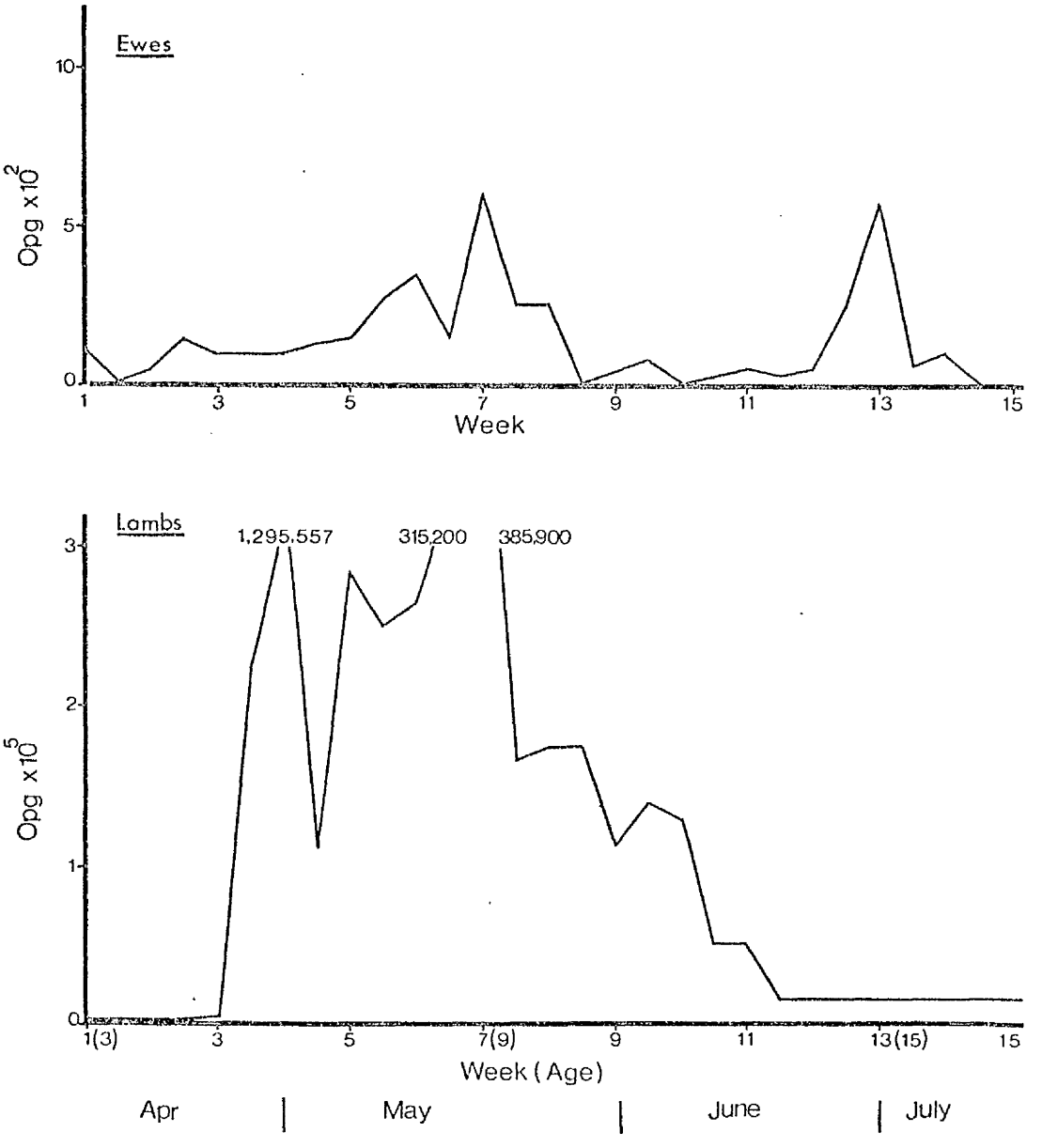


Figure 2. Mean bi-weekly Eimeria spp. faecal oocyst counts of ewes and lambs grazing in 1980.

TABLE 2 Individual bi-weekly Eimeria species of grazing ewes. 1980

Month	Week	EWE NUMBER							
		P61	P62	P66	P67	P71	P72	P74	P75
April	1	50	50	50	50	500	n.s	0	50
		0	0	0	0	50	50	0	0
	2	0	50	n.s	50	250	100	0	0
		400	50	50	300	150	150	150	50
	3	200	50	0	50	150	200	150	150
		n.s	0	n.s	100	500	0	0	n.s
May	4	200	200	50	100	0	250	0	50
		0	250	250	n.s	250	150	0	0
	5	500	50	0	400	n.s	50	50	50
		300	100	500	100	1000	n.s	0	50
	6	400	200	450	50	1050	450	0	250
		0	420	80	n.s	150	n.s	60	90
	7	190	1330	380	n.s	190	570	190	1330
		190	280	450	0	n.s	760	10	190
June	8	190	380	0	n.s	190	380	380	380
		10	40	80	50	0	50	20	100
	9	n.s	20	100	50	100	50	50	10
		20	n.s	100	10	150	200	50	50
	10	50	0	0	20	50	10	0	0
		10	20	20	100	150	10	0	0
	11	50	20	10	20	n.s	200	50	20
		30	n.s	60	20	50	n.s	30	10
	12	100	20	50	80	150	10	50	10
		0	0	400	n.s	1520	n.s	300	50
July	13	20	20	10	20	4520	50	30	50
		10	n.s	0	70	290	n.s	0	n.s
	14	10	n.s	0	0	400	180	0	0
		10	10	10	10	80	100	0	0

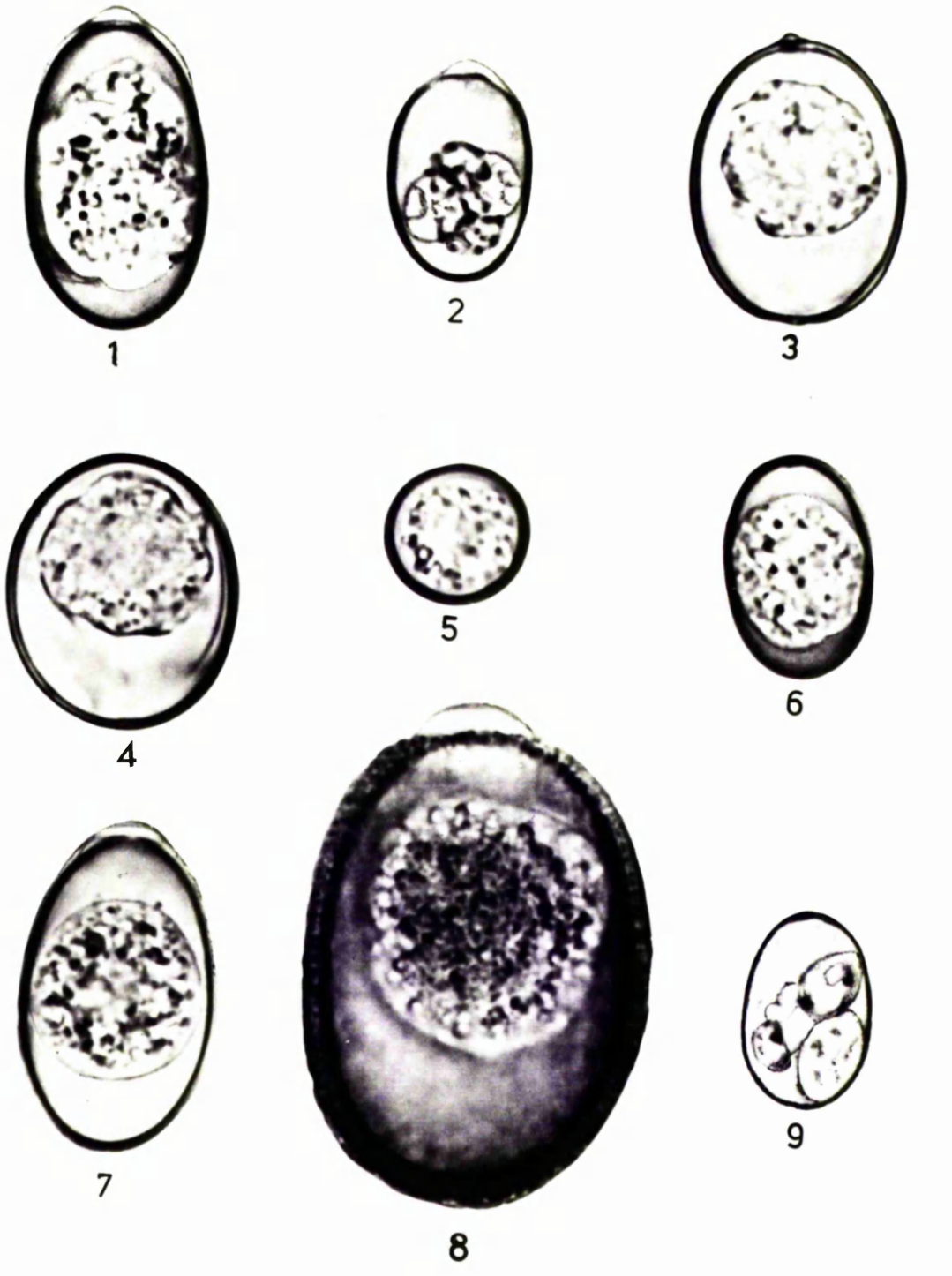


Plate 2. Eimeria species found in the faeces of the permanent lambs

1. E. ovinoidalis 2. E. crandalis 3. E. weybridgensis

4. E. ninaekohlyakimovae 5. E. parva 6. E. pallida

7. E. ahsata 8. E. intricata 9. E. marsica

25 μ

TABLE 3 Percentage of different Eimeria spp in permanent grazing lambs in 1980.

Month	Week	(E.o)	(E.c)	(E.w)	(E.n)	(E.p)	(E.p)	(E.in)	(E.a)	(E.g)	(E.f)	(E.m)
April	4	4.5 7.5	45 33	20 20	12.5 6.5	3.5 8	9.5 14.5	0 1.5	0 1.5	1 0.5	0.5 2.5	3 4.5
May	5	46 32	15 14	7 6	8 12	3 10	3 5	6 2	8 10	0 0	2 1	2 8
	6	40 48	16 17	8 12	8 5	5 1	7 3	1 0	5 10	0 0	5 2	5 3
	7	26 22	16 25	10 15	9 7	11 9	7 5	1 0	4 6	1 0	3 1	12 10
	8	25 32	35 22	21 20	6 6	2 3	3 5	0 1	2 2	0.5 0	1 3	4.5 6
	9	28 30	35 20	19 11	4 9	3 1	2 2	1 7	1 12	1 1	2 1	4 6
June	10	40 42	18 16	22 19	9 11	3 3	2 1	1 0	1 3	1 1	2 2	2 2
	11	39 35	17 18	19 18	10 10	3 3	2 3	0 1	2 3	1 1	2 1	5 7
	12	36 18	20 24	20 18	7 6	5 6	4 5	0.5 1	0.5 2	1 1	0 2	6 17
	13	20 20	22 20	20 22	7 7	5 5	7 7	0 0	2 0	0 3	2 6	15 10

Key to Eimeria spp.

E.o	=	E. oviniodalis	E.p	=	E. parva	E.g	=	E. granulosa
E.c	=	E. crandallis	E.p	=	E. pallida	E.f	=	E. faurei
E.w	=	E. weybridgensis	E.in	=	E. intricata	E.m	=	E. marsica
E.n	=	E. ninackohlyakimovae	E.a	=	E. ahsata			

NEMATODIRIASIS

Clinical findings.

Diarrhoea occurred in a few individual lambs in May and in several lambs in June ; this was attributed to *N. battus* infections since it responded to anthelmintic treatment with fenbendazole. No adverse clinical signs were noticed in the ewes.

Parasitological data.

Faecal egg counts

N. battus eggs were not usually found in the ewes, except in one animal which had 100 *N. battus* epg in mid May, and in another which had 90 epg at the end of May. The mean *N. battus* egg counts in lambs is shown in Fig. 3. *N. battus* eggs were first detected in the faeces of lambs after 4 weeks grazing. Individual epg's of lambs are shown in Appendix 3, ranging from 0-2440. The numbers of eggs present in May were low but increased markedly during early June reaching a mean maximum of 585 epg then declined to zero by late July. While this reduction was partially attributed to the treatment of individual lambs it also occurred in the untreated animals.

Nematodirus spp.

Larval herbage counts

Pasture larval numbers of *N. battus* are also shown in Fig. 3.

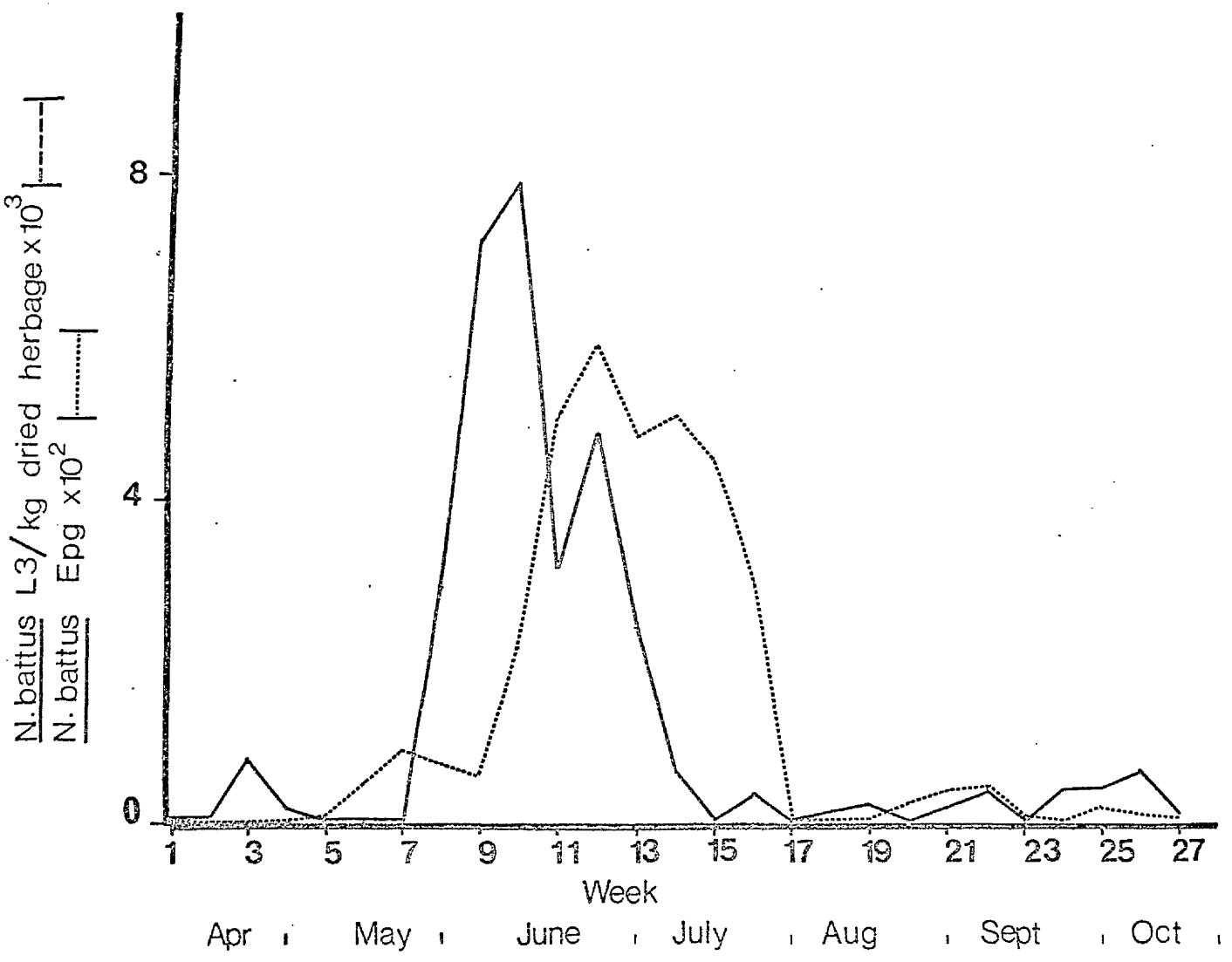


Figure 3. Weekly Nematodirus battus herbage larval recoveries plus mean N. battus faecal egg counts of lambs grazing in 1980.

These were low until late May when a sharp increase in the numbers of larvae occurred reaching a maximum of 7777 L3kg per dry herbage in early June falling to zero by the end of July. After this a second increase of Nematodirus spp (principally N. battus) larvae occurred reaching a maximum of 600 L3 in October.

Nematodirus spp Worm Burdens

Details of these are given along with those of the other trichostrongyles on page 74. N. battus burdens were heavy in the lambs killed in July (ᵔ 10,000) and this species was present in all the permanents slaughtered in November and in all the tracers. The burdens in the latter were highest in August and October. N. filicollis was also regularly present but in lower numbers.

OTHER TRICHOSTRONGYLE INFECTIONS.

Clinical findings.

Clinical signs of parasitic gastro-enteritis characterised by semi-fluid faeces were noticed in individual animals and occurred in July and August in which month Lamb No. Y45 died.

Live weight gains.

The individual liveweights of ewes and lambs are shown in Tables 4 and 5 respectively. The mean liveweight gain of the

TABLE 4 Individual fortnightly liveweights (Kg) of grazing ewes in 1980.

Month	Week	EWE NUMBER							
		P61	P62	P66	P67	P71	P72	P74	P75
April	1	47	38	55	40	39	57	46	50
	3	48	40	53	42	39	57	48	50
May	5	48	41	52	43	39	57	49	49
	7	48	42.5	55	47	40.5	58	48	54
June	9	52	43	59	49	45	59	52	58
	11	50	44	58	48	43	58	50	55
July	13	50	41	54	44	41	59	50	54
	15	49	39	54	47	43	60	51	54

TABLE 5 Individual fortnightly liveweights (Kg) of permanent grazing lambs in 1980.

Month	Week	LAMB NUMBER.											
		Y42	Y44	Y45	Y46	FR72	FR78	FR88	FR89	FR90	FR92	FR93	FR94
April	3	11	9	5	10	13	11	8	10	12	15	11	17
May	5	14	10	9	12	17	12	9	11	15	20	12	20
	7	17	15	10	15	20	15	12	16	19	23	15	25
June	9	20	20	13	19	23	16	16	18	22	28	20	28
	11	23	22	16	21	27	20	20	21	25	32	22	32
July	13	25	26	16	23	27	20	20	22	27	34	21	32
	15	28	27	14	25	30	21.5	23	24	26	37	21	33
		*			*			*					
August	17	-	27	15	-	30	20	-	25	30	40	23	35
	19	-	32	**	-	33	21	-	29	30	45	25	44
Sept.	21	-	34	-	-	36	23	-	30	35	49	26	43
	23	-	39	-	-	39	25	-	32	36	50	28	45
Oct.	25	-	41	-	-	40	26	-	35	38	53	30	47
	27	-	42	-	-	41	29	-	34	39	54	29	49

* Animal slaughtered

** Animal died

ewes was very low being 0.03kg/day. The mean liveweight gain of the lambs until the end of July,ie. at weaning, was 0.15kg/day and after post-weaning, ie. from July to the end of the experiment in October, it was 0.13kg/day.

Haematology and Biochemistry.

Packed cell volume

The fortnightly PCV's of the lambs are shown in Table 6. Individual percentages ranged from 26% - 40%. The highest mean value of 34% was recorded in early June and then a decline occurred during August to a mean level of 28% by early September.

Plasma pepsinogen levels

The mean pepsinogen levels of ewes and lambs are shown in Fig. 4. During the first 6 weeks of grazing the pepsinogen levels of the ewes increased from an average of 0.6 i.u. to reach a mean maximum of 2.3 i.u. at the end of June. The mean individual pepsinogen levels of the lambs increased from 1.2 in June to 2.1 in September and then decreased at the end of September to 0.6 ; this coincided with the second anthelmintic treatment although a minor increase occurred again in October (from 0.6 to 1.0). Individual pepsinogen values of both ewes and lambs are given in Appendix 4.

TABLE 6 Individual fortnightly packed cell volume percentages (P.C.V. %) of permanent grazing lambs in 1980

Month	Week	LAMB NUMBER											
		Y42	Y44	Y45	Y46	FR72	FR78	FR88	FR89	FR90	FR92	FR93	FR94
June	9	40	34.5	30	34.5	33	34.5	32	32	36.5	35	35.5	34.5
	11	39.5	35.5	35	34	31.5	36	31.5	33	34	35	32	33
July	13	38	33.5	36	33	32	39	33	31	33	32	30	33
	15	- *	33	33.5	-*	35.5	35	-*	32	35	36.5	28	33
August	17	-	34	34	-	33	35	-	32	35	33	30	32
	19	-	31	30 **	-	32.5	34	-	30	33	34	30	31
Sept.	21	-	33	**	-	30	26	-	26	31	31	26	28
	23	-	33	-	-	31	31	-	30	33	33	28	30
Oct.	25	-	33	-	-	31	31	-	30	32	35	30	28
	27	-	31.5	-	-	31	29.5	-	30	31	33	32	30

* Animal slaughtered

** Animal died

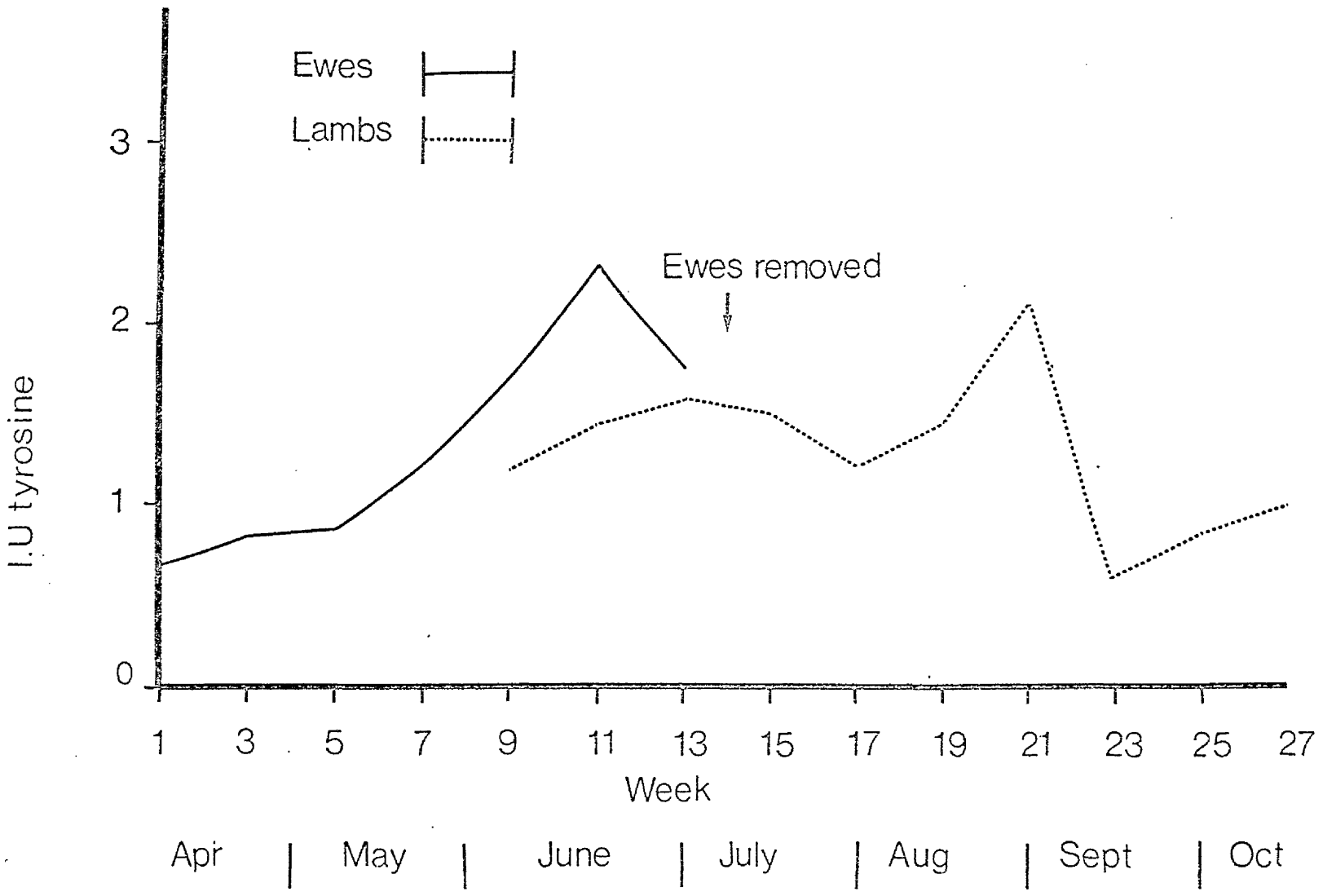


Figure 4. Mean fortnightly plasma pepsinogen levels (Iu tyrosine) of ewes and lambs grazing in 1980.

Parasitological data.

Faecal egg counts

Mean bi-weekly faecal egg counts of ewes and lambs are shown in Fig 5 and individual values in Appendix 5. Positive counts were obtained from ewes at beginning of the grazing period in April when a mean count of 337 epg was recorded ; the mean counts decreased during April and then increased to reach a mean maximum of 363 epg by late May. Thereafter, it decreased to 40 epg by July.

The lamb faeces were first positive at the beginning of May and increased to a mean of 800 epg at the end of June, following anthelmintic treatment the egg counts fell to zero and increased again to a maximum of 1105 by late August. Individual faecal egg counts ranged from 0-6060 epg. Following each anthelmintic treatment in July and September there was a sharp reduction in faecal egg counts to zero but within three weeks considerable numbers of eggs were again present in the faeces. Culture of the faeces showed a predominance of Ostertagia species in both ewes and lambs, except in late June and July when high numbers of Ch. ovina were recovered. Other parasites included H. contortus and Trichostrongylus species. The percentages of genera recovered from the ewes is shown in Appendix 6 and from the lambs in Appendix 7. Fig. 6 illustrates their seasonal fluctuation.

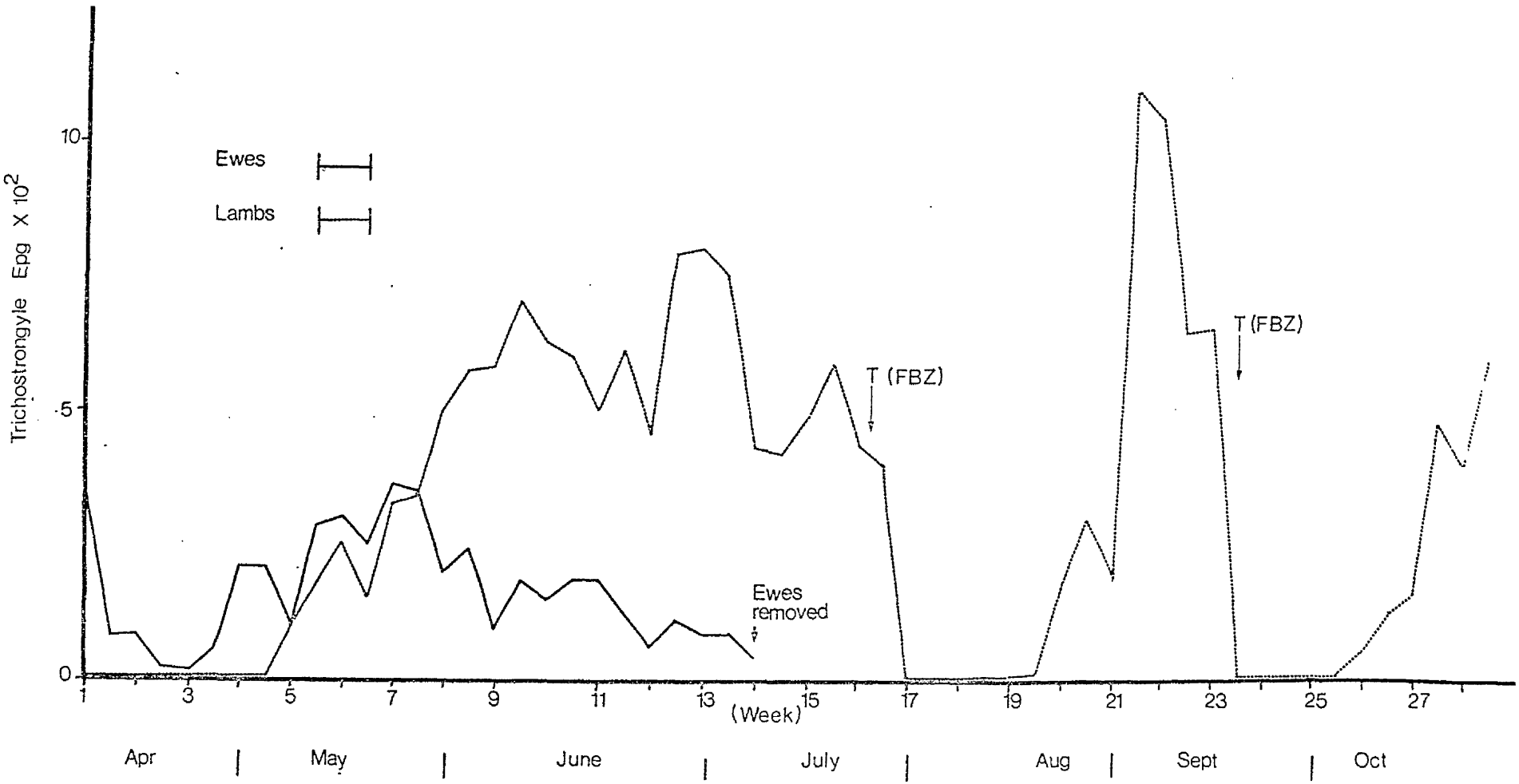


Figure 5. Mean bi-weekly trichostrongyle faecal egg counts of ewes and lambs grazing in 1980.

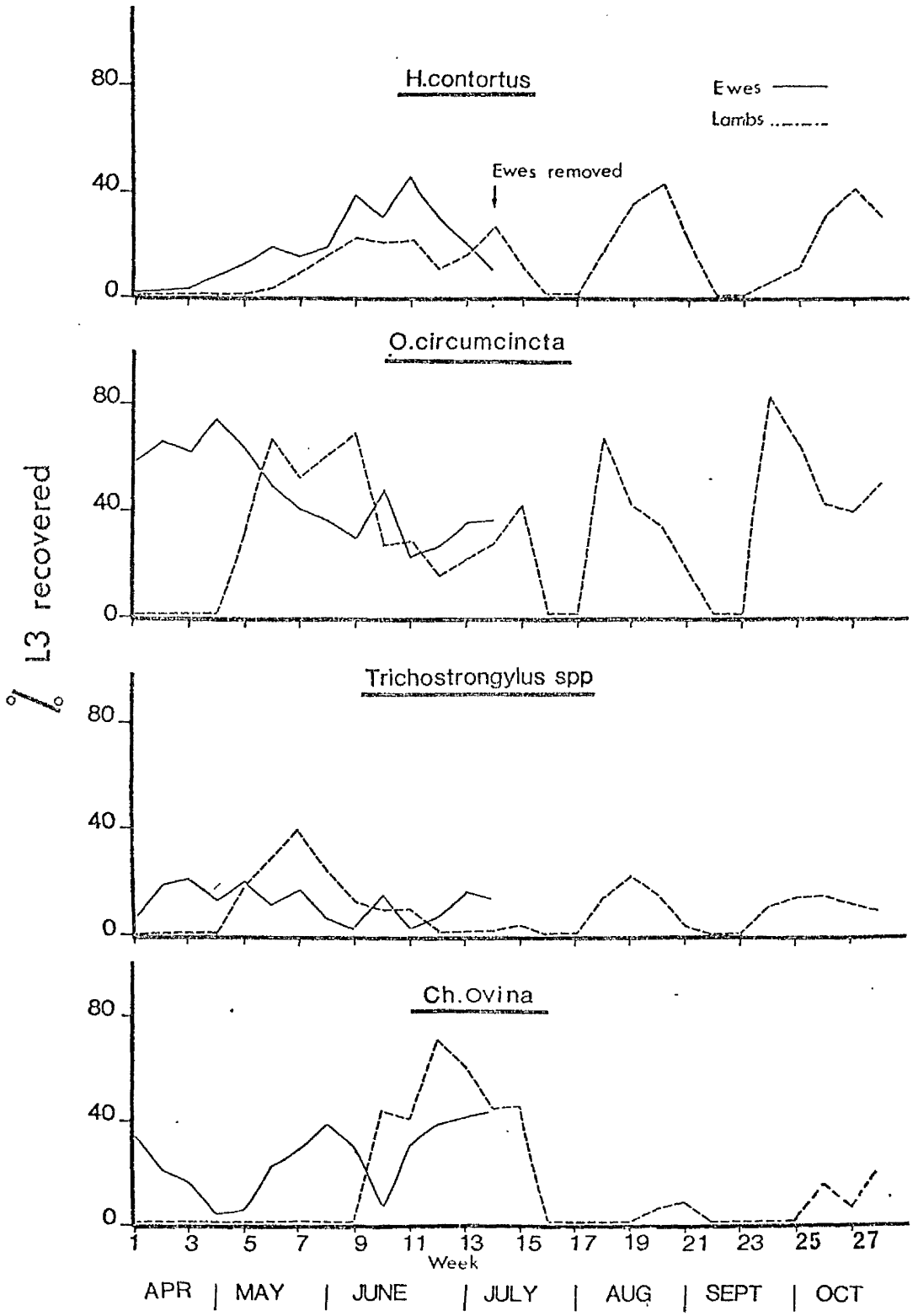


Figure 6. Percentage larval species recoveries from ewe and lamb faecal cultures 1980.

Larval herbage counts.

The total numbers of trichostrongyle L3 recovered from the pasture analysis except for N. battus are shown in Fig. 7 and those of individual genera including Ostertagia species, H. contortus, Trichostrongylus species and Ch. ovina illustrated in Fig. 8. In April the total larval population was 4,424 L3/kdh and this declined to zero by the beginning of June, thereafter a marked increase occurred reaching a maximum 40,400 in late June and then decreased to a level of 220 in late August and remained around this level until November. Appendix 8 details the weekly recoveries of infective larvae from herbage.

Apart from N. battus L3, the larvae of Ostertagia spp (entirely O. circumcincta according to worm burdens) were the most prevalent species present. The peak O. circumcincta level of 22,400 L3/kdh occurred in late June and coincided with the overall peak of other trichostrongyles. The maximum numbers of Trichostrongylus spp (which were mainly T. vitrinus according to the worm burdens) also occurred in late June and reached 16,600 L3/kdh.

Two peaks of H. contortus L3 occurred. The first, and the lesser one, coincided with those of O. circumcincta and Trichostrongylus spp was 2000 L3/kdh ; the second, and major peak of 3548 L3/kdh, occurred in late July.

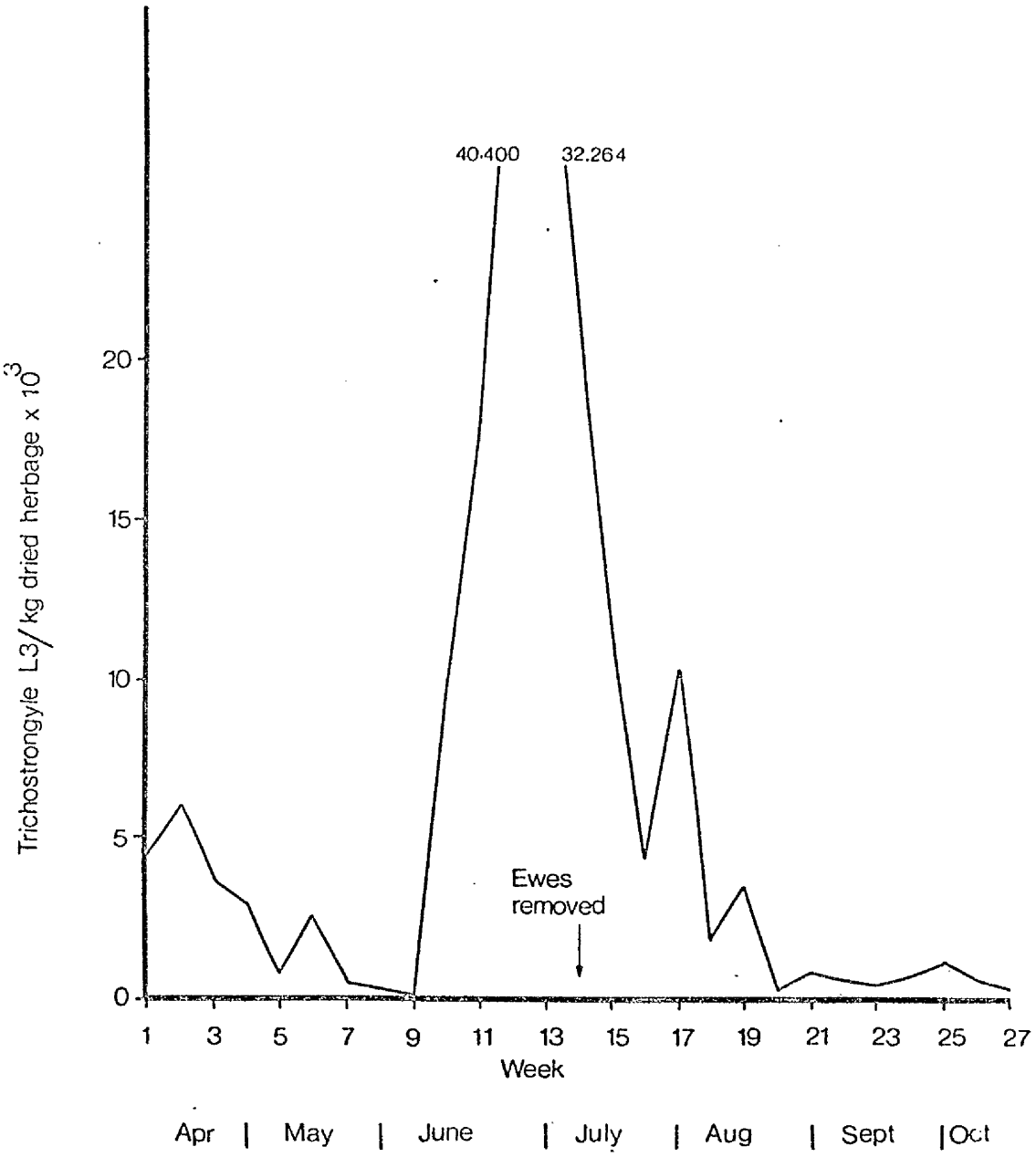


Figure 7. Weekly trichostrongyle herbage larval recoveries (excluding N.battus) in 1980.

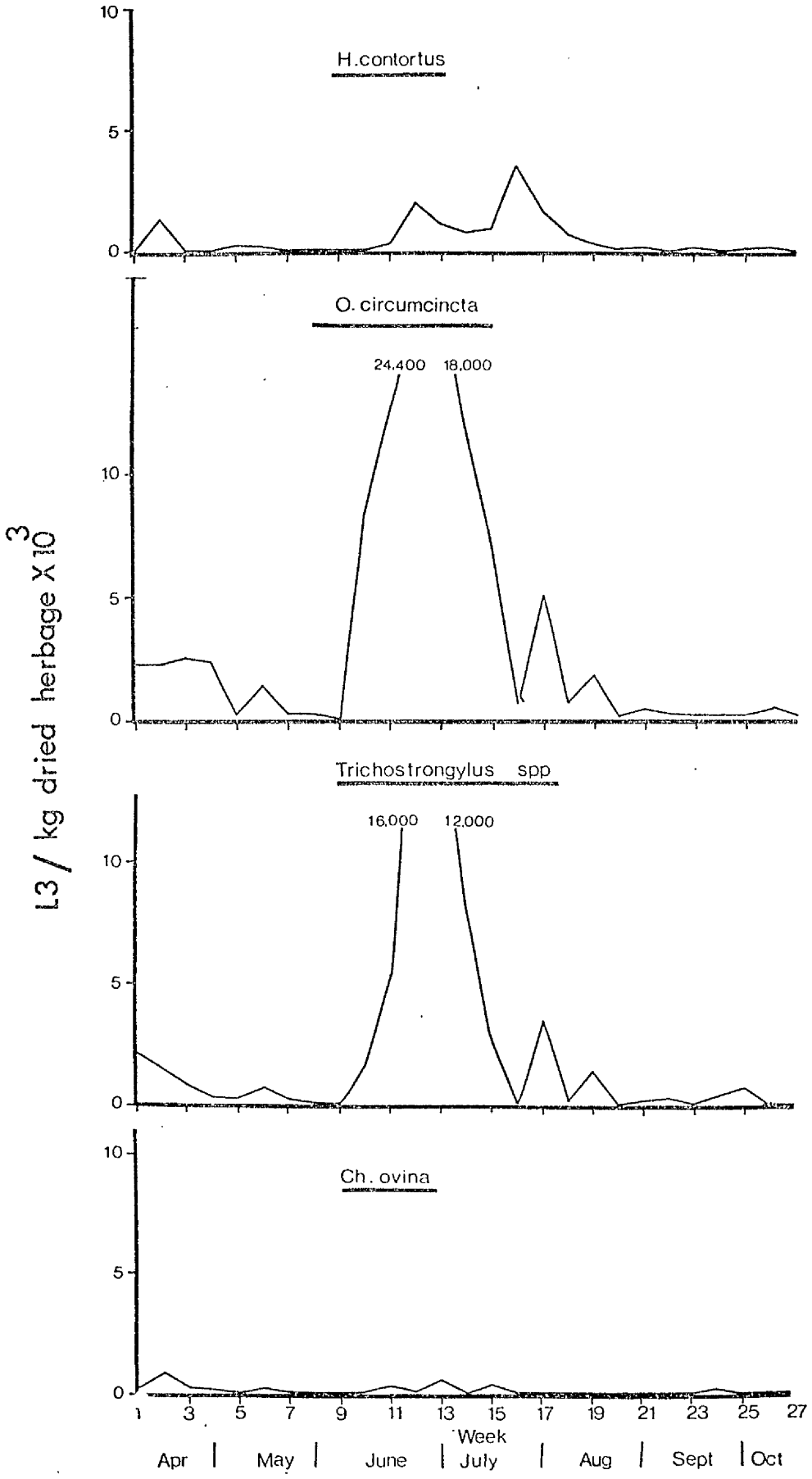


Figure 8. Weekly herbage larval recoveries of individual genera in 1980.

Ch. ovina L3 were present in the highest numbers in April, with a peak level of 887 L3/kdh. After April, the L3 of this species were present intermittently the highest number being 612 L3/kdh in late June.

Post-mortem Worm Burdens

The individual mean worm burdens of the ewes slaughtered in July are shown in Table 7. The mean total worm burden was 4248 and they ranged from 204-11,344. The individual genera included O. circumcinta, H. contortus, T. vitrinus, Ch. ovina and Tr. ovis but N. battus were not detected in the ewes ; O. circumcincta were the most prevalent species.

The worm burdens of the permanent lambs are shown in Table 8. The three lambs slaughtered at weaning in July had mean total burdens in excess of 20,000. Of this total an average of 10,599 N. battus were present (range 5700-18,500) while the other trichostrongyles accounted for a mean total of 10,123 (range 3744-16,055). Individual species included O. circumcincta, H. contortus, T. vitrinus, Ch. ovina and Tr. ovis. The worm burden of the lamb which died in August was composed of 200 N. battus and 14,000 other trichostrongyles.

The mean total worm burden of the eight permanent lambs slaughtered in November was 9872. Of this total a mean of 2937 Nematodirus spp were present. N. battus accounted for 2687 (range 200-8,900) of these N. filicollis accounted for 250 (range 0-1,300).

TABLE 7 Individual and mean worm burdens of ewes grazing in 1980

July necropsy	EWE NUMBER							
	P61	P62	P66	P67	P71	P72	P74	P75
O. circumcincta								
♂	0	700	100	500	200	200	0	300
♀	0	500	0	400	100	600	0	100
L5	200	300	0	600	600	100	0	400
DL4	200	1400	1500	2200	1000	5100	200	1000
EL4	100	600	500	600	0	1400	0	300
Total	500 (20)	3500 (17)	2100 (29)	4300 (14)	1900 (0)	7400 (19)	200 (0)	2100 (14)
			Mean	2750				
H. contortus								
♂	0	0	0	200	0	0	0	0
♀	0	0	0	100	0	0	0	0
L5	0	100	0	100	200	0	0	0
DL4	0	100	0	100	100	200	0	200
EL4	0	0	0	0	0	0	0	0
Total	0	200	0	500	300	200	0	200
			Mean	175				

Figures in brackets indicate % arrested larvae

TABLE 7 (Continued)

	EWE NUMBER							
	P61	P62	P66	P67	P71	P72	P74	P75
T. axei								
♂	0	0	100	0	200	300	0	100
♀	200	100	0	0	100	200	0	300
L5	0	0	0	0	0	0	0	0
DL4	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0
Total	200	100	100	0	300	500	0	400
			Mean	200				
T. vitrinus								
♂	0	400	0	1200	200	1800	0	200
♀	0	400	0	600	400	1000	0	0
L5	0	1600	0	200	200	400	0	0
DL4	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0
Total	0	2400	0	2000	800	3200	0	200
			Mean	1075				
C. ovina								
Adult	0	278	0	12	10	34	4	23
			Mean	45				
T. ovis								
Adult	0	0	8	0	0	10	0	9
			Mean	3.3				
Totals	700	6478	2208	6812	3310	11,344	204	2932

Mean 4248

TABLE 8 Individual and mean worm burden of permanent grazing lambs in 1980.

July necropsy	LAMB NUMBER		
	Y42	Y46	FR88
O. circumcincta			
♂	100	2300	1200
♀	0	1500	900
L5	700	3000	2400
DL4	1600	5200	3400
EL4	700	500	400
Total	3100	12500	8300
	(22.5)	(4)	(5)
	Mean 7966		
H. contortus			
♂	100	100	0
♀	0	100	100
L5	200	200	400
DL4	200	400	400
EL4	100	0	0
Total	600	800	900
	(16.5)		
	Mean 766		
T. axei			
♂	0	100	100
♀	0	100	0
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	0	200	100
	Mean 100		
N. battus			
♂	3200	7800	1800
♀	3800	10000	3200
L5	0	100	100
DL4	400	600	800
EL4	0	0	0
Total	7400	18500	5700
	Mean 10599		

Figures in brackets indicate % arrested larvae.

TABLE 8 (Continued)

	LAMB NUMBER		
	Y42	Y46	FR88
T. vitrinus			
♂	0	1000	400
♀	0	1200	1000
L5	0	200	0
DL4	0	0	0
EL4	0	0	0
Total	0	2400	1400
		Mean 1266	
Ch. ovina			
Adult	26	108	43
		Mean 59	
T. ovis			
Adult	18	47	24
		Mean 29.5	
Totals	11,144	34,555	16,467
		Mean 20,722	

TABLE 8 (Continued)

November necropsy	LAMB NUMBER								
	Y44	Y45*	FR72	FR78	FR89	FR90	FR92	FR93	FR94
Ostertagia spp									
♂	1900	3100	1700	1200	800	200	1400	200	1800
♀	1300	2900	1200	1000	1400	300	1400	600	1600
L5	0	2900	0	0	200	0	0	200	0
DL4	700	2300	0	100	0	0	100	0	500
EL4	1000	0	200	2000	200	200	500	500	300
Total	4900	11200	3100	4300	2600	700	3400	1500	4200
	(20)	(0)	(6.5)	(46.5)	(10)	(28.5)	(15)	(33)	(7)
					Mean 3087				
H. contortus									
♂	200	200	600	100	0	100	300	0	200
♀	200	300	0	100	200	0	100	200	0
L5	0	800	0	100	0	0	0	0	0
DL4	100	400	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0	0
Total	500	1700	600	300	200	100	400	200	200
					Mean 312				
T. axei									
♂	0	400	0	0	0	0	0	0	0
♀	0	0	0	0	0	0	0	0	0
L5	0	0	0	0	0	0	0	0	0
DL4	0	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0	0
Total	0	400	0	0	0	0	0	0	0
					Mean 0				

TABLE 8 (Continued)

	LAMB NUMBER								
	Y44	Y45*	FR72	FR78	FR89	FR90	FR92	FR95	FR94
T. vitrinus									
♂	2000	300	1000	1200	2200	600	2500	1800	2200
♀	4800	200	600	1800	600	1600	2400	2100	600
L5	0	100	0	0	0	0	0	0	0
DL4	0	100	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0	0
Total	6800	700	1600	3000	2800	2200	4900	3900	2800
					Mean 3500				
N. battus									
♂	100	0	800	300	0	200	2000	100	1600
♀	100	100	200	200	200	400	2500	100	2000
L5	1000	100	0	200	200	600	2700	0	4800
DL4	500	0	0	0	0	0	200	0	500
EL4	0	0	0	0	0	0	0	0	0
Total	1700	200	1000	700	400	1200	7400	200	8900
					Mean 2687				
N. filicollis									
♂	0	0	0	200	0	0	300	0	0
♀	0	0	0	400	0	100	1000	0	0
L5	0	0	0	0	0	0	0	0	0
DL4	0	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0	0
Total	0	0	0	600	0	100	1300	0	0
					Mean 250				

TABLE 8 (continued)

	LAMB NUMBER								
	Y44	Y45*	FR72	FR78	FR89	FR90	FR92	FR93	FR94
Ch. ovina									
Adult	23	0	42	26	6	89	20	30	20
					Mean 32.				
T. ovis									
Adult	2	0	0	3	2	3	3	5	2
					Mean 2.5				
Total burdens	13925	14200	6342	8929	6008	4392	17423	5835	16122
					Mean 9872				

* Died in August - not included in Mean

An average of 6994 (range 3092-12,229) other trichostrongyle were present. The individual species present included O. circumcincta, H. contortus, T. vitrinus, Ch. ovina and a few Tr. ovis. Of these species O. circumcincta was the prevalent in the permanent lambs both in July and November. The numbers of T. vitrinus were higher in the November slaughter.

The mean worm burdens of the tracer lambs are given in Table 9. From this data it can be seen that O. circumcincta, H. contortus and Nematodirus spp (N. battus, N. filicollis) and T. vitrinus were present in all tracer groups. T. axei and Ch. ovina were recovered from the August/September tracers and Tr. ovis from the October tracers.

O. circumcincta was the most prevalent species in all the groups the burdens ranging from 366-6766 except in November when N. battus was the most prevalent at 1766. H. contortus burdens were highest in July/August as were those of T. vitrinus. Individual burdens of the tracers are given in Appendix 9.

The idea of housing all the sheep prior to slaughtering for 7 days was to enable arrested larvae to be identified since arrestment usually occurs at 4 days following ingestion at the early 4th larval stage (EL4). Based on these criteria arrested EL4 stages were regularly present in the O. circumcincta populations and occasionally in H. contortus. The highest numbers of O. circumcincta

TABLE 9 The mean worm burdens of tracer lambs grazing in 1980 (3 per group)

Dates Grazed	Date Killed	O. circumcincta	H. contortus	T. axei	T. vitrinus	Nematodirus N. battus	Nematodirus N.Filicollis	Ch.ovina
28.7-11.8	18.8	6766 (1)	1200 (0)	0	1933	533	66	0
25.8- 8.9	15.9	2766 (10)	666 (0)	33	333	2400	33	11
16.9-30.9	7.10	2633 (25)	800 (4)	0	133	1266	66	0
30.9.-14.10	21.10	1133 (65)	700 (0)	0	166	666	66	0
31.10-14.11	21.11	366 (64)	66 (0)	0	33	1766	666	0

Figures in brackets indicate % arrested larvae

15 Trichuris ovis also found in October tracers

were present in the tracer lambs grazing in September, October and November, the mean percentages of the population being 25%. 64% and 65% respectively. In the permanent lambs slaughtered in November, the mean percentage of arrested O. circumcincta was 20%(range 7-46.5).

The lower percentages of arrested larvae found in the permanents compared with the tracers is due to the fact that the population of the permanents reflects larvae ingested over several weeks whereas the tracer burdens are an indication of the larvae ingested over the previous two weeks. In the ewes, permanent and tracer lambs killed earlier the mean percentages of arrested larvae were lower ranging from 1.5-14%. Arrested H. contortus were found in one permanent lamb in July and a tracer lamb in October.

DISCUSSION

For ease of presentation the discussion will be divided into the same major headings as the results ie. coccidiosis, nematodiriasis and other trichostrongyles. The 1980 and 1981 results are discussed separately and the major findings from both years assessed in a general discussion.

COCCISIOSIS 1980.

A major difference between the clinical picture reported previously for spring lambing flocks in North Britain (Boag and Thomas, 1970, 1971 ; Reid and Armour, 1975) was the occurrence

of an intermittent watery diarrhoea in the lambs towards the end of April ie. when the lambs had grazed for approximately 3 weeks and were aged only 5 to 6 weeks. In the absence of any bacterial infection coccidiosis was diagnosed as the cause of diarrhoea.

Since the acute haemorrhagic of coccidiosis described by Gregory, Joyner, Catchpole and Norton (1980a) did not occur and no lambs died, post-mortem material was unavailable for diagnostic purposes so the diagnosis of coccidiosis had to be based on the similarities with previously reported outbreaks of coccidiosis and the presence of large numbers of oocysts in the faeces.

In a survey of ovine coccidiosis in England and Wales during 1978 and 1979, Gregory, et al, (1980a) found that most cases occurred when lambs were between 4 and 7 weeks old with a peak incidence at 6 weeks ; the data obtained in the present study corresponds with the findings of Gregory and his colleagues. Gregory, et al, (1980a) also found that the oocyst counts from diseased lambs were between 10^3 and 8×10^6 opg, very similar figures to those recorded in 1980 and shown in Fig. 1 and Appendix 2. Although Pout (1969) tends to dismiss oocyst counts as being of diagnostic value the data furnished by Gregory, et al, (1980a) clearly shows that the mode of the oocyst counts of healthy lambs is 2 log doses less than those lambs with suspected clinical coccidiosis in which the diagnosis was confirmed at post-mortem. A diagnosis of coccidiosis in the 1980 lambs in late April and May therefore seems justified as based on the age of lambs, time of year and oocyst counts.

An analysis of the predominant species of coccidia which were present provided further confirmation on the diagnosis since although 11 species are known to occur in British sheep, two, E. crandallis and E.ovinoidalis are known to be particularly pathogenic causing severe lesions in the ileum and sometimes in the caecum (Gregory,et al, 1980b). As shown in Table 3 and Plate 2 all 11 species were found in the lambs in the trial but E. ovinoidalis, E. crandallis and E. weybridgensis (Cathcpole, Norton and Joyner, 1975) were the most prevalent during the clinical phase of the disease.

The source of the oocysts responsible for the coccidiosis in the lambs is particularly interesting. In previous years clinical coccidiosis had not been a feature of lambs grazing on the permanent sheep pastures in Garscube Estate, although coccidial oocysts were frequently present in the faeces (Bairden - personal communication). These lambs had all been born outdoors and from spring lambing ewes. In the current year they came from an indoor lambing flock.

The original source of infection could have been from one or other or indeed all of the following sources : -

a) the ewes

b) infection ingested by lambs during the 2 weeks of housing

c) Infection ingested by lambs at pasture ie. overwintered oocysts.

The faeces of the ewes were certainly positive for coccidial oocysts, albeit at a low level, and the predominant species were, as in the lambs, E. ovinoidalis and E. crandallis. It is interesting that the ewes continued to shed these oocysts right through to weaning of the lambs. The ewes could, therefore, have contributed to the peak infection in the lambs but it seems unlikely on sheer logistics that the low counts recorded during the first two weeks of grazing (200 opg) could have been solely responsible. The ewes are, therefore, best viewed as carriers which contribute to the build up of oocysts during the spring and possibly over the next winter.

The possibility that many oocysts were ingested by the lambs during the two weeks of housing prior to grazing must be seriously considered. Housed lambs habitually chew and lick everything in sight and depending on the construction of the sheep house, infection could readily occur. It would theoretically be less likely in houses with slatted floors (as in the farm of origin) than those with concrete or soil runs but even then lambs can often be observed licking the edges of the slats to which faeces readily adhere. In view of there being no previous history of coccidiosis in lambs grazing on these fields and the weather pattern being normal it seems reasonable to ascribe at least some of the increased prevalence of coccidiosis in 1980 to infection acquired indoors.

However, Helle (1970) in an elegant study to establish the source of infection of coccidia in Norwegian lambs found that when

those born indoors in pens with slatted floors were brought outdoors at 2 weeks of age the prevalence of coccidiosis depended on whether the pastures grazed by the lambs had also been grazed by sheep in previous years. In such circumstances the oocyst levels of the lambs increased to several millions per gram whereas those of lambs grazed on pastures which had not been grazed by sheep in the previous year had oocyst counts in the thousands. He therefore, postulated that the main source of infection was the over-wintered oocysts on the pasture. This was confirmed by inoculating lambs with soil samples taken in spring from infected and 'clean' pastures and clinical coccidiosis was reproduced with the former whereas the lambs receiving the clean soil were unaffected. Helle suggested that the chewing and licking habits acquired by the lambs while under overcrowded conditions indoors encouraged them to nibble at soil on being turned out to graze and so they acquired heavy infections within a few days.

Helle's findings contrast somewhat with studies in the U.S.A. where Deem and Thorp (1940) and Shumard (1957) showed that lambs arriving at feed lots had low oocyst counts but that these built up during the period spent in the feed lot. Conditions in the latter in terms of hygiene and congregation would probably be similar to those found in sheep houses in U.K.

It is difficult to decide whether over-wintered coccidial oocysts played as major a part in the epidemiology of coccidiosis in the curre.t study as in Helle's. Climatic conditions are

different in that there is permanent snow cover in the Norwegian situation whereas heavy winter rainfall is prevalent here and oocysts would be more rapidly washed into lower levels of soil. When one also considers the apparent absence of the problem in previous years, then over-wintered oocysts, while contributing are not the major source of infection. More likely the three sources quoted above contribute in a collective fashion and the contribution acquired indoors by both ewes and lambs has increased the level of infection above the threshold required for disease.

A final comment on the 1980 results is that the lambs appear to have acquired some immunity to the various coccidial species since there was a marked reduction in the overall oocyst output by mid-summer.

Nematodirus Infections 1980.

Although the weather conditions in the spring of 1980 were not considered ideal for the mass hatching of N. battus eggs in that temperatures were not particularly low (N. battus responds best to a prolonged cold dry spring) considerable numbers of eggs hatched L_3 during May and the pasture larval count reached 7777 early in June (Fig. 3). This figure is consistent with the numbers reported by Stamp, Dunn and Watt (1955) and Thomas and Stevens (1960) and Gibson (1963) as causing clinical nematodiriasis in lambs. Clinically evident diarrhoea occurred in some lambs in late May/early June and prior to the characteristic N. battus

eggs being present in significant numbers in the faeces. This is the classical pattern for ovine nematodiriasis since the late 4th and 5th larval stages are responsible for causing the major pathogenic effects and clinical disease occurs before the onset of patency.

As expected, the ewes were resistant to infection and very few of the ingested larvae reaching patency with only 2 ewes showing positive faecal egg counts.

The diarrhoea noted in May was probably due to the remnants of the challenge with coccidial oocysts rather than with N. battus whereas the more severe and widespread diarrhoea seen in June was caused primarily by N. battus ; as the coccidial challenge was decreasing at that time. Certainly the excellent response to anthelmintic treatment with fenbendazole suggested that N. battus was the causal agent. Also, the three permanent lambs killed in early July had a mean N. battus burden of 10,599. It is possible that the intestinal damage caused by prior coccidial infections may have increased the susceptibility to the subsequent N. battus but the response to the anthelmintic therapy suggests that the effects of the two parasites were independent and not related. This may have been because the two dominant coccidia species present, namely E. ovinoidalis and E. crandallis are located in the ileum and caecum whereas N. battus is usually found in the duodenum and ileum.

The presence of a small peak of N. battus larvae in the autumn (600 L_3/kg) is particularly interesting. This increase is mirrored by an increase in Nematodirus worms in the October tracer lambs which was primarily N. battus (mean 1766) and not the expected N. filicollis (mean 600) which is generally regarded as an autumn parasite (Gibson, 1976 ; Reid and Armour, 1975). A mean of 2687 N. battus and 250 N. filicollis were also recovered from the permanent lambs slaughtered at the end of October and the majority of these worms must have been ingested in late autumn since an effective anthelmintic treatment was given to the permanents on September 9th. All of this evidence points to a newly hatched population of N. battus appearing in the autumn which is quite contrary to the well established epidemiological picture. However, Gibson and Everett (1981) working in Southern England observed the same situation and suggested that conditions which favour a rapid disintegration of faecal material, such as heavy rain, enabling eggs to enter the favourable and more constant environment of the upper soil layers could lead to speedy development of a significant proportion of the eggs. Such conditions were present in 1980. One also wonders if the parasite is beginning to adapt or select to an 'in season' hatch to overcome the pressures imposed by grazing controls such as the alternation of sheep and cattle pastures which has been a feature of the grazing systems in Garscube Estate where the experiments took place.

Other Trichostrongyle Infections.

The epidemiological pattern shown in Figs. 5 and 7 of over-

wintered L_3 surviving until late spring then disappearing to be replaced by a new wave of infection in mid-summer is typical of that reported previously in North Britain for sheep flocks grazing in pasture known to be contaminated with trichostrongyle larvae,(Thomas and Boag, 1973 ; Reid and Armour, 1975). It is also similar to that reported by other workers in North West Europe eg. in E. England (Connan, 1968a)S. England (Gibson, 1973) Norway (Helle, 1971) and the Netherlands (Eysker, 1980).

Thus the ewes which had lambed in mid-March had positive faecal egg counts at purchase in early April despite treatment with levamisole two weeks previously. The counts fluctuated at a low but positive level of a mean less than 400 epg until the lambs were weaned early in July (Fig. 5). During April and most of May the culture of ewe faeces yielded predominantly Ostertagia spp larvae with Trichostrongylus spp the second most common species present. During June a higher proportion of H. contortus larvae (up to 40 per cent) and Ch. ovina (also up to 40 per cent) were present in some of the cultures. Despite such infections these ewes showed no clinical signs of parasitic gastro-enteritis, although their daily liveweight gains were extremely low at 0.03kg/day and this was almost certainly partly if not wholly due to the parasitic infections. However, these epg's undoubtedly made a major contribution to the heave pasture larval infections which developed in late June. The worm burdens present in the ewes at post-mortem were variable can be seen Table 7. Some with low burdens had clearly restored their immunity

and expelled the worms which had accumulated following the peri-parturient relaxation while the others with higher burdens were still susceptible.

The ewes were clearly under a larval challenge as their plasma pepsinogen values increased sharply (Fig. 4) coincident with the rise in pasture to a mean level of 2.4 i.u. with individual ewes exceeding 3.0 i.u. These figures are very similar to those recorded by Reid and Armour (1975) in hill ewes. This aspect is discussed further in Section 2.

The faeces of the lambs were first positive for trichostrongyle eggs at the beginning of May ie. after only 4 weeks of grazing (Fig. 5) and progressed to a mean maximum of 800 epg by end of June. Although Ostertagia spp L_3 were the dominant trichostrongyle species recovered from the faecal cultures high recoveries of the strongyloid Ch. ovina were also obtained, much higher than would have been expected from the pasture levels and worm burdens present ; perhaps the culture conditions favoured this parasite. Some of the lambs developed clinical parasitic gastro-enteritis at this stage and had to be treated with the anthelmintic fenbendazole ; the faecal egg counts dropped sharply to zero levels after treatment but reinfection occurred rapidly and they increased again to a mean maximum of 1105 by late August ; they again fell to zero after another anthelmintic treatment in early September to be followed by further reinfection and after this second treatment another increase in epg's in 3 weeks time.

At slaughter in November the mean epg was 500. Presumably the

constant reinfection and repeated damage to the alimentary mucosa interfered with the appetite of the lambs and the utilisation of ingested feed (Coop and Angus, 1981) and this would account for the poor daily liveweight gains of less than 0.15kg/day.

The epg figures do not quite tally with the pasture larval counts which were highest in June (see Fig. 7). At that time the lambs were still suckling their ewes and this may have had some effect on the numbers of worms established. Another possibility is that because very large numbers were established a crowding effect had caused some retardation of worm growth and egg production as has been noted with heavy experimental infections. Whatever the reason for the relatively low faecal egg counts considerable numbers of worms were present (a mean of 20,722 Table 8) and were certainly sufficient to cause clinical disease. From examination of the worm burdens present in the three lambs slaughtered at weaning N. battus infections accounted for approximately half of the total worm burdens while the rest were composed mainly of Ostertagia spp with relatively low numbers of H. contortus, T. axei, T. vitrinus, Ch. ovina and Tr. ovis, these also being the species recorded from the ewe faecal cultures (except Tr. ovis). Although the numbers of H. contortus seemed low being 600, 800 and 900 such numbers should have been sufficient to cause an anaemia in the lamb. This was not evident from the haematological data shown in Table 6 and suggests that the Haemonchus had been recently ingested and had not had sufficient time to precipitate an anaemia before these lambs were slaughtered or the other permanent lambs received anthelmintic therapy.

The mean maximum plasma pepsinogen of values 1.6 i.u. recorded from the lambs during the heavy larval challenge which occurred in June were lower than might be expected from the size of the larval challenge and also the mean Ostertagia spp burden of 10,000 in the lambs slaughtered in July. They were similar to those recorded by Reid and Armour (1975) in Scottish hill lambs but lower than those reported by Thomas and Waller (1975) in lambs (2.6 i.u) under a field challenge with 10,000 L_3/kdh. It is also interesting that the pepsinogens of the permanent lambs increased to a mean of 2.2 i.u. in August although the larval challenge as estimated by pasture sampling techniques had declined from 22,000 L_3/kg. Possibly those values were higher because on reinfection the mucosal damage is preceded by a hypersensitive state as suggested by Anderson (1973). This is discussed further in Section 2.

There are several interesting aspects of the pasture larval counts. Firstly, from Fig. 8 it can be seen that infective larvae of various species were present on the pasture at the beginning of the experiment and presumably were survivors from the previous years infection. The presence of H. contortus L_3 differs from the results of Boag and Thomas (1977) in N.E. England who found that this species did not over-winter on pasture. However, Rose (1963) in S. England found that the parasite did not over-winter in that area. The same comparisons apply to Trichostrongylus spp and Ch. ovina. Indeed maximum numbers of Ch. ovina larvae (887/kdh) were recovered in April which were presumably also survivors from the previous years infection.

Secondly, the succession of species noted by Boag and Thomas (1977) in N.E. England was not nearly so apparent. The peak of Ostertagia, H. contortus and Trichostronylus spp larval recoveries all occurred in late June whereas in Boag's work Ostertagia occurred first in June to be followed by H. contortus in July and Trichostrongylus spp in August. Unfortunately, Boag and Thomas (1977) do not record the climatic data present in 1969 when the observations were made, so direct comparisons cannot be made with the current study. It is possible that the high maximum temperatures recorded in May (Fig. 1) stimulated earlier development to the L_3 stage in the current study.

A surprising feature of the herbage larval counts which occurred in all of the main species present was the virtual disappearance during August and September of the L_3 which had accumulated in June and July and the apparent absence of new populations to replace them despite the continued deposition of eggs throughout these months. The reason for the marked reduction in pasture numbers in August and September is not definitely known but it coincided with a period of very heavy rainfall as can be seen in Fig. 1. Perhaps the larvae were washed onto the soil, a process that has been suggested as the reason for the reduction in cattle Ostertagia spp in pasture during autumn (Al Saqur, Bairden, Armour and Gettinby, 1982). However, the reduction in cattle Ostertagia L_3 is not so marked as in the case for the sheep trichostrongyle larvae since more larvae appear to develop in August or September or be recruited from the faecal reservoir. In sheep, the faecal mass is maller, pelleted and

breaks up more readily so a reservoir does not exist in the same manner and this may account for the failure to recruit more L_3 onto the pasture. Of-course, this does not explain why more L_3 did not develop in August when the temperatures were still suitable for development,or were the developing larvae also washed into the soil at that time ?

The worm burdens in the tracer and permanent lambs followed the same quantitative pattern as the pasture larval counts with one notable exception, that of the Trichostrongylus spp. In the permanent lambs slaughtered in mid-July, the worm burdens which were presumably recruited over the previous month were mainly N. battus and O. circumcincta with low but significant populations of H. contortus being present. This distribution fits very well the pasture larval pattern shown in Figs. 3 and 8. An exception is the comparatively low T. axei and intestinal Trichostrongylus infections. It has been shown by Reinecke (1974) than an interaction occurs between H. contortus and T. axei which reduces the establishment of the alternate species. It is possible that many of the Trichostrongylus spp L_3 recovered from the pasture were T. axei and that these did not establish because of the H. contortus already present. Of-course, this does not explain the relatively low numbers of intestinal Trichostrongylus found. Possibly the presence of large N. battus populations also altered the susceptibility to the T. vitrinus although American workers have shown that the presence of N. spathiger actually enhances the establishment of another Trichostrongylus spp, T. colubriformis

(Kates and Turner, 1953).

The worm burdens of the tracer lambs followed the general pattern of the pasture larval counts with steadily declining numbers being present, except for N. battus which was discussed previously. In a comparison of the pasture sampling technique and tracer lambs as indicators of the level of infection on a pasture Waller, Dobson, Donald and Thomas (1981) found that changes with time in infective larval abundance, for Haemonchus, Trichostrongylus and Nematodirus spp which were present in moderate to low numbers, followed similar trends by both techniques. However, for Ostertagia spp a larvae which were much more abundant, peak levels were defined more sharply and occurred earlier by pasture sampling than by the tracer method. The results in the current experiment seem to follow the same general pattern as those noted by Waller, et al, (1981). For example if a lamb ingests 1kg of dry matter per day over a 14 day period ie. 14kg and the average 2 weekly account of H. contortus L_3 per kdh (see Appendix 8) on the last week of July and the first of August was 1259 the lamb should have ingested approximately 17,726 L_3 (14 x 1259). The mean worm burden of the 3 tracers which grazed during the last week of July and the first of August was 1200 which represents percentage establishment of approximately 6.8%. With Ostertagia spp the mean larval count during the last week in July and first in August was 2877 (see Appendix 8) and so 39,478 Ostertagia spp should have been ingested (14 x 2877) over this period. The mean worm burden of the tracers was 6766 which represents an establishment of nearly

17% which is similar to the percentage numbers established following experimental infections of O. circumcincta (Armour, Jarrett and Jennings, 1966).

Later in the year when pasture larval levels were less abundant the relative numbers established in the tracers were higher. For example, in the first two weeks of October the mean pasture count of H. contortus L_3 was 100 per kdh and therefore the lamb should have ingested 100 x 14 L_3 ie. 1400. The mean Haemonchus worm burden was 700 which represents a 50% establishment. The mean count of Ostertagia at the same time was 331 and the lamb should have ingested 331 x 14 L_3 ie. 4634. The mean worm burden of the three tracers was 1133 ie. an establishment of approximately 25%. So in general, the tracers probably provided an underestimate at higher levels of larval abundance, possibly due to the fact that the distribution of L_3 on pasture is random (Donald, 1973) whereas the grazing pattern of lambs is not random (Donald and Leslie, 1968). At lower levels the tracers provided as good an estimate of infection levels as the pasture larval sampling techniques. This area requires further and more profound statistical evaluation.

If one assumes that the anthelmintic treatments with fenbendazole in September were effective the worm burdens found in the permanents slaughtered in November should reflect the populations acquired between then an necropsy in November. If one compares the worms present in the permanents (9872 - Table 8) and the summated worm burdens of the tracers (10,526 - Table 9) during September, October and November, there is virtually no difference which suggests

that the lambs had acquired very little immunity during their first grazing season, particularly as the burdens on the permanents may be an underestimate due to some turnover or loss of the adult worms as shown to occur by Waller and Thomas (1979).

The results of the 1980 experiments were disappointing in that there was no difference in the epidemiology of the gastro-intestinal trichostrongyles. It was hoped that by treating the ewes prior to turnout the contamination of the pasture would be delayed and the eventual level of pasture infection reduced. However, despite the administration of the anthelmintic levamisole to the ewes while indoors they were passing nematode eggs at turnout 3 weeks later and there was no reduction in contamination. This may have been due to faulty dosing of the ewes by the farmer or simply a reflection of the efficiency of levamisole. Due to the wide range of ewe bodyweights on the recommended dosage scale for levamisole the heaviest ewes would have received a dosage rate of only 3.85mg/kg which is well below the optimal rate of 7.5mg/kg. Because of this poor result the anthelmintic fenbendazole was used for the remaining treatments in 1980 and 1981.

Two positive aspects of the 1980 study were the appearance of clinical coccidiosis which may have contributed to the severe effects of the N. battus infection and the apparent hatching of N. battus eggs in the autumn.

EXPERIMENTAL DESIGN 1981.

Seven Blackface ewes and their 11 lambs were purchased at the end of April, 1981 from the same source as in 1980, the ewes again being housed from December until April. Since the ewes lambed in early April the lambs were approximately 2 weeks old at the time of purchase. On arrival at the University Estate on April 23rd seven ewes were treated with fenbendazole at 7.5mg/kg body weight (Panacur, Hoechst U.K. Milton Keynes, Bucks). The permanent sheep pasture used in 1980 was again used. In 1981 the field was sub-divided into 2 plots of 0.16 hectates and the sheep were rotated each two weeks between the plots. This was done in an attempt to overcome the over-grazing which was apparent at similar stocking rates in 1980, it also provided some information on whether rotational grazing would have any impact on the pasture levels of infection.

The lambs were weaned at the end of June, and the ewes were retained for further metabolic studies. At weaning 3 permanent lambs were removed for slaughter described in Section 2. The remaining lambs continued to graze on the same pasture until the end of October. From weaning the lambs were offered supplementary feeding of 2kg/day of lamb weaner diet (see Appendix 10 for composition). This was done to assess the influence of supplementary diet on the uptake and effect of gastro-intestinal trichostrongyles in the lambs.

In addition to the permanent lambs five groups each of 2

worm free lambs (T) were grazed for periods of 3 weeks throughout the experimental period. At the end of each 3 weeks the tracers were housed for 7 days, and then slaughtered. In October the permanent animals were housed for 7 days prior to slaughter again to allow for the differentiation of arrested larvae. All the permanent lambs were treated with fenbendazole on the 18th May because of severe nematodiriasis individual lambs with clinical nematodiriasis or parasitic gastro-enteritis in June or July were again treated with fenbendazole. The experimental design is summarised in Table 10.

OBSERVATIONS

Climatic data.

The maximum and minimum temperatures were recorded weekly using a mechanical thermograph ; weekly rainfall was also recorded using a Symons rain gauge.

Clinical.

As before the animals were examined every two weeks and signs of in-appetance and faecal consistency noted. All the animals were weighed at fortnightly intervals.

Haematology and Biochemistry.

Fortnightly blood samples were taken from the ewes (April-July)

TABLE 10 Experimental Design 1981

Animals	Management	Treatment (fenbendazole)	Slaughtered
Eight Ewes	Set stocked from 27th April to June 1981	24th April	Housed from 29th June to 23rd July.
Eleven Permanent Lambs	Set stocked from 27th April to 31st October, 1981	18th May Individuals in June and July	3 lambs housed 29th June. Slaughtered 5th July. 8 lambs housed 31st October. Slaughtered 7th November.
10 Tracer Lambs (5 x 2)	5 pairs of Tracers each grazed for a period of 3 weeks between 6th July until November.		August through November at 7 days after housing.

and from the lambs (June to the end of October). Packed cell volume percentages (PCV) and plasma pepsinogen levels were estimated as before.

Parasitological Data.

Faeces

Faecal samples were examined twice weekly by a modified McMaster technique, the results being expressed as eggs per gram of faeces (epg) and oocyst per gram of faeces (opg).

Herbage samples

Pasture larval counts were determined weekly by a method similar to that of Parfitt (1955) and the larval numbers expressed as L_3/kg dried herbage. Samples were collected from both plots.

Post-mortem worm burdens

At post-mortem the gastro-intestinal tract was removed and examined for nematodes by the method of Ritchie, et al, (1966). The nematodes were counted and identified and classified as adult (ie. mature male and female worms, 5th Larval stage (L5), (DL4) fourth larval stage and arrested (EL4) developing).

RESULTS.

The data pertaining to the climate is presented first and for ease of presentation the remaining results are given under the following headings : coccidiosis, nematodiriasis and other trichostrongyles. Coccidiosis and nematodiriasis findings will be confined to the clinical and parasitological data while the data from the other trichostrongyles will include detailed biochemical, haematological and post-mortem findings.

COCCIDIOSIS.

Climatic data.

The climatic records are given in Fig. 9. Temperatures were lower than usual for West Scotland in early spring and summer generally being better 20°C until late July. Throughout July and August rainfall was much lower than in 1980.

Clinical findings.

Severe diarrhoea was observed in some lambs after three weeks grazing ie. in May and this became intermittent in individual lambs during June. This diarrhoea may have been due to concurrent *N. battus* infection. From July onwards the occurrence of diarrhoea in the lambs diminished. No diarrhoea could be detected in the ewes.

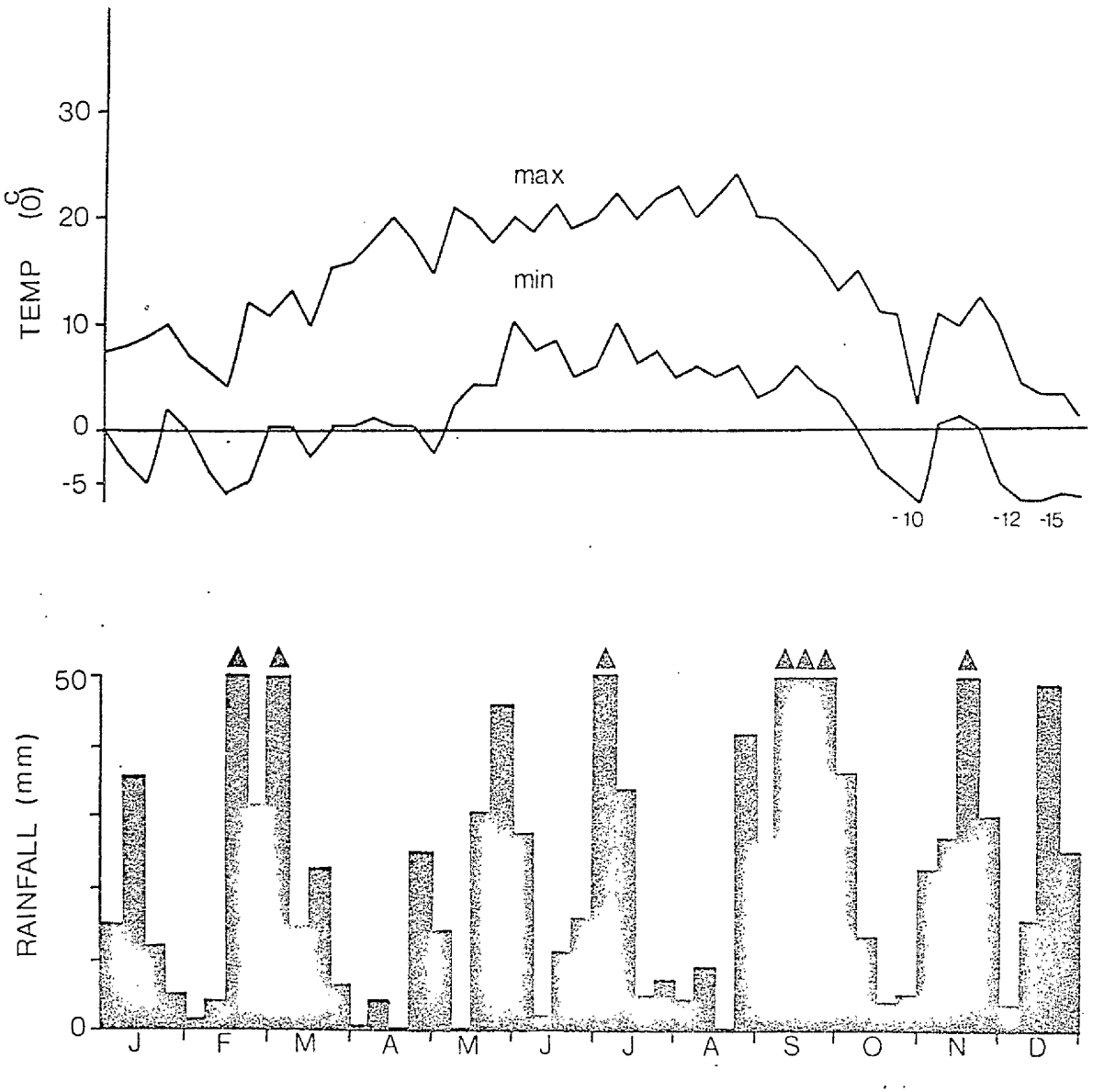


Figure 9. Weekly climatic data 1981.

Parasitological data.

Faecal oocyst counts

The mean bi-weekly oocyst counts of ewes and lambs are given in Fig.10 and the individual results shown in Appendix 11. Positive oocyst counts were recorded from ewes the mean values ranged from 50-1058 opg. The number of oocysts increased in the faeces from the end of May then declined during June. The faecal samples from the lambs became positive for coccidial oocysts after 3 weeks grazing when they were 4-5 weeks old and during the next 2 weeks reached a peak of 196,900 ; this was coincident with the clinical diarrhoea seen in the lambs. Thereafter, the counts fell, fluctuating during June then declined to very low levels in July when the lambs were 15-16 weeks old. The individual numbers of Eimeria spp in lambs ranged from 0-188,000. The predominant spp were again E. ovinoidalis and E. crandallis. The range and percentage of Eimeria spp present are detailed in Table 11.

NEMATODIRIASIS.

Clinical findings.

Severe diarrhoea probably due to N. battus infections occurred in lambs particularly during May. Most of the lambs responded well to anthelmintic treatment with fenbendazole. No diarrhoea was noticed in the ewes.

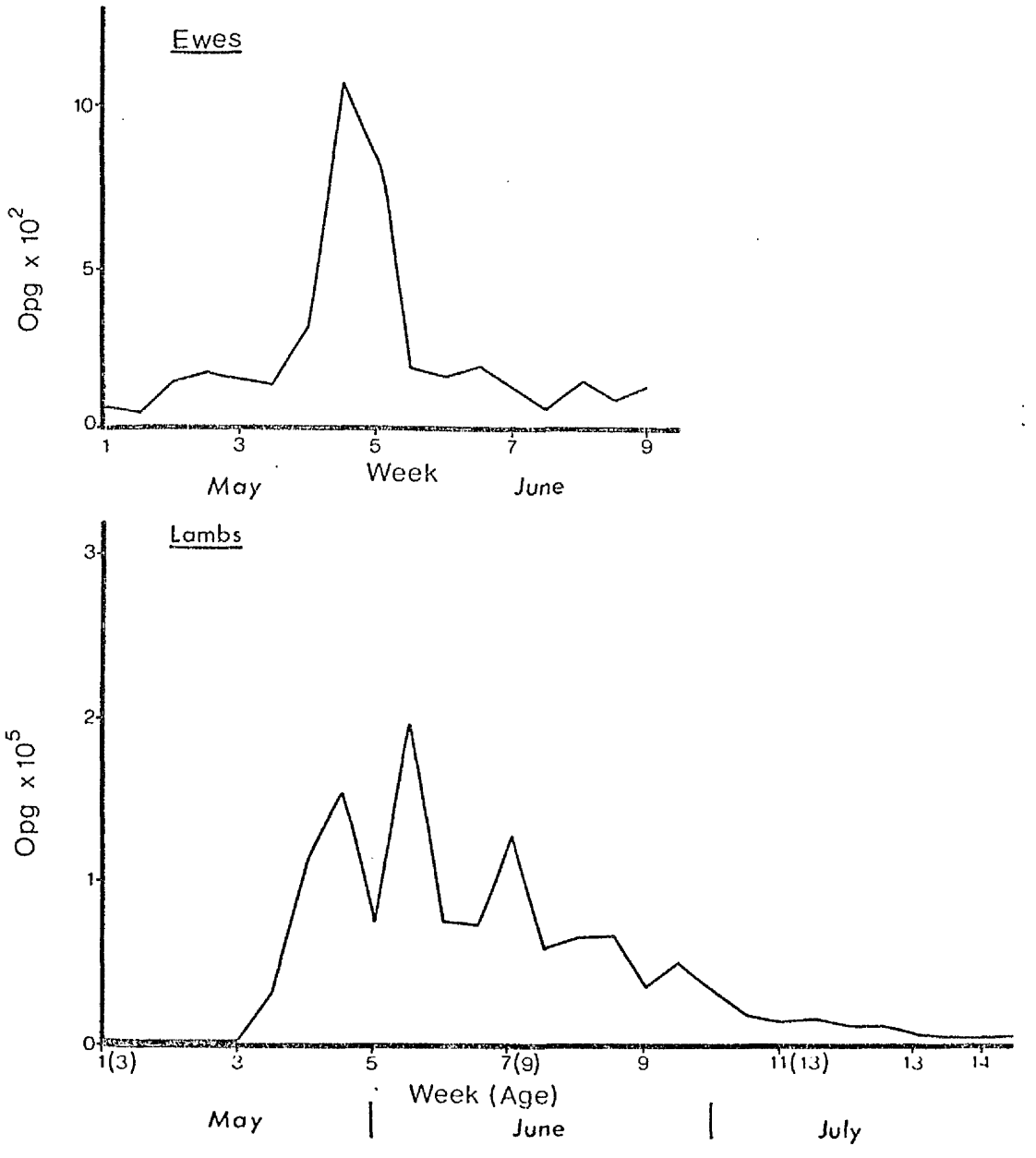


Figure 10. Mean bi-weekly Eimeria spp. faecal oocyst counts of ewes and lambs grazing in 1981.

TABLE 11 Percentages of different Eimeria spp in permanent grazing lambs 1981.

Month	Week	(E.o)	(E.c)	(E.w)	(E.n)	(E.p)	(E.p)	(E.in)	(E.a)	(E.g)	(E.f)	(E.m)
April	1	0	0	0	0	0	0	0	0	0	0	0
May	2	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0
		27	26.5	13	10	7	9	0.5	2	0	0.5	4.5
	4	28.5	34.5	12.5	9.5	4.5	6	0	1.5	0	0.5	2.5
		25	28.5	16.5	8	5	10	1	4	0.5	1.5	5
	5	23	27	4.5	6.5	8.5	15	0	5	0	1.5	9
		32.5	26.5	8.5	10.5	3	9	0.5	3.5	1.5	1.5	3
June	6	28	33	8	10	5	10	0	1.5	0.5	2	3
		33	41	9	7	1	4	0	2	0.5	0	2.5
	7	30	35	5	14	4	5	0.5	3	0	1	3
		31	30	7	14	7.5	3	0	1.5	0	2.5	3.5
	8	28	40	5	7	3	9	1.5	2	0	0	4.5
		47	25	7	10	1	0.5	1	4.5	0	0	44
	9	38	26	5	9	1.5	2	0.5	13	0.5	0.5	4

Key to
Eimeria spp.

E.o = E. ovinoidalis
E.c = E. crandallis
E.w = E. weybridgensis
E.n. = E. ninaekohlyakimovae
E.p. = E. parva
E.p. = E. pallida
E.in. = E. intricata
E.a = E. ahsata
E.g = E. granulosa
E.f = E. faurei
E.m. = E. marsica

Parasitological Data.

Faecal egg counts

N. battus eggs were only found in two ewes their epg's being 50 and 100 during May. The individual bi-weekly egg counts of the lambs shown in Appendix 12. The mean weekly egg counts of the lambs are shown in Fig. 11 from which it can be seen that positive N. battus egg counts were recorded after just 3 weeks grazing, individual values ranging from 0-3000. The numbers of eggs present in May were initially high, declined after anthelmintic treatment and increased again in the beginning of June reaching a mean maximum of 675. A decline to zero followed during July and August although N. battus eggs reappeared in October. Despite low numbers of N. Filicollis worms being recovered at post-mortem, the eggs of this species were not seen in the faecal samples examined.

N. battus larval herbage counts

Mean weekly larval counts of N. battus recovered from both plots are shown in Fig. 11. These were combined since the differences between plots were negligible. The numbers of L_3 increased in early May reaching a maximum of 14,112/kdh ; the number then declined to zero during August. N. battus larvae reappeared again in October reaching a maximum of 636.
N. filicollis L_3 were not recovered from any of the samples

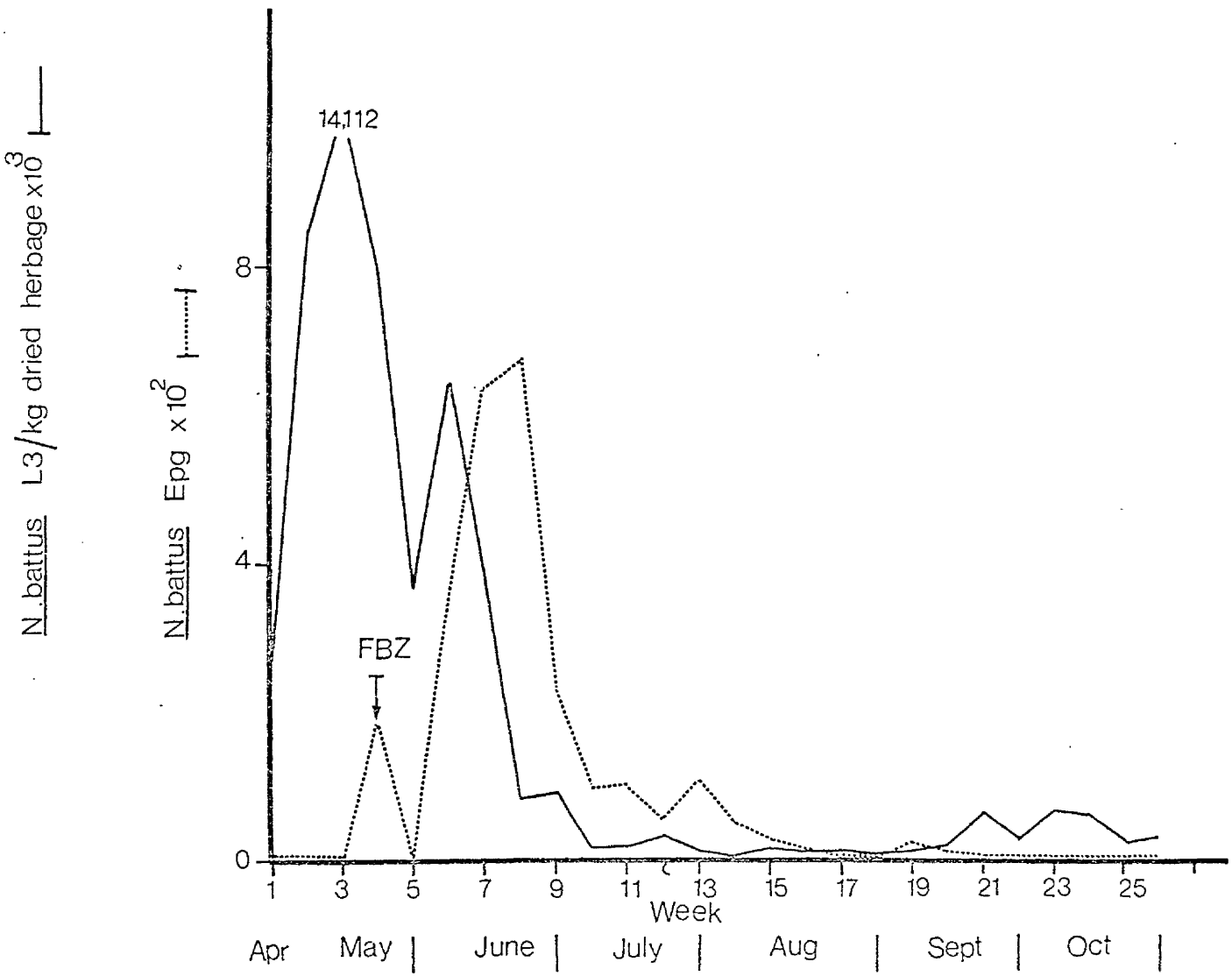


Figure 11. Weekly Nematodirus battus herbage larval recoveries plus mean N. battus faecal egg counts of lambs grazing in 1981.

although some must have been present as worms of this species were found at post-mortem.

OTHER TRICHOSTRONGYLE INFECTIONS.

Clinical findings.

Intermittent diarrhoea was noticed in individual animals during July.

Liveweight gains

The individual liveweight of ewes and lambs are given in Tables 12 and 13. The mean liveweight gain of the ewes from April until the end of June was 0.16kg/day. The mean liveweight gain of the lambs up to weaning was 0.14kg/day ; after weaning ie. from July until the end of the experiment in October it was 0.12kg/day.

Haematology and Biochemistry.

Packed cell volume percentages

Individual (PCV's) of the ewes and lambs are shown in Appendix 13. The mean PCV's of ewes ranged from 23% - 32% ; and the mean values of lambs ranged from 29% -35% .

TABLE 12 Individual fortnightly liveweights in (Kg) of ewes grazing in 1981.

Month	Week	EWE NUMBER						
		633	754	P40	263	515	382	P33
April	1	49	39	38	38	45	45	50
May	3	56	47	42	45	47	51	49
	5	61	52	47	50	55	56	55
June	7	64	53	46	51	59	60	57
	9	65	53	48	51	58	55	55

TABLE 13 Individual fortnightly liveweights in (Kg) of Permanent Lambs grazing in 1981.

Month	Week	LAMB NUMBER										
		Y13	Y14	Y16	Y17	Y22	Y3	Y7	Y8	Y9	Y10	Y19
June	7	15	15	12	14	9	15	19	18	17	16	18
	9	19	18	15	15	11	19	21	19	20	18	20.5
July	11	20	19	16	17	*	*	23	22	21	*	24
	13	21	22	18	21	-	-	25	24	25	-	25
August	15	25	25	20	24.5	-	-	29	26	25	-	25.5
	17	25	26	20	26	-	-	30	26	28	-	28
Sept.	19	27.5	29.5	22	30	-	-	37	33.5	34	-	31
	21	30	30	23	31.5	-	-	36	33	32	-	31
October	23	32	32	23.5	32	-	-	35	33	31.5	-	32
	25	32	35	24	35	-	-	38	38	34	-	35

* Animal Slaughtered

Plasma pepsinogen levels.

The pepsinogen levels of ewes is shown in Table 14. During the first 5 weeks of grazing periods the mean levels rose from an average of 0.65 i.u. to a mean maximum of 1.3 i.u. at the end of June. The mean values of ewes and lambs are shown in Fig. 12 and the individual lamb results shown in Appendix 14. The individual lamb values ranged from 0.3 to 4.2. The mean pepsinogen levels increased from 0.54 in June to 2.57 in September. They then declined at the end of October to 1.8 i.u.

Parasitological data.

Faecal egg counts

Mean bi-weekly trichostrongyle faecal egg counts of the ewes and lambs (including Ch. ovina and excluding N. battus) are shown in Fig. 13 and individual figures are shown in Appendices 15 and 16. Positive counts were not obtained from the ewes until after 3 weeks of grazing ie. in late May. The counts increased to reach a mean maximum of 410 by late June. The individual faecal egg counts ranged from 0 to 700.

The mean trichostrongyle faecal egg counts of the lambs started to increase from June reaching a maximum 435 during August ; they then declined to approximately 156 epg and increased in September to reach 406. The individual faecal egg counts ranged

TABLE 14 Individual fortnightly plasma pepsinogen levels (i.u. tyrosine) of ewes grazing in 1981.

Month	Week	EWE NUMBER						
		663	754	P40	363	515	382	P33
April	1	0.4	0.6	0.7	0.6	0.6	0.8	0.9
May	3	0.6	1.0	0.6	0.7	0.5	0.8	0.6
	5	1.2	1.5	0.9	1.5	0.9	0.9	0.8
June	7	0.8	1.4	0.8	0.9	0.6	0.7	0.6
	9	1.8	1.7	0.9	1.3	1.0	1.5	0.9

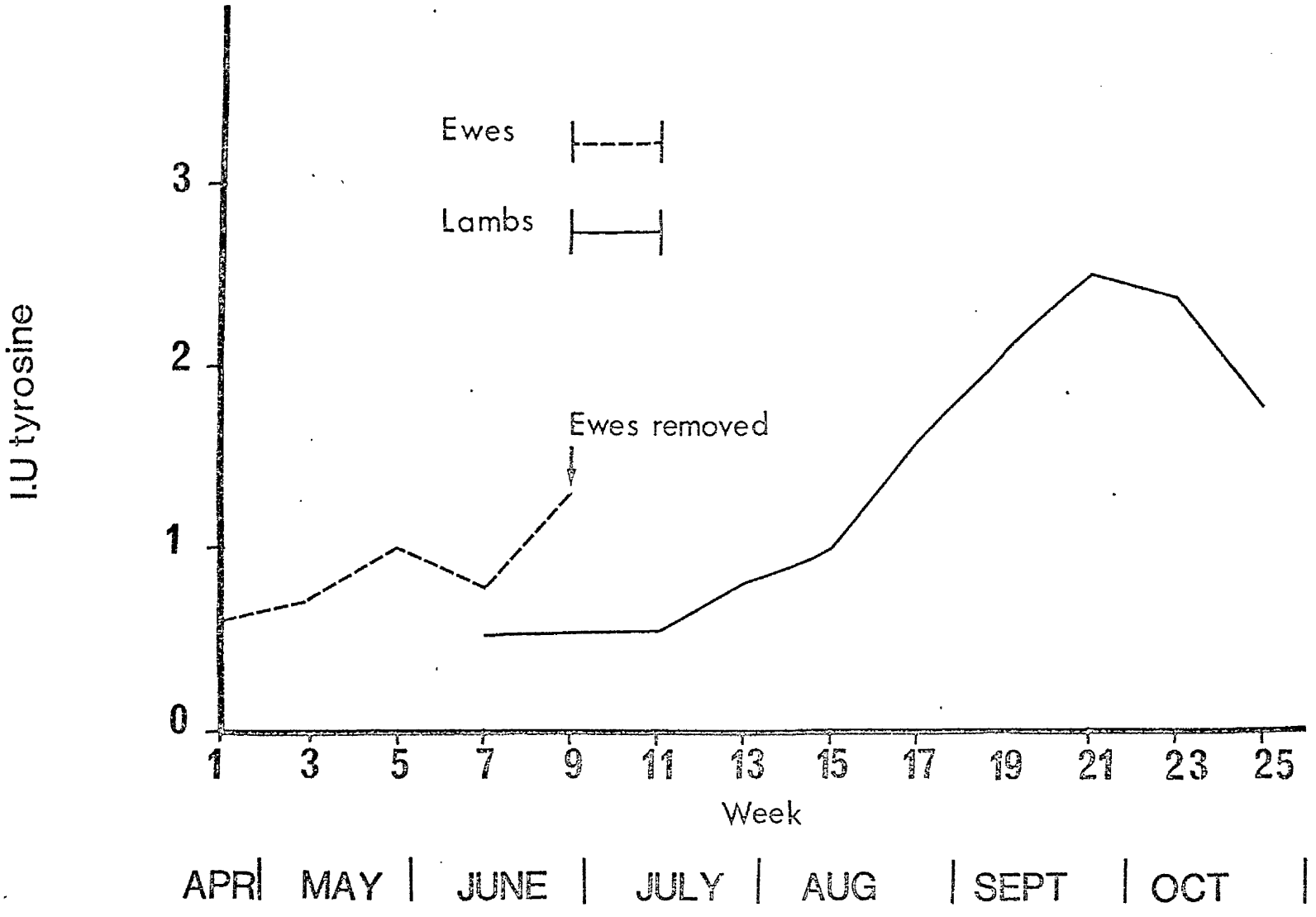


Figure 12. Mean fortnightly plasma pepsinogen levels (Iu tyrosine) of ewes and lambs grazing in 1981.

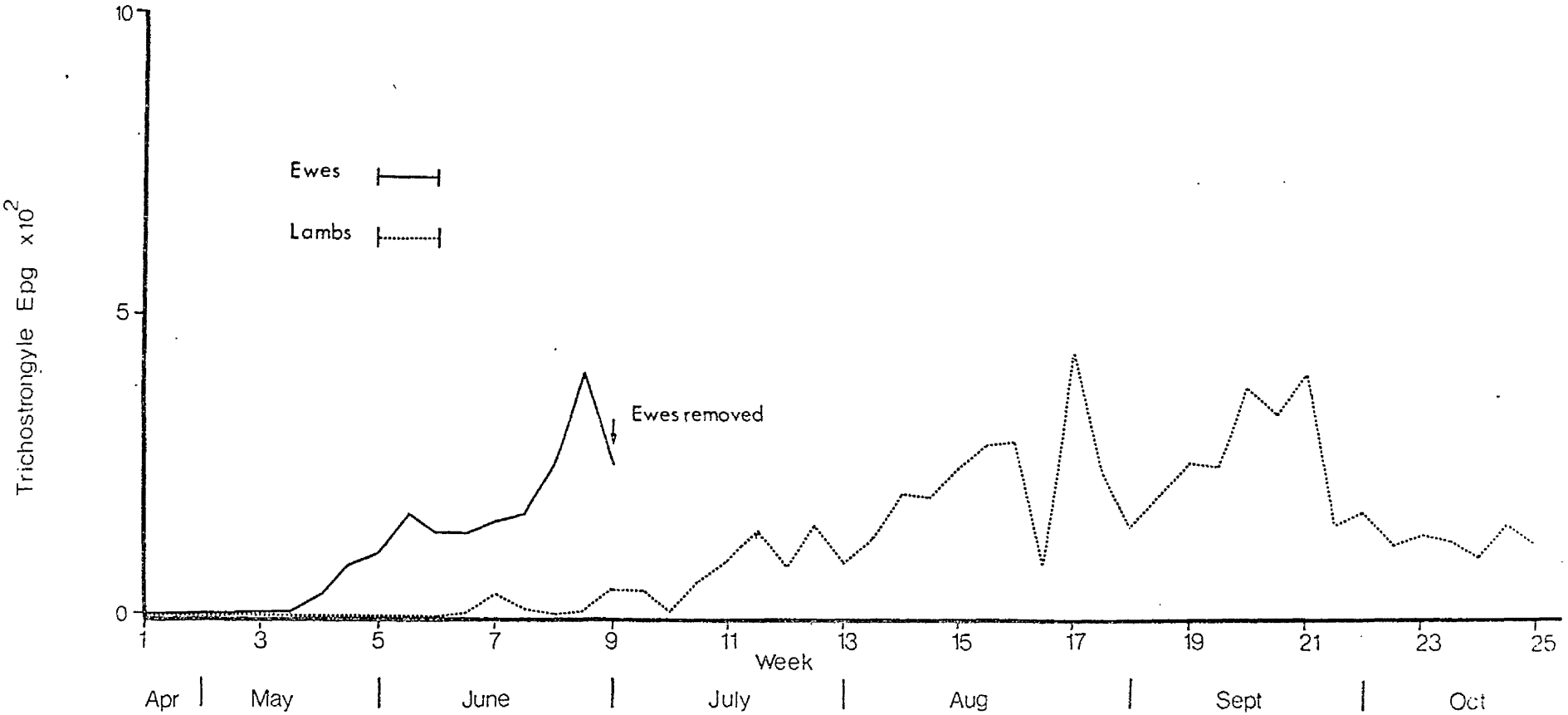


Figure 13. Mean bi-weekly trichostrongyle faecal egg counts of ewes and lambs grazing in 1981.

from 0 to 1850. Culture of lamb faeces revealed that Ostertagia spp (O. circumcincta), H. contortus and Trichostrongylus spp, were all present the former being the dominant species. The percentages of individual genera is given in Appendix 17 and the seasonal fluctuation in Fig. 14.

Larval herbage counts

As with the N. battus counts the data from the two plots showed very minor differences and they have been combined. The total numbers of trichostrongyle L_3 recovered from the herbage of paddocks are shown in Fig. 15 and those of individual genera including Ostertagia spp, H. contortus and Trichostrongylus spp, are given in Fig. 16.

The trichostrongyle pasture larval counts were 600 L_3/kdh at the beginning of April. A decline in numbers occurred during May and following some fluctuation in larval numbers in June a marked increase occurred in July reaching a maximum of 4492 L_3/kdh in early August. The larval numbers then decreased to a level of 174 by late August only to increase again to 3925 L_3/kdh at the end of October. The predominant species present in the herbage was Ostertagia and this species was responsible for the two main peaks which occurred in July/August and October.

H. contortus L_3 were present in relatively low numbers during the year though peaks of infection occurred in both plots in May

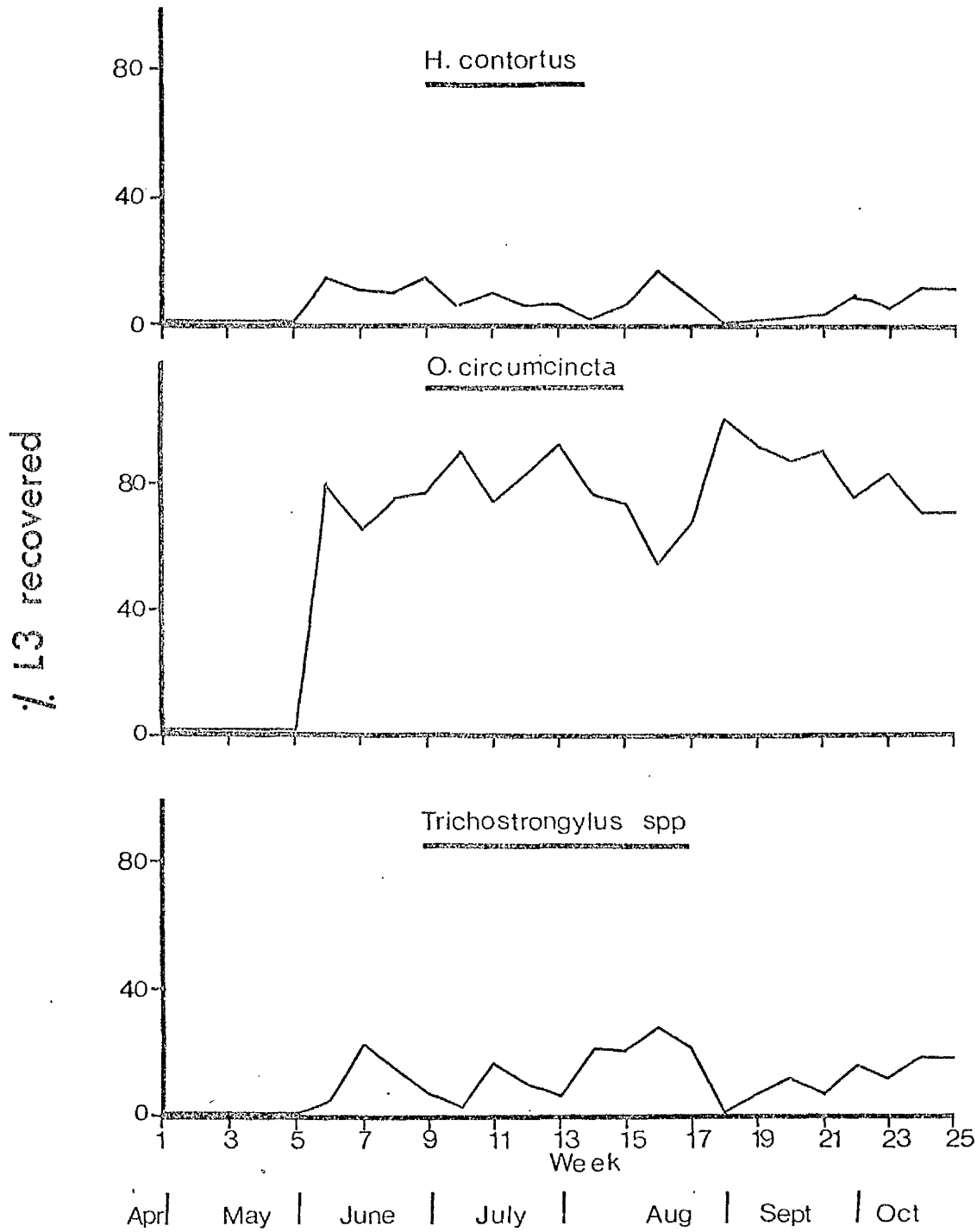


Figure 14. Percentage larval species recoveries from lamb faecal culture in 1981.

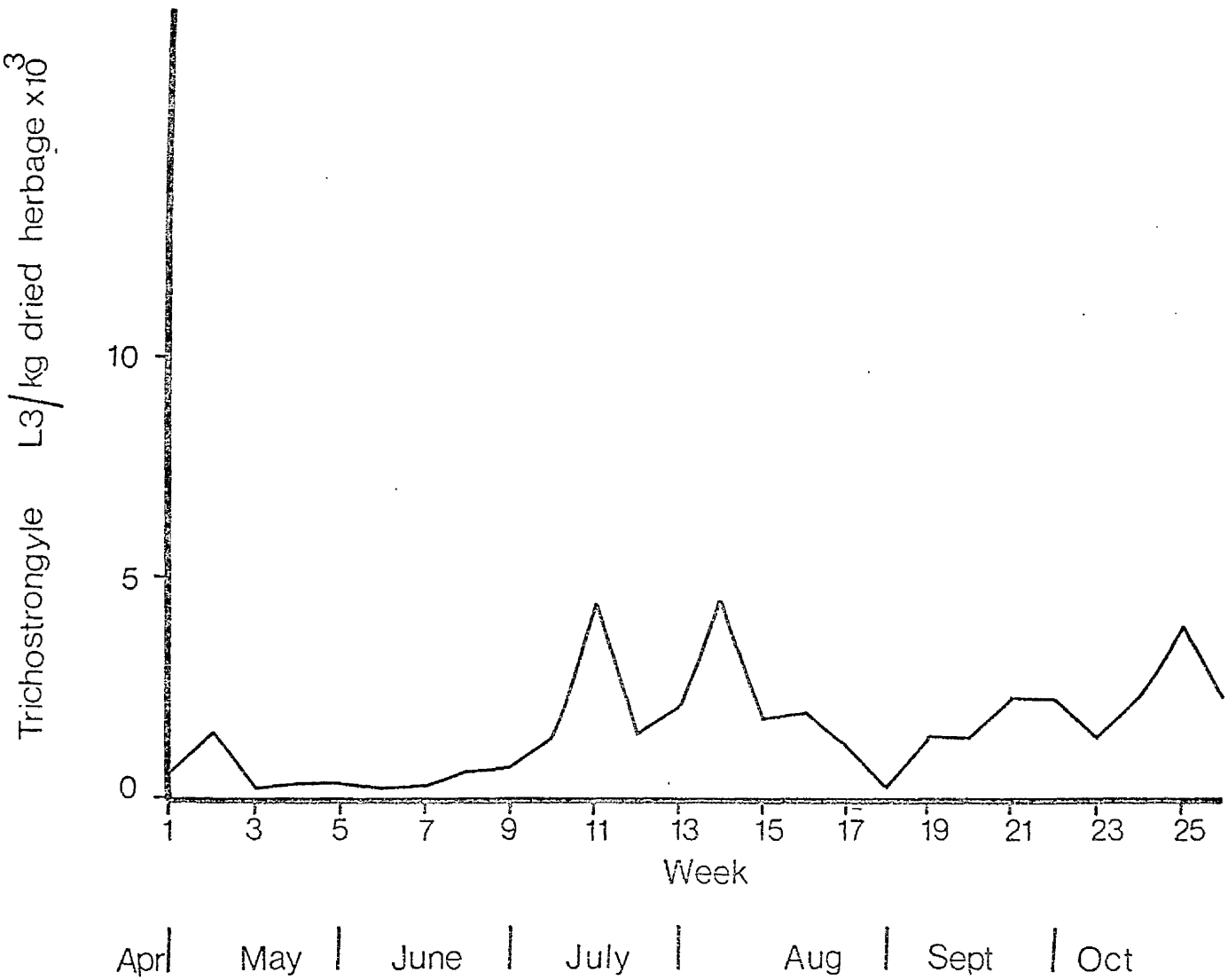


Figure 15. Weekly herbage larval recoveries in 1981.

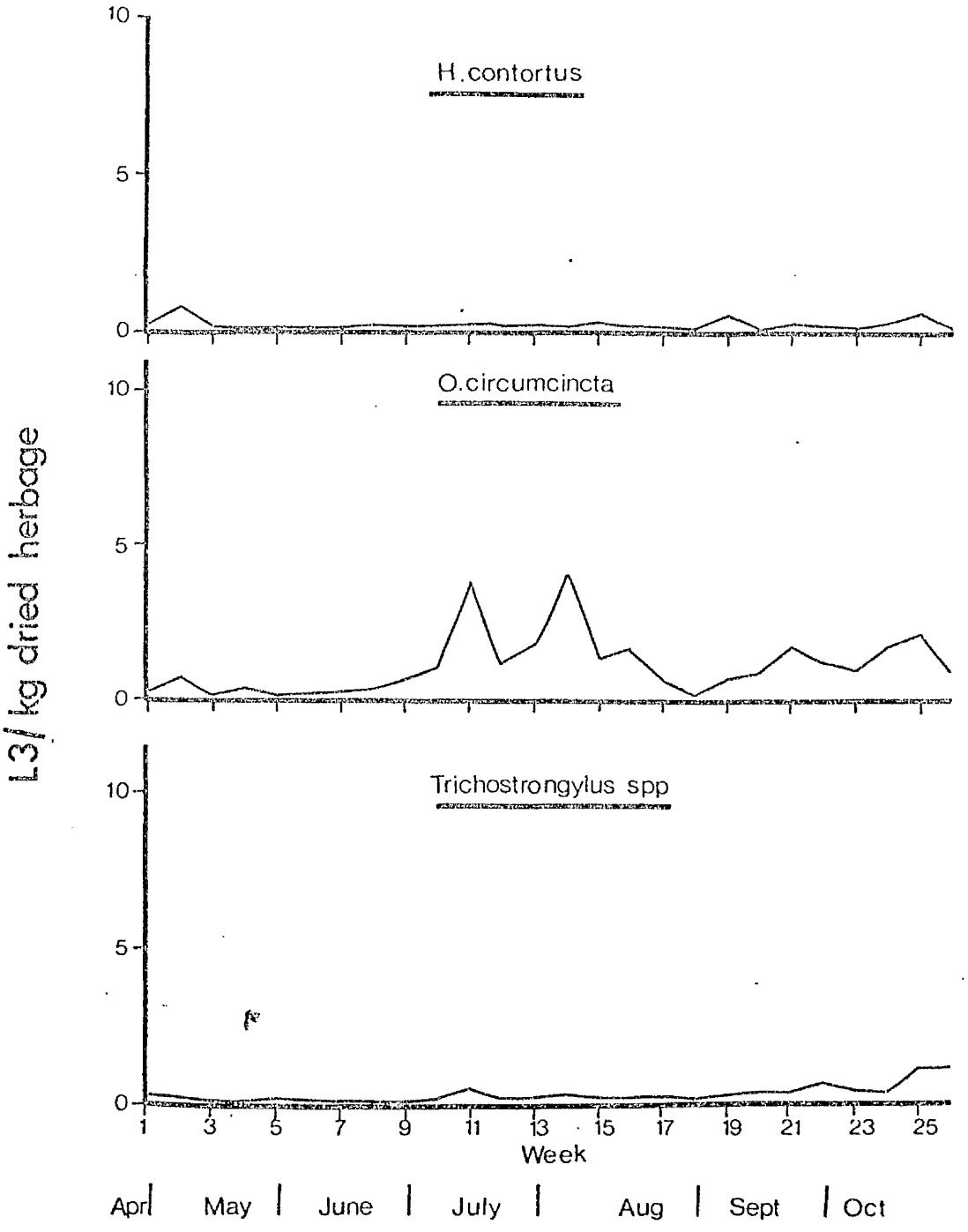


Figure 16. Weekly herbage larval recoveries of individual genera (excluding N. battus) in 1981.

and October to 692 and 580 L_3/kdh respectively. The other species commonly present was Trichostrongylus spp and though the actual numbers were relatively small an obvious increase in the numbers of larvae of this species occurred in October to 1210 L_3/kdh. Low numbers of Ch. ovina were recovered on four occasions during the year. Weekly numbers of trichostrongyle L_3 recovered from the herbage are shown in Appendix 18.

Post-mortem worm burdens.

The ewes were retained for further metabolic studies and their worm burdens are detailed in Section 2. However, the mean burdens of the unchallenged ewes at slaughter in late July are shown in Table 15. These are very low (150 O. circumcincta) indicating that their immunity had been restored following weaning of the lambs.

The individual worm burdens of the permanent lambs are shown on Table 16. The three lambs slaughtered at weaning at the beginning of July had total worm burdens of 9,200, 3,800 and 3,800 respectively. These burdens were made up predominantly of N. battus together with low numbers of O. circumcincta and H. contortus.

The mean total worm burdens of the eight permanent lambs slaughtered in November was 8273 and as can be seen from Table 16 the dominant species was O. circumcincta with a mean of 6812. The next most common species were N. battus at 825 and T. vitrinus at

TABLE 15 Individual worm burdens of unchallenged ewes grazing 1981.

	EWE NUMBER			
July necropsy	P40	363	663	754
O. circumcincta				
♂	300	0	0	100
♀	200	0	0	0
L5	0	0	0	0
DL4	0	0	0	0
Total	500	0	0	0
	Mean 150			
H. contortus	Negative			
Trichostrongylus spp	Negative			
Ch. ovina				
Adult	8	0	0	0
	Mean 2			

TABLE 16 Individual worm burdens of Permanent Lambs grazing in 1981.

	LAMB NUMBER		
July necropsy	Y3	Y10	Y22
O. circumcincta			
♂	100	100	100
♀	0	0	0
L5	0	100	0
DL4	0	0	0
EL4	0	0	0
Total	100	200	100
		Mean 133	
H. contortus			
♂	0	100	0
♀	0	0	0
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	0	100	0
		Mean 33	
N. battus			
♂	900	1300	4300
♀	2590	1000	4000
L5	200	1100	400
DL4	100	100	400
EL4	0	0	0
Total	3700	3500	9100
		Mean 5433	
Total Burdens	3800	3800	9200
		Mean 5600	

TABLE 16 (continued)

November necropsy	LAMB NUMBER							
	Y13	Y14	Y16	Y17	Y7	Y8	Y9	Y19
O. circumcincta								
♂	800	700	1700	1800	800	1200	1100	1500
♀	600	1300	1500	3000	1700	700	2400	800
L5	200	600	0	0	100	300	200	300
DL4	1200	1900	600	1400	1900	1700	1300	1200
EL4	2200	4600	2600	1000	2000	2800	3600	1200
Total	5000	9100	6400	7200	6500	6700	8600	5000
	(44)	(50)	(40)	(14)	(30)	(42)	(42)	(24)
				Mean 6812				
H. contortus								
♂	0	0	0	0	100	0	0	100
♀	0	0	100	0	0	0	0	0
L5	0	0	0	0	0	0	0	0
DL4	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0
Total	0	0	100	0	100	0	0	100
				Mean 37.5				

TABLE 16 (continued)

	LAMB NUMBER							
	Y13	Y14	Y16	Y17	Y7	Y8	Y9	Y19
T. vitrinus								
♂	300	0	200	0	200	0	200	0
♀	300	400	200	400	200	0	200	200
L5	400	200	0	0	200	0	800	0
DL4	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0
Total	1000	600	400	400	600	0	1200	200
				Mean 550				
N. battus								
♂	0	0	0	0	0	200	200	0
♀	0	0	100	100	200	200	200	0
L5	0	0	100	100	0	200	0	0
DL4	0	0	0	800	2200	800	1000	200
EL4	0	0	0	0	0	0	0	0
Total	0	0	200	1000	2400	1400	1400	200
				Mean 825				

TABLE 16 (continued)

	LAMB NUMBER							
	Y13	Y14	Y16	Y17	Y7	Y8	Y9	Y19
N. filicollis								
♂	0	0	0	0	0	0	0	0
♀	0	0	100	100	0	200	0	0
L5	0	0	0	0	0	0	-0	0
DL4	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0
Total	0	0	100	100	0	200	0	0
	Mean 50							
Total Burdens	6000	9700	7200	8700	9600	8300	11,200	5500
	Mean 8273							

Figures in Brackets indicate % of arrested larvae

550. Only low numbers of H. contortus and N. filicollis were present and no Ch. ovina were recovered.

The individual worm burdens of the groups of tracers which each grazed for period of three weeks between July and November are shown in Table 17. O. circumcincta were present in every tracer lamb, the highest burden of 3700 4100 being found in the August grazing lambs. N. battus were present in all the tracers and the numbers fluctuated between 100 and 1200. There was some indication that the numbers of N. battus present in the October grazed tracers were relatively higher than before being more than 1000. The numbers of H. contortus, T. axei, T. vitrinus and N. filicollis were very low and often negative (ie. less than 50 which is the dilution used in the counting technique and only exceeded 100 worms on two occasions. The worm burdens of tracers from both paddocks are shown in Table 18.

Arrested larvae (EL_4 stage) of O. circumcincta were recovered from all of the sheep which grazed from August onwards. Prior to that date only one ewe had 10% of arrested larvae present in the post-mortem worm burden. From August onwards the numbers of arrested larvae increased and in the last tracers which grazed at the end of October the O. circumcincta present consisted of 97.5% EL_4 stages.

No arrested larvae of other worm species were recovered.

TABLE 17 Individual worm burdens of Tracer Lambs grazing in 1981

	LAMB NUMBER									
	Early August		Late August		September		October		November	
	B89	B91	B42	B46	B95	B96	B88	B90	B87	B85
O. circumcinta										
♂	500	800	1000	1700	1700	300	200	400	0	0
♀	600	500	800	500	800	800	200	300	0	0
L5	300	300	1000	1000	400	200	600	0	100	0
DL4	600	900	800	700	0	0	0	0	0	0
EL4	0	0	100	100	200	100	600	700	1800	1500
Total	2000 (0)	2500 (0)	3700 (3)	4100 (2.5)	3100 (6.5)	1400 (7)	1600 (37.5)	1400 (50)	1900 (95)	1500 (100)
Mean	2250		3900		2250		1500		1700	
H. contortus										
♂	0	0	0	100	0	0	0	0	0	0
♀	0	0	200	0	0	0	0	0	0	0
L5	0	0	200	100	0	0	0	0	0	0
DL4	0	0	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0	0	0
Total	0	0	400	200	0	0	0	0	0	0
Mean	0		300		0		0		0	

Figures in Brackets are % of arrested larvae.

TABLE 17 (continued)

	LAMB NUMBER									
	Early August		Late August		September		October		November	
	B89	B91	B89	B46	B95	B96	B88	B90	B87	B85
T. axei										
♂	0	0	0	0	0	0	0	0	0	0
♀	0	0	0	100	0	0	0	0	0	0
L5	0	0	0	0	0	0	0	0	0	0
DL4	0	0	0	0	0	0	0	0	0	0
FL4	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	100	0	0	0	0	0	0
Mean	0		0		50		0		0	
T. vitrinus										
♂	0	0	0	0	0	0	0	0	0	0
♀	0	0	0	100	100	0	0	0	0	0
L5	0	0	0	0	0	0	0	0	0	0
DL4	0	0	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	100	100	0	0	0	0	0
Mean	0		0		100		0		0	

TABLE 17 (continued)

	LAMB NUMBER									
	Early August		Late August		September		October		November	
	B89	B91	B42	B46	B95	B96	B88	B90	B87	B85
N. battus										
♂	800	0	200	0	400	600	200	0	0	0
♀	400	100	200	100	100	300	600	0	0	0
L5	0	0	100	200	100	100	0	100	0	0
DL4	0	0	0	100	100	200	0	0	1100	1000
EL4	0	0	0	0	0	0	0	0	0	0
Total	1200	100	500	400	700	1200	800	100	1100	1000
Mean	650		450		950		450		1050	
N. filicollis										
♂	0	0	200	0	0	0	0	0	0	0
♀	0	0	400	0	0	0	0	0	0	0
L5	0	0	200	0	0	0	0	0	0	0
DL4	0	0	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0	0	0
Total	0	0	800	0	0	0	0	0	0	0
Mean	0		400		0		0		0	
Total Burdens	3200	2600	4600	4700	4800	2700	2400	1500	3000	2500

Mean 3200

TABLE 18 Combined worm burdens of Tracers in 1981.

Dates Grazed	Dates Killed	*Ostertagia* spp	*Haemonchus*	*T.axei*	*T.vitrinus*	*N.battus*	*N.filicollis*
6.7-27.7	2.8	2000	0	0	0	1200	0
		2250				650	
		2500 (0)	0	0	0	100	0
4.8-25.8	30.9	3700	400	0	0	500	0
		3900	300			450	
		4100 (3)	200	0	0	400	0
30.8-20.9	28.9	3100	0	100	100	700	800
		2250		50	100	950	400
		1400 (7)	0	0	100	1200	0
30.9-21.10	28.10	1600	0	0	0	800	0
		1500				450	
		1400 (43.7)	0	0	0	100	0
30.10-20.11	27.11	1900	0	0	0	1100	0
		1700				1050	
		1500 (97.5)	0	0	0	1000	0

( ) = % arrested larvae

DISCUSSION 1981.

To facilitate the comparison of data from 1980 to 1981 the most relevant epidemiological events are summarised on Table 19. The data is arranged in sequence commencing with that pertaining to coccidiosis, then nematodiriasis and finally other trichostrongyles.

Coccidiosis 1981

In 1981, both the timing and magnitude of the coccidial oocyst output in both ewes and lambs were different to that in 1980. This may have been due to the fact that the lambs were born 2 weeks later and therefore, turned out to graze 2 weeks later than in 1980 or more likely it was a reflection on the different weather pattern. In 1981 the spring was much wetter than in 1980 and this may have resulted in faeces being rapidly broken down and oocysts washed into the soil. Whatever the reason the peak opg's in the lambs did not occur until the middle of May in the lambs and at the end of May in the ewes, furthermore the levels of maximum coccidial output in the lambs were about one fifth of that recorded in 1980. Those of the ewes were similar. Severe diarrhoea undoubtedly occurred in the lambs during the peak oocyst output but since this also coincided with the ingestion of many *N. battus* larvae and severe nematodiriasis the influence of the coccidial infection is difficult to assess. However, since the clinical diarrhoea abated following treatment with an anthelmintic effective against *N. battus*, coccidiosis *per se* appeared to be a minor problem in 1981 although it may have contributed to the severity of the nematodiriasis as

TABLE 19 Important Epidemiological Events.

	1980		1981	
	Date	Number	Date	Number
Eimeria oocysts First appeared in Lambs	April 19th	-	May 13th	-
Peak oocyst production	April 24th	1,295,557	May 24th	196,900
First *N. battus* eggs	May 3rd	-	May 17th	-
Peak egg production	June 24th	630	June 17th	800
N. battus L_3 First appeared	April 17th	769	April 26th	2,600
N. battus L_3 Peak	June 10th	7,777	May 10th	14,112
Trichostrongyle eggs first appeared in (1) ewes	April 14th	-	May 24th	-
Peak epg	May 17th	363	June 24th	410
first appeared in (2) lambs	May 2nd	-	June 17th	-
Peak epg	August 31st	1,105	August 23rd	435

TABLE 19 (continued)

	1980		1981	
	Date	Number	Date	Number
Trichostrongyle L_3 Over-wintered	April 4th	4,424	April 26th	600
Peak	June 21st	40,400	August 5th	4,492
O. circumcincta L_3 Over-wintered	April 4th	2,222	April 26th	200
Peak	June 21st	22,400	August 5th	4,045
H. contortus L_3 Over-wintered	April 9th	1,348	April 26th	200
Peak	July 17th	3,548	October 17th	580
Trichostrongylus spp L_3 Over-wintered	April 4th	2,000	April 26th	200
Peak	June 21st	16,000	October 24th	1,210
Ch. ovina L_3 Over-wintered	April 4th	202	June 3rd	58
Peak	April 14th	887	Seprember 24th	136

discussed below.

Nematodirus Infections 1981

In 1981 the pattern of infection was different. A much eaelier hatch of N. battus eggs occurred and pasture larval counts of L_3 soared near to 15,000 in early May. At this time severe diarrhoea occurred in the lambs and anthelmintic treatment with fenbendazole had to be undertaken on the 18th May ie. over a month earlier than in 1980. Rapid reinfection occurred and N. battus faecal egg counts were again high during June and individual lambs had to be re-treated. As in 1980 a small peak of N. battus L_3 occurred on the herbage in late autumn again suggesting that some eggs had developed and hatched in late summer. This late infection was also evident (Tables 16,18) from the burdens found in the permanent and tracer lambs and was again due to N. battus and not to N. filicollis.

The presence of a few thousand N. battus in the permanent lambs slaughtered in October suggests that the immunity to this parasite is most certainly not absolute by 6 month as shown by Gibson (1963). He found that lambs over 6 months were difficult to infect experimentally and suggested that on age immunity was operating which enhanced any acquired immunity. The results of the present experiments suggest that this is not always so although the issue may have been complicated by intercurrent infections with other trichostrongyles and coccidia since Gibson used parasite - naive

lambs in his studies.

Other Trichostrongyle Infections 1981.

The epidemiological pattern in 1981 was also different to that of 1980 with variations occurring in the timing and magnitude of events.

To deal with the similarities first of all. As in 1980 the liveweight gains of both ewes and lambs, despite the latter receiving supplementary feeding, were again very poor with the mean daily liveweight gains never exceeding 0.16kg/day. This may be a reflection of the heavy stocking rate (21 ewes and 33 Lambs/hectare up to July and 24 lambs/hectare after July) combined with the indifferent quality of the permanent pasture. It has been estimated that to make sheep farming a profitable business lamb growth rates at double those obtained in this experiment are necessary (Rutter, 1975) while maintaining similar overall stocking rates. The interaction of parasitism and poor growth rates in relation to stocking rates in sheep requires much more study such as has been carried out in cattle by Hansen (1982).

Packed cell volume percentages of ewes and lambs were again just below the normal figures and since the numbers of H. contortus were relatively few in 1981 these figures probably reflect the nutritional plane of the sheep rather than any parasitic problem.

Apart from the nematodiriasis episode the lambs in 1981 did not suffer from clinical trichostrongylus on a flock basis and only individual lambs had to be treated.

The course of plasma pepsinogen changes followed a similar temporal course in both years although in 1980 the ewes had a mean maximum pepsinogen of 2.3 at the end of June and the mean maximum of ewes in 1981 was only 1.25 i.u. in early July. The elevation of pepsinogen levels in ewes is a subject currently under much discussion and is dealt with in more detail in Section 2. However, it does seem to be related to the level of larval challenge and there is no doubt that the challenge in 1980 was higher than in 1981. Strangely enough although the rise in pepsinogen levels in the lambs was delayed in 1981 they did eventually increase in September to similar levels to those recorded in July in the lambs of the previous year (2.1 i.u in 1980 and 2.5 i.u. in 1981). This occurred despite much lower levels of infection in 1981 and it contrasts to the findings of Anderson (1973) and Thomas and Waller(1975) there was no apparent correlation between the level of challenge and pepsinogen readings. However, the larval challenge in the present experiment was three times as high as those reported by the workers and perhaps in lambs under very heavy challenge the zymogen cells as well as the parietal cells are replaced by undifferentiated cells ; so pepsinogen production is limited and in consequence the plasma levels of the enzymes despite the changes in mucosal permeability wrought by this parasite. This has been shown to occur in very heavy infection with O. ostertagi in cattle(Stringfellow and Madden, 1979).

The major differences occurred in the parasitological data. At first glance it affects that there are not any differences between the two years until the end of June. Thus in 1980 the mean trichostrongyle epg's of the ewes during May was 363 and in 1981 it was 233 (see Appendix 15). Allowing for the considerable variation which occurs in the faecal egg counting techniques this is a very small difference. There are variations however, in the species responsible for this egg count with many Ch. ovina being present in 1980, according to the faecal culture, and virtually no Ch. ovina being present in 1981. The percentages of H. contortus and Trichostrongylus spp were also higher in 1980. The reason for these species changes is almost certainly the greater efficacy against these species of the anthelmintic used in 1981 namely fenbendazole, when compared with the efficancy of levamisole which was used to treat the ewes in 1980.

In 1980, towards the end of June there was a vast increase in the pasture levels of trichostrongyle L_3 (principally Ostertagia spp) from virtually zero to 40,400 L_3/kdh followed by a marked increase in the lambs epg's to a mean 723 ; this did not occur in 1981 during June but was delayed to mid-July ie. a similar timing to that noted in Northeast England by Boag and Thomas (1972) and Waller and Thomas (1978). Apart from the difference in timing there was a vast difference in magnitude since the pasture levels in 1981 only reached 4730 L_3/kdh and the lamb's epg's a mean of 400. Furthermore while the pasture levels in 1980 dropped rapidly in August and September and thereafter remained low a second peak in

L_3 numbers and lambs epg's occurred in October, 1981

The virtual absence of Ch. ovina in 1981 and the much later appearance of H. contortus are particulary interesting. A possible explanation is that the main source of these infections in 1980 were the ewes and that the levamisole treatment had not been very successful in removing these species whereas fenbendazole had been so in 1981. As a result the main source of infection in 1981 of these species were surviving over-wintered larvae which were ingested and cycled by the lambs in July and August ; hence the delayed appearance of these species.

It is generally accepted (Boag and Thomas, 1977) that pasture L_3 populations originate from two sources, namely the ewes and the lambs. In 1980, the failure to control the ewe epg's and the early infections in the lambs meant that there was no clear distinction between the pasture populations originating from either of these sources. In 1981, the ewe epg's were controlled better and the lamb infections occurred later so the characteristic two peaks occurred, the first in July originating from ewe contamination and the second in September/October originating from the lamb contamination.

The reason for the vast difference in the magnitude of pasture levels of trichostrongyle L_3 at the end of June are not clear. Thus the faecal egg counts of the ewes were similar in the spring of each year when the important contamination is known to take place (Michel, 1969) ; the stocking rates were only slightly lowere and yet there

was a one log greater increase in larval numbers at the end of June in 1980 compared with 1981.

As mentioned previously some differences occurred in the relative numbers of the different species present in the faecal cultures, on the pasture and at post-mortem. Thus in 1981 H. contortus and Trichostrongylus spp numbers were much lower and occurred very much later while Ch. ovina were absent from the cultures and were only recovered occasionally from the pasture. However, Ostertagia L_3 (O. circumcincta) were again the dominant species present and the absolute numbers of this species were also one log scale lower than in 1980. So it is unlikely that the differences could be attributed to any species trend.

Although the pastures were alternated each fortnight the differences in timing and magnitude of the L_3 levels in each area were so small that the results have been combined and the alternation is therefore, also unlikely to have been a contributing factor to the between year differences in larval levels.

A likely cause is the difference in weather patterns and examination of Figs. 1 and 12 do in fact show that maximum temperatures (and therefore, the mean day/night temperatures) were much higher in May 1980 and slightly higher in June of 1980. During the latter half of May in 1980 temperatures reached 26°C whereas they did not exceed 20°C until late July in 1981.

The general rainfall pattern was similar in both years though some differences in magnitude did occur. Thus although rainfall was higher in the spring of 1981, there was sufficient moisture in 1980 to promote larval migration. So the major climate difference was in the late spring temperatures which appear to have been ideal in May and June of 1980 with maximum temperatures ranging from $20^{\circ}C$ - $26^{\circ}C$.

However, a major contributing factor to the differences between the two years seems to have been the efficiency of the two anthelmintic treatments. By using the highly effective broad spectrum anthelmintic fenbendazole in 1981 the ewe epg's remained at zero until 4 weeks post treatment whereas in 1980 eggs continued to be shed in the faeces following the less effective levamisole treatment. Also in 1981, the mid-May treatment with fenbendazole resulted in the lambs faeces being clear of trichostrongyle eggs until the third week in June whereas they were being shed from mid-May in 1980.

If one examines the effect of these treatments in a quantitative fashion and multiplies the mean trichostrongyle eggs produced per sheep (Appendices 4,5,15 and 16) by the quantity of faeces per ewe and lamb until mid-June, then the following figures for egg deposition are obtained assuming that the ewes produce 2kg of faeces per day and lambs 0.5kg.

In 1980 - 8 ewes produced 32.0×10^6 eggs

-12 lambs - 18.4×10^6 eggs

In 1981 - 7 ewes - 6.9×10^6 eggs

-11 lambs - 40.4×10^3 eggs

In 1981, the total eggs deposited on each pasture would also be reduced by half due to the rotation although the availability would be increased by the higher concentration of sheep per unit area.

When these figures are compared and the relatively cold conditions existing in 1981 are also considered then the reason for the between year differences becomes clearer and the possible benefits of winter housing and effective anthelmintic therapy apparent.

As in 1980 the worm burdens found in the permanent lambs killed in July and in the tracers reflected the seasonal fluctuations in pasture L_3. However, the worm numbers were somewhat lower than would have been expected from the pasture levels. For example the mean Ostertagia L_3 from mid-June values up to the first week of July when the first permanents were killed were negligible (see Fig. 7 ; Appendices 17 and 18) being only 627. Because these snimals had received anthelmintic treatment in May and June it is not possible to according compare the availability of L_3 and the worm burdens present. However, if the tracer burdens are examined

a comparison is possible. Thus the mean Ostertagia L_3 on the pastures beyween 6th and 27th July was 2014 L_3/kdh and the mean worm burden of the tracers at that time was 2250. Assuming again an intake of 1kg dry matter per day over the 3 week period the lambs should have ingested 21 x 2014 L_3 ie. 42,294. At this level of infection a worm burden of 2250 established in the tracers represents a 'take' of about 5% . In the October tracers the mean burden was 1500 Ostertagia and the larval availability was 1623 L_3/kdh. Using the same calculation this represents an establishment of similar proportions ie. 4.4% .

These calculated percentage establishment figures are very much lower than those obtained in 1980 despite the tracers grazing for an extra week in 1981. It is possible that the introduction of supplementary feeding limited the uptake of grass and therefore, of L_3 particularly as the tracer lambs had been reared indoors but this could not account for the low burdens in the permanents slaughtered in July. A more likely explanation is that the pasture sampling technique is a more sensitive indicator of pasture infectivity due to the random distribution of the L_3 and since lambs have a non random grazing pattern. In these studies the correlation between the pasture L_3 numbers and the worm burdens in the tracers was much poorer than that obtained by Waller, et al, (1981). Possibly, a better and closer result would have been obtained had a greater number of tracers been used or if the tracers had been grazed on other 'clean' pasture before their introduction.

In 1981 the arrested larvae recovered were all O. circumcincta. The seasonal pattern was generally similar to 1980 with higher proportions of the worm burdens from lambs slaughtered in the autumn containing EL4 stages. Indeed, the levels were somewhat higher than in 1980, the November tracer burdens of O. circumcincta consisting almost entirely of EL4 stages (97.58).

The absence of arrested H. contortus and Ch. ovina is surprising since these have been consistently found during late summer and autumn by workers in Cambridge (Connan, 1968) and Newcastle (Waller and Thomas, 1975) and this is generally regarded as the method by which these species survive the winter in sheep. Possibly, the strains existing on the University farm are capable of surviving the winter as L_3 in sufficient numbers and do not require an arrested phase although the evidence in these experiments is that the numbers which do survive are not high.

Arrested Trichostrongylus spp have been reported as occurring in autumn by European workers (Eysker, 1978) and the point at which the arrestment occurs is the exsheathed L_3 stage. It is possible that these very small stages have not been detected at post-mortem in the current experiment or alternatively the strains obtaining here in Glasgow do not become arrested in response to autumn conditions.

The influence of the supplementary feeding to the weaned lambs made little difference to the epidemiology since the important

period of contamination is prior to July and there were no marked differences in the respective levels of egg deposition in each year. Individual trichostrongyle faecal egg counts of the permanent lambs were variable in the summer and early autumn ranging from 50-1700 although the mean numbers of trichostrongyle worms present at post-mortem were not particularly high being 7398. It is possible that the extra feeding limited the uptake of the larvae but as judged by the haematology and liveweight gains it did not seem to influence the effects of the parasites established.

In summary, in 1981 the climatic pattern was less favourable to the development and migration of trichostrongyle L_3 than in 1980. As a result of this and the effective anthelmintic treatments of ewes and lambs in the spring the pasture levels were much lower as were the worm burdens acquired by both permanent and tracer lambs. The introduction of supplementary feeding did not significantly alter the epidemiology although it may have indirectly reduced the uptake of larvae by reducing the time spent grazing. The technique of sampling pasture to estimate the levels of L_3 present proved a more sensitive indicator of infection levels than the tracer lamb technique.

GENERAL DISCUSSION.

The principal objectives of these epidemiological studies were to assess the impact of winter housing of ewes on the subsequent prevalence of coccidiosis and its interaction with nematodiriasis caused by N. battus. It was also hoped to evaluate the effect of an anthelmintic treatment before turnout to the ewes on the epidemiology of parasitic gastro-enteritis.

Clinical coccidiosis occurred in 1980 although this disease had not been noticed in previous years. On first analysis it therefore, appears that the effect of being congregated indoors had facilitated the ingestion of large numbers of oocysts which were responsible for the clinical disease which occurred within 2 weeks of treatment. However, the same sequence of events was not seen in 1981 and since the indoor conditions had not altered whereas the outdoor environment had due to climatic variations it seems more likely that infection occurred principally outdoors although some may have originated indoors. This agrees with the findings of Helle (1971) working in Norway.

The interaction between coccidiosis and nematodiriasis is difficult to assess. In 1980 the former clearly occurred prior to the hatching of N. battus eggs and in the second year where the two diseases occurred contemporaneously the level of coccidial as judged by oocyst counts was relatively low. So the question whether a positive interaction occurs between the protozoa and

the nematode remains unanswered. However, one useful observation was the finding in Scotland of all the common coccidial species previously noted by the Weybridge workers in South England including the two most pathogenic E. ovinoidalis and E. crandallis.

The timing of the peak N. battus infection varied occurring primarily in June in 1980 and May in 1981. Pasture levels of N. battus L_3 were high despite the optimal climatic conditions of a cold dry spring not being fulfilled. A most interesting finding was that significant numbers of N. battus larvae appeared on the pasture in late autumn apparently from recently ingested eggs. Also, considerable burdens of N. battus were found in the 7 month - old lambs at slaughter thus confounding the long held opinion that sheep of that age were immune to N. battus.

The pattern of trichostrongyle infection differed in each year. It was hoped that by treating ewes while still indoors with an effective anthelmintic that egg deposition would not occur until reinfection for suppressing over-wintered larvae had occurred. In 1980 this did not happen due to the infectiveness of the anthelmintic used, namely levamisole. In retrospect this might have been due to the wide range of bodyweights which are catered for by the recommended dosage rate and so the heavier ewes received a sub-therapeutic dose. As a result the epidemiological pattern in 1981 was similar to the widely accepted pattern for sheep grazing on known contaminated pasture (Gibson, 1965 ; Boag and Thomas, 1970).

However, in 1981 when the more effective anthelmintic fenbendazole was used the ewes epg's remained negative until after 4 weeks grazing and so pasture contamination was limited. This reduction in contamination was aided by the anthelmintic treatment for N. battus infection which had to be given in May and so limited virtually any significant contamination by these lambs until the end of June. The success of the anthelmintic treatment was aided by two facts, first, that the ewes were turned out to graze 2 weeks later in 1981 which allowed more time for the surviving over-wintered L_3 to succumb to rising spring temperatures. Secondly, the comparatively low temperatures which persisted throughout spring and early summer in 1981.

From the results of 1981 there is every likelihood that where ewes are housed until April and treated effectively pasture contamination can be drastically limited. If the drug used has a residual effect or is given in the form of a slow release bolus it should be possible to prevent parasitic gastro-enteritis by such prophylaxis of the ewe combined with lamb treatments in May and June to eliminate ingested over-wintered L_3. The reason for the apparent failure of similar treatments to control PGE on out-wintered flocks, Boag and Thomas (1970) is presumably that considerable numbers of eggs can be deposited within the first part of the peri-parturient rise in epg's, which commences at 4 to 5 weeks prior to the post lambing anthelmintic treatment being administered. The proposed control scheme based on the 1981 findings requires further study.

The introduction of supplementary feeding to the lambs in 1981 does not appear to have had any real effect on their liveweight gain ; despite the absolute and relative numbers of worms established in both tracers and permanents being lower. Possibly, the damage induced by the prior N. battus infection had a prolonged effect on the functioning of the alimentary tract.

Although the normal seasonal occurrence in autumn of arrested O. circumcincta EL_4 stages was observed in both years in permanent and tracers. The percentages occurring in the tracers were higher reflecting the arrested status of the most recently ingested larvae. Strangely, arrested larvae of other worm species were absent or present only in negligible numbers. This is different to previous reports from other parts of Britain including West Scotland (Reid and Armour, 1975) and again raises the question of strain differences within the various species.

Finally, the technique of collecting pasture samples and counting the numbers of L_3 present proved to be a more sensitive indicator of larval population than helminth-naive tracer lambs. This aspect should be re-examined using tracer lambs which have been reared at pasture and then treated with an anthelmintic prior to the test period ; too many helminth-naive tracers spend their time standing apart from the flock and only graze intermittently.

SECTION 2.

STUDIES ON SOME PATHOPHYSIOLOGICAL CHANGES ASSOCIATED WITH OVINE GASTRO-INTESTINAL HELMINTHIASIS.

INTRODUCTION TO SECTION 2.

This section consists firstly of a literature review of studies carried out on the pathophysiology of ovine gastro-intestinal helminthiasis. Although this review refers to studies on some helminths which occur most frequently in the tropis the main empahsis is on parasites found in more temperate climates.

Secondly, it reports and discusses the results of experimental investigations into the plasma protein loss which occurs in the alimentary tract of a) susceptible lambs naturally infected with gastro-intestinal nematodes and b) immune ewes given an experimental larval challenge with these nematodes.

Radioisotopic methods are used to monitor these changes and to facilitate the reading of this section there specialised techniques may have been incorporated into the text of each experiment. For details of other techniques reference should be made to the Material and Methods section.

Since the type of data in this section lends itself particularly well to statistical analysis, in contrast to the more variable parasitological results in section 1, much of it is presented as means and standard errors. The raw data is available for inspection in the laboratory.

THE PATHOPHYSIOLOGICAL EFFECTS OF GASTRO-INTESTINAL NEMATODIASIS IN SHEEP.

A LITERATURE REVIEW.

Nematode infections in sheep are invariably of mixed species and the dominant species may vary with the time of year. The predilection sites of infection in temperate areas of the world are the abomasum and the proximal small intestine although in sub-tropical and tropical zones some important parasites occur in the large intestine.

PATHOGENESIS

The precise pathological lesions and their effect on the host vary with the stage of larval development and can often be attributed directly to the degree of mucosal penetration and the feeding habits of the parasite.

The gastro-intestinal nematodes responsible for most outbreaks of ovine parasitic gastritis in temperate regions are in the genus Ostertagia (Thomas, 1973 ; Reid and Armour, 1973 ; Waller and Thomas, 1978). In the abomasum O. circumcincta develops within the gastric gland but does not penetrate the lamina propria. The pathological lesion produced is a discrete raised nodule visible on the surface of the gastric glands which are dilated by the growing worm. In heavy infections the nodules coalesce to produce a nodular morocco-leather like appearance (Armour, Jarrett and Jennings 1966). It appears that while the larvae are developing in the gastric gland there are no significant alterations in the biochemical values of either the abomasal fluid or the blood, but when the larvae emerge from the glands of the abomasum a sequence of pathological and biochemical changes occur. These include hyperplasia and a loss

of cellular differentiation, particularly of the parietal cells which are replaced by undifferentiated cells. These changes spread and affect the neighbouring uninfected glands which in heavy infections results in a reduction in HCl secretion and an increase in the pH of the abomasal contents. Also because of failure of the junctional complexes of the cells to form properly a leakage of pepsinogen into the plasma occurs.

The principle consequences of these structural and biochemical changes are :

1) The elevation of the pH of the abomasal fluid. This results in a failure to activate pepsinogen secreted by the zymogen cells to pepsin and a failure to denature proteins in the abomasum. A loss of bateriostatic effect also occurs with a resultant increase in the number of bacteria in the abomasum.

2) The enhanced permeability to macromolecules results in elevated plasma pepsinogen levels. Hypoalbuminaemia can develop in cases where the mucosa is severely damaged as plasma proteins, particularly albumin can leak from the circulation into the lumen of the abomasum via the open epithelial cell junctions (Murray, 1974). Any loss of protein macromolecules is usually accompanied by a loss of electrolytes mainly sodium and chloride and the onset of diarrhoea further increases this loss of

electrolytes. In heavy infections the clinical consequences of these changes are loss of appetite, impaired abomasal digestion and diarrhoea.

Some investigators have also reported that O. circumcincta infections caused a moderate anaemia (Todd, Arbogust, Wyant and Ston, 1951 ; Horak and Clark, 1964) while other workers have reported insignificant haematological changes in Scottish Blackface sheep infected with 100,000 or 300,000 O. circumcincta larvae Armour, et al, 1966 ; Holmes and MacLean, 1971). However, Bezubik, Sinski and Wedrychowicz (1975) working with Polish Long Wool sheep obtained a significant drop in the packed cell volume percentage (PCV) with an infective dose of 50,000 or 400,000 larvae. This may be an example of the importance of breed in determining the pathogenic effect of parasitic infections.

In O. circumcincta infected lambs, each fitted with an abomasal cannula and with a separate fundic pouch, marked changes in abomasal secretion were observed such as a decrease in acid secretion leading to an elevation in the abomasal pH, altered electrolyte concentration and this was accompanied by an increased plasma pepsinogen (McLeavy, Anderson, Bingley and Titchen, 1973). At the same time as these changes occurred, there was a marked increase in the secretory activity in the separated abomasal fundic pouch. A hypergastrinaemia occurred in the fundic pouch possibly related to increased levels of circulating gastrin or other contributory influences occurring locally in the part of the abomasum in which the parasites were established

(Anderson, Blake and Titchen, 1976 ; Titchen and Anderson, 1977). More recent studies by Anderson, Hansky and Titchen (1981) showed that the presence of the parasite is critical for the development of a hypergastrinaemia as the gastrin levels return to normal following therapy with an anthelmintic. Furthermore, the occurrence of a marked hypergastrinaemia in abomasal parasitism is not necessarily related to the presence of diarrhoea since in infections with T. colubriformis where diarrhoea is present, gastrin levels are not elevated (Titchen and Anderson, 1977).

Other parasites in the abomasum are H. contortus and T. axei. With H. contortus most physical damage is caused by the larvae and adult worms which in the course of feeding lacerate the mucosa with the aid of a lancet in their buccal cavity ; they may cause a severe anaemia by direct haematophagia and haemorrhage from the feeding point (Veglia, 1915 ; Fourie, 1931 ; Andrews, 1942 ; Whitlock, 1950 ; Richards, Shumard, Pope, Philps, and Herrick, 1954).

When large numbers of immature or young adult parasites are present changes occur in the mucosa which lead to an increase in pH (Allonby, 1974). Thus Coop, (1971) and Christie (1970) found that a massive dose of H. contortus larvae in sheep produced a remarkable change in the abomasal pH within 3-5 days and the plasma pepsinogen levels also increased within 5 days and were still elevated 27 days after infection. Recently Dakak, Fioramonti and Bueno (1982) have shown that in H. contortus infected lambs, each fitted with an abomasal cannula, significant increases in pH and Na^+ and decreases

in K^+ and CL^- concentrations occurred 2-4 days after infection.

Ross, Purcell and Todd (1969) studied T. axei infections in lambs and demonstrated that in acute fatal infections there was a consistent increase in pH of the abomasal fluid to above 6.0 from the 14th to 21st day as a result of cellular changes leading to a non-functional abomasal mucosa. This was associated with an increase in the abomasal fluid Na^+ and a decrease in the abomasal fluid K^+. The dry matter content in the faeces dropped from 45% to 25% and there was evidence of haemoconcentration ; plasma pepsinogen levels increased at the 4th week following infection.

Several species occur in the small intestine and the pathological effects are proportional to the level of mucosal penetration. For example T. colubriformis and T. vitrinus cause extensive villous atrophy or flattening with elongation of crypts. In the duodenum and anterior jejunum these changes lead to a reduction in the surface area available for absorption of water and electrolytes and nutrients (Reid and Murray, 1973 ; Sykes, 1978 ; Coop, Angus and Sykes, 1979 ; Taylor and Pearson, 1979).

In nematodiriasis which is caused by N. battus and usually seen in lambs 6-10 weeks old, the pathological changes occur when the young adults are emerging from the mucosa. Inflammatory changes occur with severe degeneration of the mucosal surface associated with a stunting of microvilli and a decrease in absorptive capacity, (Thomas and Steven, 1956 ; Baxter, 1957 ; Gibson, 1973 ; Reid and Murray,

1973 ; Sykes. 1978).

In the large intestine Oe. columbianum and Ch. ovina are both plug feeders and the adults of both species cause ulceration of the mucosal wall of the colon as a result of their feeding habits, (Horak and Clark, 1966 ; Dobson, 1967 ; Bawden, 1969a ; Herd, 1971). Experimental infections of lambs with 1000-200,000 L_3 of Ch. ovina have been shown to produce a severe anaemia between the 1st and 6th weeks which was associated with diarrhoea and the presence of blood in the faeces Ross, et al, (1969) . Tr.ovis which is found primarily in the caecum of young animals causes erosions and the resultant bleeding may be reflected by blood in the faeces.

Apart from the obvious clinical and pathological effects outlined above gastro-intestinal parasitism is normally attended by one or a combination of the following : - reduced feed intake, anaemia and hypoproteinaemia (Allonby and Dargie, 1973, Dargie 1980 ; Symons, Steel and Jones, 1981).

REDUCTION IN FOOD INTAKE.

This is a common finding in alimentary parasitism and can lead to a serious impairment of wool growth and body weight gain. Infections produced by a single large experimental dose of infective larvae have been shown to cause a reduction in voluntary feed intake for example in ostertagiasis of sheep (Holmes and MacLean, 1971) and T. colubriformis infections of sheep (Steel, 1972 ; Roseby, 1973 ;

Reveron Topps MacDonald and Pratt, 1974). Persistent reduction of appetite was observed following a large single dose of infective larvae of O. circumcincta (300,000 or more) while a continuous daily intake of between 1000 and 3000 larvae over 7 weeks was sufficient to significantly reduce feed intake and liveweight gain in weaner lambs (Coop, Sykes and Angus, 1977). In further experiments (Sykes and Coop, 1976 ; Sykes and Coop, 1977) it was found that the physiological effects of infection were still present after 14 weeks of continuous dosing with 4000 O. circumcincta daily, although there was some evidence of recovery at this stage. Similar findings were reported by Horak and Clark (1964), Holmes and MacLean (1971), Parkins, Holmes and Bremner (1973), Sykes (1978) and Coop and Angus (1981).

Intestinal Trichostrongylus spp infections caused a progressive reduction in voluntary food intake in sheep subjected to daily dosing of 2500 L_3 per day from 4 weeks post-infection and resulted in lower weight gains (Sykes and Coop, 1976 ; 1977 ; Sykes, 1978). A similar course of events has been noted in H. contortus infections Dargie, 1973),in single heavy infections of T. colubriformis (Gordon, 1958 ; Horak and Clark, 1966),and Cooperia spp (Andrews, 1939 ; Andrews, Kuffman and Davis, 1944) and in mixed populations of gut nematodes (Southcott, Heath and Langlands, 1967). Though various reports conclude that the reduction in food intake accounted for a significant proportion of the weight differences between infected and control, the etiology of such a loss in appetite is not known. Dargie (1980) was of the opinion that although the pathological lesions produced by the parasites could make an animal disinclined to

to eat, the fact that the reduction in intake often fell progressively as the infection progressed (especially if the food was of poor quantity) mad him conclude that the patho-physiological condition of the animal as manifested by blood and serum protein changes (which fell progressively with increasing duration of infection) were more important causes of the reduced intake.

ANAEMIA.

This is a sequel of infection with several parasites including H. contortus which can cause a severe anaemia (Veglia, 1915 ; Fourie, 1931 ; Andrews, 1942 ; Whitlock, 1950 ; Richards et al 1954 ; Clark, Kiesal and Goby, 1962 ; Dargie and Allonby, 1975 ; Anosa, 1977 ; Ogunsusi, 1978 ; Roberts and Swan, 1982). Other gut nematodes implicated in the production of a milder anaemia are T. axei (Gibson, 1954a ; Kate and Turner, 1960 ; Ross et al 1969) and T. colubriformis (Horak, Clark and Gray, 1968 ; Barker, 1973). Anaemia may also result from the blood sucking activities of the hookworms B. trigonocephalum and Gaigeria pachyscelis (Ortlep, 1937 ; 1939 ; Roche, Perez-Giminez and Levy, 1957 ; Miller, 1966). Anaemia may also be due to the removal of plugs of tissue with resulting ulceration and haemorrhage following infection with the large intestinal nematode Ch. ovina (Watzel, 1931 ; Ross et al 1969 ; Herd, 1971) and Oe. columbianum (Horak and Clark, 1966 ; Dobson, 1967 ; Bawden, 1969a).

The anaemia of haemonchosis mainly results from haemorrhage

into the host abomasum due to the blood sucking activities of the immature and adult parasites and heavy infections produce a macrocytic hypochromic anaemia whereas light infections are characterised by a normochromic response which can also progress to the macrocytic stage. Both acute and chronic infections are eventually marked by reductions in serum iron levels, in the percentage saturation of transferrin and in depleted iron stores (Dargie and Allonby, 1975).

The haematophagic activity has been studied by the use of ^{59}Fe and ^{51}Cr labelled red cells and the estimated daily blood loss per parasite was 0.5ml per day. There was also an increased faecal clearance of red cells from 6-12 days after infection prior to the appearance of eggs in the faeces (Whitlock, 1950 ; Baker and Douglas, 1957, 1966 ; Clark, et al 1962 ; Georgi, 1964 ; Georgi and Whitlock, 1965).

Dargie and Allonby (1975) made a detailed study of the pattern of erythrocyte loss exhibited by sheep during the course of single and challenge infections with H. contortus. Using radioisotopic methods they correlated the clinical course of anaemia and the development of the parasite populations. They suggested that there are essentially three stages in the development of the anaemia in infected sheep. In the first, the sheep may lose large amounts of blood in the first 3 weeks of the infection and because the erythropoeitic system requires time to respond and increase its output of red cells, deaths due to acute haemonchosis may occur. In the second phase from about 1-2 months, although there may still be

a substantial loss of red blood cells, haematocrit values may not decrease any further as the host is now able to compensate due to increased erythropoeisis. In the final phase the haematocrit reading may decline rapidly as the erythropoeitic system becomes defective due to iron deficiency and possibly to a reduction in the availability of amino acids (Allonby 1973 ; Schillhorn Van Veen, 1978).

Studies by Allonby (1974) on the bone marrow in the femur demonstrated differences between acute and chronic haemonchosis ; in acute haemonchosis the red marrow changes its distribution and extends along the medullary cavity, leading eventually to a complete replacement of the white marrow ; in chronic cases there is only a scanty red marrow and it is replaced by a yellow gelatinous substance ; there is also thinning of the shaft of the medullary bone and the area adjacent to the epiphysis is rarefied.

HYPOPROTEINAEMIA.

Most parasites cause alterations in the plasma protein composition of their host. The general picture is one of a reduction in albumin concentration accompanied by an increase in one or more of the globulins (Allonby and Dargie, 1973). The application of radioisotopes to the study of plasma protein turnover rates and losses into the gut has led to the observation that the plasma proteins are in a state of dynamic equilibrium between the intravascular and extravascular pools due to continuous synthesis and catabolism. A change in concentration may indicate altered

distribution or a change in the rates of synthesis and catabolism.

Most investigations on protein metabolism in parasitised animals have tended to concentrate on albumin metabolism (Holmes, Dargie, MacLean and Mulligan, 1968 ; Mulligan, 1973 ; Dargie, 1975 and Obasaju, 1981). Albumin labelled in vitro with radioiodine ^{125}I or ^{131}I is most suitable for metabolic studies, particularly the measurement of rates of catabolism ; . the label remains firmly attached to the protein under normal physiological conditions and is liberated only when the protein is degraded. The labelled albumin behaves metabolically similar to the animals' own unlabelled proteins.

The other great advantage of this label is that the iodine is not reutilized in protein synthesis and is quantitatively excreted when the thyroid has been blocked by prior administration of inactive iodine (Sterling, 1951 ; McFarlane, 1958 ; Holmes, 1969 ; Mulligan, 1973 ; Dargie, 1975). Gray and Sterling (1950) first demonstrated that ^{51}Cr chloride could be used to label albumin, and Waldman (1961) first introduced the isotope as a quantitative tool for diagnosing intestinal protein loss. ^{51}Cr labelled albumin has the disadvantage that slight denaturation of the protein molecules occur and this is reflected by the very short plasma half-life ($t\frac{1}{2}$) (Hofer, Schatz and Thumb, 1968). Nevertheless, ^{51}Cr-albumin can be used as a reliable indicator of the plasma loss into the gastro-intestinal tract, at least in sheep, where a fair correlation has been found to exist between the higher catabolism of albumin and the loss of plasma protein into the gastro-intestinal tract (Holmes,

1969). Van Tongeron and Major (1966) found that the rate of disappearance of ^{51}Cr from the plasma was the same whether the isotope was administered as ^{51}Cr chloride or ^{51}Cr-albumin. For convenience, carrier-free $^{51}CrCl_3$ was injected to allow *in vivo* labelling of the plasma protein to occur.

Polyvinylpyrrolidone (PVP) can also be used for the measurement of protein loss from the plasma to the gut by labelling with either ^{125}I or ^{131}I as first demonstrated by Gordon (1959). Recently, Holmes (1969), Mulligan (1973) and Dargie (1975) found a good correlation between faecal excretion of PVP and gastro-intestinal loss. However, the chief objection to its use appears to be its dissimilarity as a molecule to plasma protein (Mulligan, 1973).

Other isotopes used for quantifying enteric plasma leak include ^{95}Niobium-labelled albumin (Jeejeebhoy, Singh, Mani and Sanjana, 1964), ^{67}Cu-labelled ceruloplasmin (Waldman and Wochner, 1965) and ^{59}Fe-labelled iron dextran (Jarnum, Westergoad, Yssing and Jensen, 1968). The ^{59}Nb-labelled albumin was unsuitable for turn-over studies while the high cost and short half-life of ^{67}Cu-ceruloplasmin has precluded its routine use. The ^{59}Fe iron dextran was shown to give a better correlation between faecal excretion and turn-over rate. Studies by Holmes (1969) in which (^{125}I and ^{51}Cr) were used together revealed statistically significant correlation between faecal plasma clearance and plasma albumin concentration ; simultaneous administration of ^{125}I and ^{131}I-PVP also revealed a significant correlation between serum

albumin concentration and both the faecal excretion of PVP and gastro-intestinal loss.

Gastro-intestinal loss of protein as a cause of hypoproteinaemia especially hypoalbuminaemia has been observed in many helminth infections of Sheep (Dargie,1975), and the results of many workers support this conclusion.

Holmes and Maclean(1971) used a heavy single experimental infection of sheep with 300,000 or 900,000 O. circumcincta and a double isotope technique using ^{125}I-albumin and $^{51}CrCl_3$-labelled plasma albumin to monitor albumin turn-over and gastro-intestinal plasma leak.

These authors related the development of hypoalbuminaemia to a marked increase in the catabolic rate of albumin and loss of plasma into the gut. Such changes were most noticeable between the 1st and 3rd weeks of infection. The route of this excessive leakage was thought to be through the junctional complexes in the hyperplastic mucosa which failed to seal properly in the parasitised abomasa thereby permitting macromolecules to pass through (Armour et al, 1966 ; Neilson, 1968 ; Reid and Murray, 1973).

Holmes and MacLean (1971) showed that albumin turn-over values were higher in diarrhoeic than non-diarrhoeic animals but that faecal clearance values were similar and high turn-over and increased faecal radioactivity occurred in the absence of diarrhoea. Perhaps some

losses of plasma from increased endogenous catabolism into peri-vascular spaces (not into the gut) occurred in the severely affected animals or diarrhoea per se was important in precipitating the protein leakage (Neilson, 1966).

In recent studies by Symons et al (1981) it was noticed that enteric plasma loss and albumin turn-over rates were significantly increased following the administration of 120,000 infective larvae of O. circumcincta, albumin concentrations being most severely depressed during the 4th weeks of infection.

When Dargie (1973) studied the hypoalbuminaemia in H. contortus infections he used ^{131}I-labelled albumin and ^{131}I-labelled PVP to measure albumin turn-over and enteric plasma respectively in Merino sheep and he concluded that the hypercatabolic hypoalbuminaemia could be attributed to the blood sucking activities of the parasites and eventual passage of the plasma constituents into the gut. Dargie (1975) also suggested that an amount of plasma in excess of that expected from losses due to haemorrhage probably passed into the gut similar to that which was observed in fasciolasis by Dargie, Holmes, MacLean and Mulligan (1968).

Although hypoproteinaemia was observed in Trichostrongylus species infections of ruminants as early as 1939, by Andrews and later by Horak, et al, (1968), recent work has shown that infection with T. colubriformis markedly increased plasma albumin turn-over rate and loss into the intestine of guinea pigs (Symons, Jones

and Steel, 1974).

Most gastro-intestinal parasites cause some dereangement of function which ultimately gives rise to metabolic disturbances and poor productivity of the host ; these include depressed food consumption impaired digestibility and efficiency of utilisation of dietary nitrogen and energy, impaired calcium and phosphorus metabolism, curtailed deposition of fat and protein (Sykes and Coop, 1976, 1977 ; Sykes, 1978) and lowered productivity which affects carcase quality.

Production losses due to the above alterations can be manifested by reduction in wool growth eg. up to 59% reduction has been described in artificial infections with gastro-intestinal nematodes and F. hepatica infections of sheep (Donald, 1979) and a 40% reduction has been reported by Southcott et al (1967), Roseby (1970) Barger, Southcott and Williams(1973) and Edwards, Alsaigh, Williams and Chamberlain (1976). Recent studies by Steel, Jones and Symons (1982) found that in sheep infected with both O. circumcincta and T. colubriformis wool growth was reduced by up to 66%, this loss of productivity in concurrent infections being greater than single infections. These changes may result from an increased energy requirement associated with an increased rate of turn-over of body protein, presumably in response to the leakage of plasma protein through the gastro-intestinal tract (Holmes and MacLean, 1971 ; Holmes and Bremner, 1971).

An important study of infection of sheep with O. circumcincta was described by Parkins, et al (1973) who found that a negative nitrogen balance and significantly higher urinary nitrogen excretion developed after infection ; they also reported that marked negative nitrogen balances occurred in animals fed low levels of crude protein which supports the results of some work done with N. brasiliensis in the rat by Steel (1974) and Symons and Jones (1975).

Other workers Andrews, et al (1944) Cauthen and Landram (1958) Ross, et al (1969 a,b) and Reveron,et al (1974) have also shown that lambs and calves infected with T. colubriformis and T. axei went into negative nitrogen balance, and Steel, Symons and Jones (1980) showed that lambs infected with T. colubriformis excrete more urinary nitrogen retention. However, in infections also with T. colubriformis Poppi, Macrae and Corrigall (1981) found that although ileal nitrogen flow was increased, faecal nitrogen levels were unchanged suggesting that reabsorption of the N had taken place. Later in a similar study with H. contortus, albeit on abomasal parasite, Rowe, Abbott, Dargie and Holmes (1982) could find no differences in N. flow.

Thus, apart from diseases causing damage to the lower intestinal tract from which no reabsorption can occur, distrubances in nitrogen balance appear to result from a failure of the host to conserve amino acids derived from excessive degradation of tissue and blood proteins rather than from impaired digestion or absorption (Dargie, 1975).

Production losses can also occur in immune sheep under natural challenge with O. circumcincta. Anderson (1972) found that resistant adult sheep had higher plasma pepsinogen values when they were subjected to increasing larval intake by natural infection although their resultant worm burdens were low. Anderson suggested that this might be associated with a hypersensitive state in the gut mucosa and that in such situations, leakage into the gut of amino acids essential for wool could account for the impaired wool growth recorded. Barger and Southcott (1975) using varying levels of experimental infection with the intestinal parasite T. colubriformis in resistant adult sheep also found reduced wool production.

Studies of guinea pigs and sheep infected with T. colubriformis by Symons and Jones (1975) have also revealed a reduction in muscle protein synthesis while liver protein synthesis showed an increase. The increased liver synthesis was correlated with protein leakage into the gut (Symons, et al, 1974 ; Jones and Symons 1978). Thus the distribution of synthesis between and the extent of catabolism within the various body pools of protein differ in parasitised and normal animals with much of the available amino acids in the former being diverted to organs involved with the synthesis of those amino acids essential for survival, eg. haemoglobin and plasma proteins. These changes in distribution of protein synthesis between the liver and musculature could possibly be correlated with the increased corticosteroids and decreased insulin and thyroxine concentration noted in the plasma of sheep infected with T. colubriformis (Pritchard, Hennessy and Griffiths, 1974 ; Sykes, 1978).

MINERAL METABOLISM AND SKELETAL GROWTH.

Poor calcium and phosphorus retention has been reported in infections with O. circumcincta and T. colubriformis in sheep (Barger, 1973 ; Reveron, et al, 1974 ; Sykes, Coop and Angus, 1975, 1977 ; Sykes and Coop, 1976, 1977). Bone size and bone quality were severely reduced, the latter because of reductions in the density of bone matrix and on its degree of mineralisation leading to osteoporosis. In T. colubriformis infections hypophosphataemia was a constant finding while in O. circumcincta infections the serum levels of Ca and P were normal. In both infections the reductions in bone size and/or circulating P could not be accounted for by reductions in food intake. Reduced digestibilities of either both Ca and P (Sykes and Coop, 1976, 1977 ; Sykes, et al, 1977) or of P alone (Reveron, et al, 1974) were reasons advanced for the bone abnormalities. These workers concluded that in trichostrongylosis this was an induced Ca and P deficiency while in ostertagiasis there was an induced energy or protein deficiency. However, no attempt was made to assess the relative digestibilities or the relative contribution of dietary and endognous losses to the faecal mineral losses, particularly as energy and protein intake in infected animals was lowered by 20%. Because of these deficiences Dargie (1980) expressed the opinion that the observed differences between the two infections merely reflected differences in the degree of infection.

The role of cobalt supplementation in the diet in the

pathogenisis of abomasal parasites was studied by Downey (1965, 1966a,b) in lambs infected with H. contortus. A low level of cobalt in the diet was found to be detrimental to the worms,as lambs given a coblat supplement had a higher faecal egg count and more severe clinical signs of haemonchosis than the coblat deficient lambs. In contrast in O. circumcincta infections lambs on a coblat deficient diet showed higher egg counts and more severe clinical signs and weight loss than those on a coblat supplemented diet. The latter was confirmed in field observations by Reid and Armour (1977).

EFFECT ON GUT MOTILITY.

The level of parasitism may alter the gut motility and digesta flow. Thus it has been demonstrated that in animals carrying moderate burdens of T. colubriformis the amounts of liquid and solid materials in the small intestine were increased whereas in the rumen both were markedly reduced (Roseby, 1977). In animals infected simultaneously with T. axei and Ch. ovina altered gut activity has also been reported associated with changes in the electrical activity of the gut wall of infected sheep. Since diarrhoea is at best transient or even absent in many of the common parasitic conditions of sheep, alterations in gut motility, if they occur, can only be localised in certain regions of the gut and are not generalised. The overall contribution of altered gut motility on digesta flow and to lowered productivity therefore remains doubtful.

INTERACTIONS OF NUTRITIONAL PLANE AND PARASITISM.

The interactions of nutritional plane and parasitism have been studied from two main aspects. The first being the influence of the nutritional level on the establishment, growth and survival of parasites has been considered while in the second the influence of nutrition on the pathogenic effect of the parasite was investigated.

Gibson (1954a,b) has reported that higher numbers of T. axei were present when animals were kept in a poor plane of nutrition. In contrast (Brunsdon, 1964) found that the nutritional plane had no effect on the numbers of gastro-intestinal parasites (O.circumcincta, T. axei, H. contortus) established and this was later confirmed by Goldberg (1965), Scroggs (1968), Downey, Connolly and O'Shea (1972), and Sykes and Coop (1977). However, as pointed out by Sykes (1978) these conclusions may not always be justified since the criteria used in that formulation ie. faecal egg counts or worm recoveries at post-mortem do not necessarily reflect the initial worm burdens established.

Bawden (1969a,b) and Dobson and Bawden (1974) working with the large intestinal nematode Oe. columbianum also found that the plane of nutrition did not affect the numbers establishment but that a higher protein diet did cause a delay in the development of 4th stage larvae and their emergence from the gut wall. They concluded that this was because of the animals on the better diets had mounted a more efficient immune response which affected the development of the worms.

The studies of Gordon (1948, 1950, 1964) and Stewart and Gordon (1953) provided many of the early observations on the effect of nutrition on the pathogenesis of intestinal parasites. It was constantly observed that diet had no effect on the magnitude of worm burdens but did affect the resistance of the host to the effect of the established worms. Protein supplementation of grazing calves has also shown to reduce the pathogenic affects of gastro-intestinal parasitisms (Ciordia, Bizzelli, Baird, McConnell, 1962) resulting in fewer mortalities and ealier maturity of the calves.

In Section 11 of this thesis two aspects of the pathophysiological effects of gastro-intestinal helminthiasis reviewed above are investigated.

In the first, radioisotopes are used to study if a loss of protein occurs into the intestinal tract of lambs under a natural challenge with helminths, previous studies having been conducted with experimental infections.

In the second, the loss of protein into the intestinal tract is studied in immune ewes under experimental challenge with O. circumcincta. Previous observations with this forant have been with natural infection.

EXPERIMENT 1

EXPERIMENTAL STUDIES ON PLASMA PROTEIN LOSS ASSOCIATED WITH GASTRO-INTESTINAL HELMINTHIASIS IN GRAZING LAMBS.

INTRODUCTION

As discussed and demonstrated in Section 1 ovine gastro-intestinal parasitism in temperate climates is usually associated with mixed infection by a number of genera but predominantly Ostertagia spp and in lambs N. battus (Thomas, 1959 ; Gibson, 1965 ; Boag and Thomas, 1970). In the West of Scotland these species are particularly prevalent and account for most clinical outbreaks of parasitic gastro-enteritis (Reid and Armour, 1975).

Studies on pathogenesis of ostertagiasis have shown that the hypoproteinaemia which characterises this disease occurs essentially as a result of the loss of plasma proteins into the gastro-intestinal tract. This was first established using the radioactive tracers $^{51}CrCl_3$ and ^{125}I-albumin to determine gastro-intestinal protein loss and alterations in albumin metabolism respectively (Holmes and MacLean, 1971). However, these studies were carried out using heavy single experimental infections in housed animals and the direct relevance of these findings to grazing animals under conditions of continuous natural infection has not been assessed.

More recent studies have examined the influence of ostertagiasis on the intake and utilisation of food (Sykes and Coop, 1977) and skeletal growth (Sykes, et al, 1977) in lambs given daily doses of O. circumcincta.

The present studies were designed to compar gastro-intestinal plasma protein loss in lambs grazing on pasture which was either heavily or lightly contaminated with trichostrongyle larvae.

EXPERIMENTAL DESIGN.

Grazing History.

From early April 1980, two groups of Blackface ewes and their lambs were grazed on two separate fields, each of 0.33 hetares of permanent pasture. One group (A) consisted of 8 ewes and 12 lambs grazed on pasture known to be heavily contaminated with trichostrongyle larvae(L_3) and was the group used for the 1980 epidemiological studies in Section 1. The other group (B) consisted of 7 ewes and 11 lambs which occupied a nearby pasture which had not been grazed by sheep for 12 months and was contaminated with only a low number of L_3 ; in mid-July the lambs in both groups were weaned, the ewes removed and the lambs continued to graze the same pasture until the end of October, with the exception of one lamb in group A which died in August. At weaning 3 lambs from each group were removed for slaughter and parasitological examination. Subsequently, at regular intervals until October, groups of 3 worm-free tracer lambs (total of 5 groups) were grazed for periods of 2 weeks with each flock and then removed, housed for 7 days and slaughtered to assess the level of larval intake.

The following anthelmintic regime was adopted for the lambs : - in group A, individual lambs showing clinical signs of nematodiriasis were treated in May and the whole group in July and September, with fenbendazole, the latter regimen of treatment being common practice in this area. Group B lambs were treated with fenbendazole at

fortnightly intervals from mid-May onwards to minimise contamination of their pasture.

OBSERVATIONS.

Parasitological.

Faecal samples were collected twice-weekly from the lambs and analysed using flotation and McMaster methods (MAFF, 1971). These were expressed as eggs per gram (epg) and total egg output per day calculated by multiplying the epg by the estimated daily total faecal weight. At weekly intervals all the sheep were weighed. Herbage samples were collected at weekly intervals and examined for the presence of trichostrongyle L_3 by the technique of Parfitt (1955), the numbers of larvae present being expressed as L_3 per kilogram of dried herbage (kdh).

At the end of the appropriate grazing period, each lamb was housed for 1 week prior to slaughter. The gastro-intestinal tract was removed and processed by the techniques of Ritchie, et al, (1966) and MAFF (1971). The worms present in aliquots from the abomasal small intestinal and large intestinal contents and a digest of the abomasal mucosa were counted and identified as in Section 1.

Pasture Analysis.

Representative grass samples from each grazing area were

obtained at monthly intervals during the later part of the experiment and analysed for crude protein (CP) content by an automated standard Kjedahl technique and for crude fibre (CF) by a standard weak acid/ alkali method.

Radioistotopic Measurements.

Plasma protein losses into the gastro-intestinal tract were measured using $^{51}CrCl_3$ in both groups of sheep on three separate occasions, viz : late July (Period 1), late August (Period 11) and October (Period 111). On each occasion six lambs in each group received 800 uCi $^{51}CrCl_3$ by intravenous injection. Plasma samples were then obtained at 10 minutes post-injection and thereafter daily for ten days. Daily faecal samples were also collected during the ten day period and two 10g. aliquots prepared for radioactivity measurement.

Radioactivity determinations of plasma and faecal samples were carried out in a Packard automatic gamma scintillation counter. The radioactivity of each sample was plotted on semi-logarithmic graph paper as a percentage of the 10 min. sample and the half life obtained by regression analysis. The total feacal radioactivity was obtained by multiplying the mean activity (cpm) in 1g. of faeces (obtained from the 10g. samples) by the estimated total daily faecal output (vide infra). The total injected radioactivity for each sheep was calculated using the radioactivity of known standards multiplied by the weight of $^{51}CrCl_3$ injected into each animal (Holmes and MacLean, 1971). The daily faecal clearance of plasma (ml/day) was obtained

by dividing the total daily faecal radioactivity (cpm) by the radioactivity per ml of plasma (cpm/ml) at the beginning of each 24 hour period.

Estimation of total faecal output.

Six days before the injection of $^{51}CrCl_3$ and thereafter daily for the duration of each study period each lamb received 1g. of chromic oxide (Cr_2O_3) per os in a gelatin capsule. Faecal samples were obtained each day from male lambs by fitting them with a harness and faecal bag for 3-4 hours per day, and from female lambs by twice daily rectal sampling. Dry matter content was determined by drying in a forced air-draught oven at 80°C for 48 h. and chromium concentration in the dried faeces determined by atomic absorption spectroscopy (Williams, David and Iismaa, 1962).

Blood biochemistry.

Serum samples were collected weekly from the lambs throughout the grazing season and analysed for total serum protein concentration by a modified Biuret method, and for albumin concentration by a bromocresol green technique (Technicon Autoanalyser 111). At fortnightly intervals plasma samples were analysed for levels of pepsinogen by a modification of the method described by Edwards, et al, (1960) and expressed as international units of tyrosine (i.u. of tyrosine).

Statistics.

The standard 't' test was used to determine the levels of significance (Fisher, 1934).

RESULTS.

Clinical findings.

Clinical signs of nematodiriasis ie. diarrhoea, were seen in individual lambs of group A in May and June and parasitic gastro-enteritis characterised by semi-fluid faeces occurred also in individuals in July and one lamb died in August from severe parasitism. In the remaining lambs clinical signs abated following each of the anthelmintic treatments, and the faeces regained their normal consistency. Live weight gains did not differ significantly (0.16 kg/day in groups A and B respectively).

Parasitological data.

This has been presented in detail in Section 1 but to facilitate reading of this section may be summarised as follows :- Trichostrongyle eggs, including those of *N. battus* were first detected in faeces of group A lambs after four weeks grazing, ie. early May. Initially the number of eggs present was low but those of *N. battus* increased markedly during May reaching a maximum of 585 epg during June and then declined rapidly to zero by late July.

The mean trichostrongyle epg (excluding N. battus) rose to 800 at the end of June and reached a mean maximum of 1,105 at the end of August. Following each anthelmintic treatment there was a sharp decrease in faecal egg count to zero but within three weeks, eggs were present in the faeces again and gradually and gradually increased until the next treatment. The faecal egg count of the lambs from group B which were treated each fortnight remained negative throughout.

Low numbers of N. battus L_3 were present on the pasture grazed by group A lambs in May and a sharp increase in the numbers of these larvae occurred, reaching a maximum of 7777 L_3/kdh early in June, and declined to a level of 322 by mid-July. The numbers of L_3 from other trichostrongyle species were also low early in the grazing season and remained so until June when the numbers began to increase steadily, reaching a maximum of 40,400 in late June. Thereafter, there was a marked decrease in the numbers present to a level of only 725 by late August. The numbers of larvae on the pasture grazed by group B lambs remained low throughout. A maximum count of 8,800 L_3 trichostrongyle larvae were recorded in late June, of which approximately 90% were N. battus.

The mean total worm burden of the three permanent lambs from group A killed in July was 20,722 and the mean burdens of groups of three tracers which each group grazed for periods of two weeks in each month from July through November were 10,500, 6,245, 4,900, 2,731 and 2,833 respectively. The mean total worm burden of the

8 permanent lambs in group A slaugthered at the end of October was 9,872. The permanent lamb which died in August had severe lesions of ostertagiasis and a worm burden of 14,200 O. circumcincta species were the most common nematodes present in these burdens followed by N. battus ; smaller burdens of H. contortus, T. axei and T. vitrinus were also present, together with a few Ch. ovina. The faecal egg counts from the lambs of group B remained negative throughout the entire grazing season and no worms were detected at their post-mortem.

Haematological and Biochemical findings.

A slight but significant hypoalbuminaemia was observed in the lambs of group A during Period 1, 11 and 111 (Tables 20, 22 and 24). The mean plasma pepsinogen levels of group A are shown in Fig. 4. They rose progressively during June and July from less than 1.0 i.u. to 1.5 i.u. Following anthelmintic treatment in July, it decreased to 1.2 and increased again to 2.2 in early September. Following the treatment in September, a marked recuction to less than 1.0 occurred. The mean values in group B remained below 1.0 i.u. throughout the experiment.

Faecal output and dry matter content.

During all three study periods the fresh faecal output was greater in group A than in group B and this was closely associated with the reduced faecal dry matter content in the former group

as can be seen from Tables 20, 22 and 24.

Plasma loss into the gut.

The plasma loss which occurred at the different sampling periods is given in Tables 21, 23 and 25. It was consistently higher in the lambs of group A than those of Group B. This difference was detectable regardless of whether the plasma leak was expressed as the total faecal plasma clearance (ml/d) or plasma clearance per gram of fresh or dried faeces (ml/d). However, differences were detected between these parameters in the three study periods. In period 1 all the lambs in group A had higher total faecal clearances than those in group B (Table 21). A similar effect was observed if the plasma loss was expressed as ml of plasma per g. of dry faeces.

In Period 11 the plasma faecal clearances as ml/d of both group A and group B had increased above those of Period 1, although again group A had higher values than group B (Table 23). However, when the plasma losses were expressed as ml/g of faeces, both group A and group B were lower than those observed in Period 1. This apparent discrepancy is probably brought about by the higher faecal output during Period 11 compared with Period 1 (Tables 20 and 22).

In Period 111 the differences between groups A and B were less marked than during previous period (Table 25) and this was related to the low faecal egg counts of group A at this time (Table 24).

TABLE 20 Body Weight, Faecal Egg Count, Faecal Output, Total Daily Faecal epg, Output and Faecal Dry Matter Content of lambs grazing heavily (Group A) and lightly (Group B) contaminated pasture. July - Period 1.

Group	Lamb No.	Sex	Body Wt (kg)	Faecal egg Count epg	Faecal Output Kg/day	Total daily faeces egg Output x 10^3	Faecal Dry Matter %
A	Y45	o	14.5	680	1.413	960	12.8
	FR96	o	21	1260	0.457	575	34.5
	FR93	♀	21.5	1036	1.141	1182	18.1
	Y44	o	26	200	2.443	448	14.8
	FR94	o	33	806	1.719	1138	19.2
	FR92	o	37	470	1.558	732	23.2
	Mean		25.5	742	1.455	887	20.4
	± SE		± 3.4	± 156	± 0.27	± 144	± 3.1
B	612	♀	21.5	0	0.862		34.9
	Y61	o	22	0	1.466		21.8
	FR85	o	23	0	1.551		22.3
	FR86	♀	27	0	1.343	N.1	28.8
	FR97	o	29	0	0.748		32.6
	FR95	o	34.5	0	1.250		25.6
	Mean		26.2	0	1.203		27.7
	± SE		± 2.0		±0.13		± 2.2

TABLE 21 Serum albumin concentration, Mean Daily faecal plasma clearance and $^{51}CrCl_3$ plasma half-lives (t1/2) in lambs grazing heavily (Group A) and lightly (Group B) contaminated pasture.

Group	Lamb No.	Serum Albumin (gm/Lit)	Total faecal clearance ml/day	Faecal clearance ml/gm/day (dry faeces)	Plasma half-lives (hrs)
	Y45	24	241	0.164(1.281)	64
	FR96	24	163	0.363(1.052)	95
	FR93	27	175	0.165(0.912)	88
A	Y44	31	2	0.086(0.581)	75
	FR95	28_t	177.6	0.108(0.563)	88
	FR92	33	213	0.144(0.621)	85
	Mean	27.8	197	0.172(0.835)	83
	± SE	± 1.4	± 12	± 0.04 (± 0.12)	± 5
	612	35	71	0.146(0.418	71
	Y61	36	98	0.076(0.346)	89
	FR85	35	124	0.076(0.341)	79
B	FR86	37	104	0.080(0.278)	117
	FR97	36	103	0.146(0.448)	80
	FR95	40	113	0.094(0.367)	86
	Mean	36.5^{xxxx}	102^{xxxx}	0.103(0.366)	86
	± SE	± 0.8	± 7	± 0. 01(± 0.24)	± 7

xxxx P 0.001

TABLE 22 Body Weight, Faecal Egg Count, Faecal Output, Total Daily Faecal epg Output and Faecal Dry Matter Content of lambs grazing heavily (Group A) and lightly (Group B) contaminated pasture. August - Period 11

Group	Lamb No.	Body Wt (kg)	Faecal Egg Count epg	Faecal Output Kg/day	Total daily faeces egg Output x 10^3	Faecal Dry Matter %
A	Y45 *	-	-	-	-	-
	FR96	22.5	5220	0.414	216	29.5
	FR93	26	1293	1.563	2020	23
	Y44	34.5	120	4.916	589	16.4
	FR94	42.5	1333	3.467	4621	20.7
	FR92	48.5	440	3.187	1402	19.9
	Mean	34.8	1681	2.709	2159	22
	± SE	± 4.8	± 914	± 0. 78	± 675	± 2.0
B	612	27.5	0	1.401	-	28.8
	Y61	30.5	0	3.225	-	19.8
	FR85	29.5	0	2.170	-	22.7
	FR86	35	0	2.353	-	21.7
	FR97	33.5	0	1.150	-	27.8
	FR95	41.5	0	3.898	-	24.3
	Mean	33	0	2.366	-	24.3
	± SE	± 2.0	0	± 0. 42	-	± 1.4

* Animal Died

TABLE 23 Serum albumin concentration, Mean Daily faecal plasma clearance and $^{51}CrCl_3$. plasma half-lives (t1/2) in lambs grazing heavily (Group A) and lightly (Group B) contaminated pasture. August - Period 11.

Group	Lamb No.	Serum Albumin (gm/Lit)	Total faecal clearance ml/day	Faecal clearance ml/gm/day (dry faeces)	Plasma half-lives (hrs)
A	FR96	26	134	0.239(0.810)	127
	FR93	29	199	0.122(0.531)	92
	Y44	33	317	0.666(0.402)	78
	FR94	30	275	0.078(0.377)	107
	FR92	34	319	0.099(0.497)	91
	Mean	30.4	249	0.121(0.523)	99
	± SE	± 1.4	± 36	± 0. 03(± 0.08)	± 8
B	612	34	95	0.067(0.233)	103
	Y61	34	214	0.067(0.338)	78
	FR85	33	123	0.059(0.260)	114
	FR86	36	173	0.074(0.341)	97
	FR97	33	105	0.089(0.321)	102
	FR95	38	139	0.077(0.310)	102
	Mean	34.7^{x}	142^{x}	$0.072(0.301)^{xx}$	99
	± SE	± 0.8	± 18	± 0. 00(± 0.0.)	± 5

x $P < 0.05$

xx $P < 0.01$

TABLE 24 Body Weight, Faecal Egg Count, Faecal Output, Total Daily Faecal epg Output and Faecal Dry Matter content in lambs grazing heavily (Group A) and lightly (Group B) contaminated pasture. October - Period 111.

C oup	Lamb No.	Body Wt (kg)	Faecal Egg Count epg	Faecal Output Kg/day	Total daily faeces egg Output x 10^3	Faecal Dry Matter %
A	FR96	28.5	455	1.124	511	28.6
	FR93	29.5	35	2.167	75	22.7
	Y44	41.5	195	3.126	609	17.6
	FR94	48.5	320	3.226	1032	22.0
	FR92	53.5	395	2.871	1134	19.1
	Mean	40.3	280	2.503	672	22
	± SE	± 4.9	0	± 0. 39	± 191	± 1.8
B	612	32.5	0	1.413	-	31
	Y61	34.5	0	2.478	-	21.6
	FR85	32.5	0	1.512	-	28.3
	FR86	35	0	1.477	-	30.7
	FR97	38	0	1.224	-	29.8
	FR95	43.5	0	1.433	-	20.3
	Mean	36	0	1.590	-	27
	± SE	± 1.7	0	± 0. 18	-	± 1.9

TABLE 25 Serum albumin concentration, Mean Daily faecal plasma clearance and $^{51}CrCl_3$ plasma half-lives (t1/2) in lambs grazing heavily (Group A) and lightly (Group B) contaminated pasture. October - Period 111.

Group	Lamb No.	Serum Albumin (gm/lit)	Total faecal clearance ml/day	Faecal clearance ml/gm/day (dry faeces)	Plasma half-lives (hrs)
	FR96	31	135	0.133(0.465)	97
	FR93	32	136	0.061(0.269)	98
	Y44	34	181	0.058(0.329)	83
A	FR94	35	146	0.046(0.207)	112
	FR92	37	167	0.059(0.309)	86
	Mean	33.8	153	0.071(0.316)	95
	± SE	± 1.0	± 9	± 0.01(± 0.04)	± 5
	612	38	89	0.060(0.194)	95
	Y61	36	90	0.036(0.166)	90
	FR85	39	183	0.124(0.438)	109
B	FR86	39	135	0.091(0.298)	104
	FR97	36	91	0.076(0.255)	91
	FR95	34	101	0.067(0.330)	123
	Mean	37	115	0.076(0.280)	102
	± SE	± 0.8[x]	± 15	± 0.01(± 0.04)	± 5

x $P < 0.05$

TABLE 26 Pasture Analysis (Mean Values)

Date	Sample	Crude Protein g/kg	Crude Fibre g/kg	Predicted Nutritive Value MJME/kg.DM
7/8/80	BP2	243.27	260.10	9.9
	BP4	217.78	258.75	9.9
4/9/80	BP2	210.75	264.10	10.57
	BP4	185.75	269.10	10.61
9/10/80	BP2	160.4	228.49	10.48
	BP4	186.57	233.52	10.36
5/11/80	BP2	128.11	220.96	11.28
	BP4	147.31	228.77	11.16

Nutritive value of pasture.

Predicted values of metabolisable energy (MJME/kg DM) and digestible crude protein (gDCP/kg DM) were obtained from analyses of the CF and CP contents of the pasture samples shown in Table 26 (MAFF, 1975). Plots grazed by both groups were very similar in ME content throughout. For example, values of 9.9 MJME/kg DM were obtained in early August with the regrowths rising steadily to about 10.7 MJME/kg DM by early November. The predicted DCP content in the early part of each of the months of August, September, October and November for group A was 185, 155, 110 and 80 gDCP/kg DM respectively, which was comparable to values obtained for the group B grazing of 162, 132, 133 and 97 gDCP/kg DM respectively for the same times.

DISCUSSION.

The clinical picture observed in the group A lambs was similar to that often seen in lambs set-stocked on permanent sheep pastures in the West of Scotland ie. diarrhoea and weight loss caused by N. battus in May and early June, followed by poor thriving and intermittent diarrhoea from July through September and attributable to parasitic gastro-enteritis. However, it is interesting that, despite these clinical episodes in the group A lambs the fact that the group B lambs were clinically normal throughout, their mean live weight gains were similar. This may respect the rapid response of the two groups to each anthelmintic treatment and/or the relatively poor daily mean live weight gain of the group B lambs, ie. 0.13kg/day.

Pasture analysis showed the available grazing to be similar in nutritive content with respect to energy protein. The protein content was high at the beginning of August (about 170 gDCP/kg DM) and fell steadily to a mean value of about 90 gDCP/kg DM by the early part of November when grass growth ceased. The grazings contained a protein concentration in excess of the animals' maintenance and maximum potential growth requirements where grazing was unrestricted.

The pattern of elevated pasture larval counts of N. battus in May and other trichostrongyle larvae in July is typical of that previously reported in Northern Britain (Boag and Thomas, 1971 ; Reid and Armour, 1975). However, the very rapid drop in numbers of L_3 present at the end of July from 10,293 to 725/kdh in late August was unexpected. Although the reason is not definitely known, it may be related as suggested in Section 1 to the continuous heavy rainfall in August and September causing a downward larvage of larvae into the soil ; since the temperatures were decreasing at this time, further recruitment of larvae from recently deposited eggs would also be interupted.

The tracer burdens generally reflected the pasture larval counts and confirmed that the challenge to the group A lambs was diminishing from the end of July. Since the lambs were treated with fenbendazole on September 9th the worm burdens of the permanent lambs at post-mortem in November (mean 9872) reflected the numbers established over the 7 week period prior to housing and slaughter.

When the cumulative tracer burdens over that period are considered they come to a similar figure of 10,526. If allowance is made the turn-over infection known to occur in continually exposed animals (Michel, 1969 ; Whitlock, Crofton and George, 1972). It would, therefore, appear that these permanent lambs had acquired little or no immunity to infection by the end of October.

It is interesting that group B lambs were apparently clear of adult infections throughout the year, as evidenced by their consistently negative faecal egg counts and also the negative worm burdens at post-mortem. However, the presence of nodules in the abomasal mucosa of these lambs at post-mortem confirmed that some of the larvae recovered from pasture B must have become established prior to their removal by the fortnightly anthelmintic treatments.

The infection with Ostertagia spp and H. contortus from the end of June also resulted in an elevation in plasma pepsinogen levels to 2.2 i.u. This figure is lower than that recorded by Thomas and Waller (1975) despite the larval challenge and tracer burdens in the present study being similar to those recorded by the Newcastle workers.

The other related biochemical finding is that a slight but significant hypoalbuminaemia was observed in group A lambs during all three experimental periods. Such a finding is commonly observed in a variety of infections with gastro-intestinal parasites and its aetiology has been intensively investigated in sheep infected with experimental infections of O. circumcincta (Holmes and MacLean,

1971) and H. contortus (Dargie, 1975). As a result of these laboratory investigations, it has been clearly demonstrated that the hypoalbuminaemia observed in such infections is essentially the result of a protein-losing gastro-enteropathy from the feeding activities and damage caused by these parasites.

Similarly, in the present experiment there was a close association between the degree of hypoalbuminaemia and the level of plasma loss into the gastro-intestinal tract as measured by ^{51}Cr-plasma proteins. Such a study therefore, confirms that, under grazing conditions typical of the West of Scotland, parasitised sheep can suffer a considerable loss of plasma proteins into the gut.

In assessing the full significance of the gastro-intestinal plasma loss in grazing sheep, it is important to consider a number of factors. First, the values in the present study for the daily faecal clearance of plasma as ml per day in sheep grazing lightly contaminated pasture are considerably higher than those obtained from parasite-free sheep in previous experiments with caged sheep (Holmes and MacLean, 1971 ; Dargie, 1975). There are several possible reasons for this difference. One important consideration is that in the current experiment total direct faecal collections could not be made and indirect assessments had therefore, to be made using the inert marker Cr_2O_3 . Despite day to day variations, the values obtained in the present study are comparable to others obtained by direct daily collections of the faecal output in caged

sheep (Kendall, 1977).

It is also important to bear in mind that the lambs in group A also consistently showed high daily faecal plasma clearances on the basis of ml of plasma per gram of faeces. Such calculations do not involve the figure for the estimated total daily faecal output and hence avoid possible inaccuracies associated with the calculation of the total faecal clearance.

A more likely explanation for the relatively high faecal clearance values in group B lambs compared with caged parasite-free sheep is that their elevated plasma loss was due to their exposure to low levels of parasite infection throughout the grazing season. Certainly the evidence that larvae could be detected on their pasture and the finding of nodules in the abomasal mucosa at post-mortem supports such an explanation.

Secondly, it is important to appreciate that the estimates of faecal plasma clearance obtained in the present study represent plasma protein loss into the gastro-intestinal tract but not necessarily lost to the animal in the faeces. Indeed there is now considerable evidence from other studies that the excretion of faecal N is seldom increased in animals with gastro-intestinal helminthiasis (Parkins, et al, 1973 ; Dargie, 1980). However, urinary N levels are commonly increased in such conditions and are associated with infected animals entering negative nitrogen balance. Nevertheless faecal N levels may be increased in diarrhoeic animals

or in cases where the lower gastro-intestinal tract is parasitised. It is only recently that the nitrogen flow between different parts of the tract has been investigated in parasitised animals. It is, therefore, of interest that in sheep infected with the small intestine roundworm T. colubriformis, Poppi, et al, (1981) found that, although ileal nitrogen flow was increased, faecal nitrogen levels were unchanged. However, in similar recent study in sheep infected with the abomasal parasite H. contortus (Rowe, et al, 1982), it was found that despite a large increase in duodenal N flow, there was no difference in ileal N flow. Such a finding indicates that blood proteins lost in the abomasum may be efficiently reabsorbed from the small intestine, even in heavy infections.

Thirdly, there is now considerable evidence from a variety of previous studies (Sykes and Coop, 1976 ; 1977 ; Sykes, et al, 1979) that in gastro-intestinal parasitic infections depressed productivity, and death in extreme cases, results primarily through reduced feed intake and increased energy utilisation in tissue and blood regeneration rather than through a protein drain on the animal from the loss of blood proteins into the gastro-intestinal tract. In the present experiment it was not possible to measure feed intake and so this factor could not be evaluated. However, two factors suggest that anorexia was not significant in the current study. First, the overall weight gains in the lambs in group A were slightly higher than those of group B, though this trend is partly due to the death of the lightest lamb in group A during August, and secondly, faecal dry matter output per kilogram body weight was not markedly different

between the two groups during any of the three study periods.

It is of interest that, despite the latter finding, there was a consistent increase in the faecal moisture content of the lambs in group A. This increased faecal water loss was clearly related to the level of parasitism and reflects the increased intestinal flow rate of ingesta commonly associated with gastro-intestinal parasitism.

In conclusion, the results presented here are the first demonstration that a significant loss of plasma protein occurs in grazing sheep naturally infected with gastro-intestinal trichostrongyles and thereby confirms previous data obtained from laboratory studies.

EXPERIMENT 2.

EXPERIMENTAL STUDIES ON PLASMA PROTEIN LOSSES AND CHANGES IN PLASMA PEPSINOGEN LEVELS ASSOCIATED WITH A NEMATODE LARVAL CHALLENGE OF IMMUNE EWES.

INTRODUCTION.

In most experimental studies on the pathophisiology of ovine helminthiasis, parasite-naive lambs have been used and the possible influence of immunity to reinfection following repeated challenge has not been a complicating factor.

However, there have been indications from several recent studies that older animals previously exposed to field (Anderson, 1973) and experimental (Barger and Southcott, 1975) trichostrongyle infections, though showing low or negative faecal egg counts and low worm burdens, suffer production losses when under subsequent larval challenge. In Anderson's studies where the larval challenge was primarily with an abomasal parasite O. circumcincta, the plasma pepsinogen levels were also markedly elevated.

The latter findings may be of significance since it has been accepted for a considerable time that elevated plasma pepsinogen values reflect histopathological changes in the host due to an increased permeability of the parasitised abomasal mucosa which permit an increased diffusion of pepsinogen into the blood and a leakage of plasma proteins into the gastro-intestinal tract (Jennings, Armour, Lawson and Roberts, 1966) Holmes and MacLean, 1971).

It is, therefore, possible that the production losses noted in previously exposed sheep by Anderson (1973) may have occurred

primarily as a result of plasma protein losses into the gastro-intestinal tract.

The current study was, therefore, conducted to determine whether a rise in plasma pepsinogen levels in immune ewes under larval challenge is associated with increases in plasma protein losses into the gastro-intestinal tract and could be used as a marker for such losses.

EXPERIMENTAL DESIGN.

Nine Blackface ewes aged 5-7 years were removed from pasture in July and divided into 3 groups each of three animals, housed for 7 days and then placed in sheep metabolism cases for 14 days.

Group A, after housing and prior to caging, were challenged with 7,000 mixed trichostrongyle larvae. After caging larval challenge ceased for five days and was then reintroduced daily for the remaining nine days, so that each ewe received a total of 112,000.

Group B received no larval challenge and acted as non-challenged infected controls, while Group C,also received no larval challenge and, in addition, were treated 5 days prior to caging with fenbendazole at 7.5 mg/kg and served as non-challenged, non-infected controls (Panacur, Hoechst, Milton Keynes, England).

Groups A and B had previously grazed pasture heavily contaminated with a mixed trichostrongyle larvae infection (90% *O. circumcincta*); and were the same ewes used in the 1981 epidemiological study reported in Section 1. The mean larval population on the pasture over the week prior to housing was 664 L_3/kdh.

Group C had grazed an adjacent lightly contaminated pasture (100 larvae/kdh) and had received fortnightly treatment with fenbendazole for the previous 3 months. All animals were killed 21 day sfter the first larval challenge.

OBSERVATIONS.

Parasitological techniques.

Preparation of larval inocula

Larvae harvested from faecal cultures of a lamb grazing the pastures previously occupied by the ewes of Groups A and B. The larvae were differentiated, counted and aliquots prepared for inoculation. The larvae consisted of 90% O. circumcincta, 9% Trichostrongylus spp and 1% Ch. ovina.

Faecal examination

Faeces were examined for the presence of nematode eggs before the experiment and thereafter faecal samples were collected twice-weekly ; flotation and McMaster methods were used to detect the presence of nematode eggs (MAFF Technical Bulletin No. 18 (1971) and these were reported as eggs per gram (epg).

Post-mortem examination

The gastro-intestinal tract was removed and examined for the presence of nematodes using the method of Ritchie, et al, (1966). The nematodes present were identified, counted and classified as adult, male and female, fifth larval stages and fourth larval stages

as outlined previously.

Blood analysis

Serum samples were collected every 3 to 4 days and analysed for total serum protein concentration by a modified Biuret method and for albumin concentration by a bromocresol green technique (Technicon Autoanalyser III). Also at regular intervals plasma samples were analysed for levels of pepsinogen by a modification of the method described by Edwards, et al,(1960).

Packed cell volumes were measured by the microhaematocrit method (Hawksley & Son Ltd., London, England).

Radioisotope techniques.

Preparation of labelled materials

Commercial sheep albumin (Cohn Fracton U, Pentex Incorp., Kankakee, Illinois, U.S.A.) was trace-labelled with ^{125}I by the method of McFarlane (1958).

Injection and treatment of samples

To ensure rapid excretion of ^{125}I the thyroid was blocked by daily dosing per os with 10ml of 0.75% K.I. beginning 4 days prior

to the injection of ^{125}I albumin and thereafter continuously throughout the experiment. Each sheep received 10ml. of labelled ovine albumin containing approximately 1 mCi ^{125}I and approximately 850 uCi $^{51}CrCl_3$ at the beginning of the experiment.

The ^{125}I and $^{51}CrCl_3$ were injected via the jugular catheter. The first blood sample was taken 10 min. post-injection and further samples were taken daily for the duration of the two week study period. At each bleeding a heparinised sample was taken from which 1ml. of plasma was obtained for radioactivity determinations. The total urine and faeces excreted during each 24 hr. period were collected and the ^{51}Cr and ^{125}I activity of representative samples were determined using a Packard automatic Y scintillation counter.

Calculations and expression of results

The faecal clearance of plasma as ml. lost per day into the gut was determined from the daily total ^{51}Cr activity of the faeces, divided by the radioactivity as counts/sec./ml. of the plasma at the beginning of each 24 hr. colecction period.

The fraction of the intravascular pool of albumin catabolised per day (F CA) was obtained from the ^{125}I data using the method of Campbell, et al,(1956). This method, based on the daily activity excreted in the faeces and urine, assumes that following catabolism of the labelled protein the liberated isotope is rapidly

and quantitatively excreted.

$$\text{hence F CA} = \frac{\text{Total excreted activity in urine and faeces/day}}{\text{Total plasma activity}}$$

RESULTS.

Clinical findings.

In group A, one ewe showed depression of appetite 9 days after the first larval inoculation and this continued until the termination of the experiment. Very soft faeces were noticed in all the ewes in this group, and one ewe developed diarrhoea 15 days post-infection. The ewes of group B, ie. unchallenged but previously infected had intermittent softenings of the faeces, while those of the ewes of group C were of normal consistency throughout the study.

Parasitological data.

Faecal examination

Eggs were consistently present in the faeces of all ewes in group A and two ewes in group B. In group A the epg reached a mean maximum of 883 on day 15 after challenge and ranged from 100-883 . The relatively high mean count of 883 was due to an individual value of 1600 from a sheep which was inappetent and

produced considerably less faeces than to control. In group B a mean maximum epg of 183 was recorded in day 18 range 50-183. The faeces of those in group C remained negative.

Post-mortem worm burdens

Total worm burdens of the ewes in group A (challenged infected) were 302,2119,3302 ie. an establishment of 0.27, 1.89 and 2.77% respectively from the 112,000 administered. These burdens consisted of approximately 25% mature adult worm and 75% developing larval stages. The worm burdens of group B (non-challenged infected) were 0, 0, and 508 adult worm. O. circumcincta species were the most common nematodes present (90%) in these burdens and only low numbers of T. vitrinus and a few Ch. ovina were present. No worms were detected in the ewes of group C at post-mortem . Worm burdens are shown in Table 27.

Blood Biochemical and Haematological findings.

The pepsinogen levels are shown in Fig. 17. The levels in groups A and B were similar at the beginning of the experiment exceeding those of group C by approximately 0.5 i.u. The pepsinogen levels of group A rose during the first administration of larvae from 1.2 to 1.7 then temporarily declined to 0.9 i.u. when the larvae were withdrawn and then increased to 1.8 when larval inoculation was resumed. Group B values declined steadily to a level of 0.5 at the end of the experiment. Normal plasma pepsinogen

TABLE 27 Individual worm burdens of ewes grazing in 1981

July necropsy	EWE NUMBER								
	GROUP A			GROUP B			GROUP C		
	P33	382	514	P40	363	663	123	784	790
O. circumcincta									
♂	0	500	100	300	0	0	0	0	0
♀	0	400	200	200	0	0	0	0	0
L5	0	900	300	0	0	0	0	0	0
DL4	300	1000	1300	0	0	0	0	0	0
EL4	0	300	0	0	0	0	0	0	0
Total	300	3100 (10)	1900	500	0	0	0	0	0
		Mean 1766			Mean 166.6			0	
H. contortus									
♂	0	0	0	0	0	0	0	0	0
♀	0	0	0	0	0	0	0	0	0
L5	0	200	0	0	0	0	0	0	0
DL4	0	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0	0
Total	0	200	0	0	0	0	0	0	0
		Mean 66.6			0			0	

TABLE 27 (continued)

	EWE NUMBER								
	GROUP A			GROUP B			GROUP C		
	P33	382	514	P40	363	663	123	784	790
T. vitrinus									
♂	0	0	100	0	0	0	0	0	0
♀	0	0	100	0	0	0	0	0	0
L5	0	0	0	0	0	0	0	0	0
DL4	0	0	0	0	0	0	0	0	0
EL4	0	0	0	0	0	0	0	0	0
Total	0	0	200	0	0	0	0	0	0
		Mean 66.6			0			0	
Ch. ovina	2	2	19	8	0	0	0	0	0
		Mean 8			Mean 2.5			0	
Total burdens	302	3302	2119	508	0	0	0	0	0
		Mean 1908			Mean 169			Mean 0	

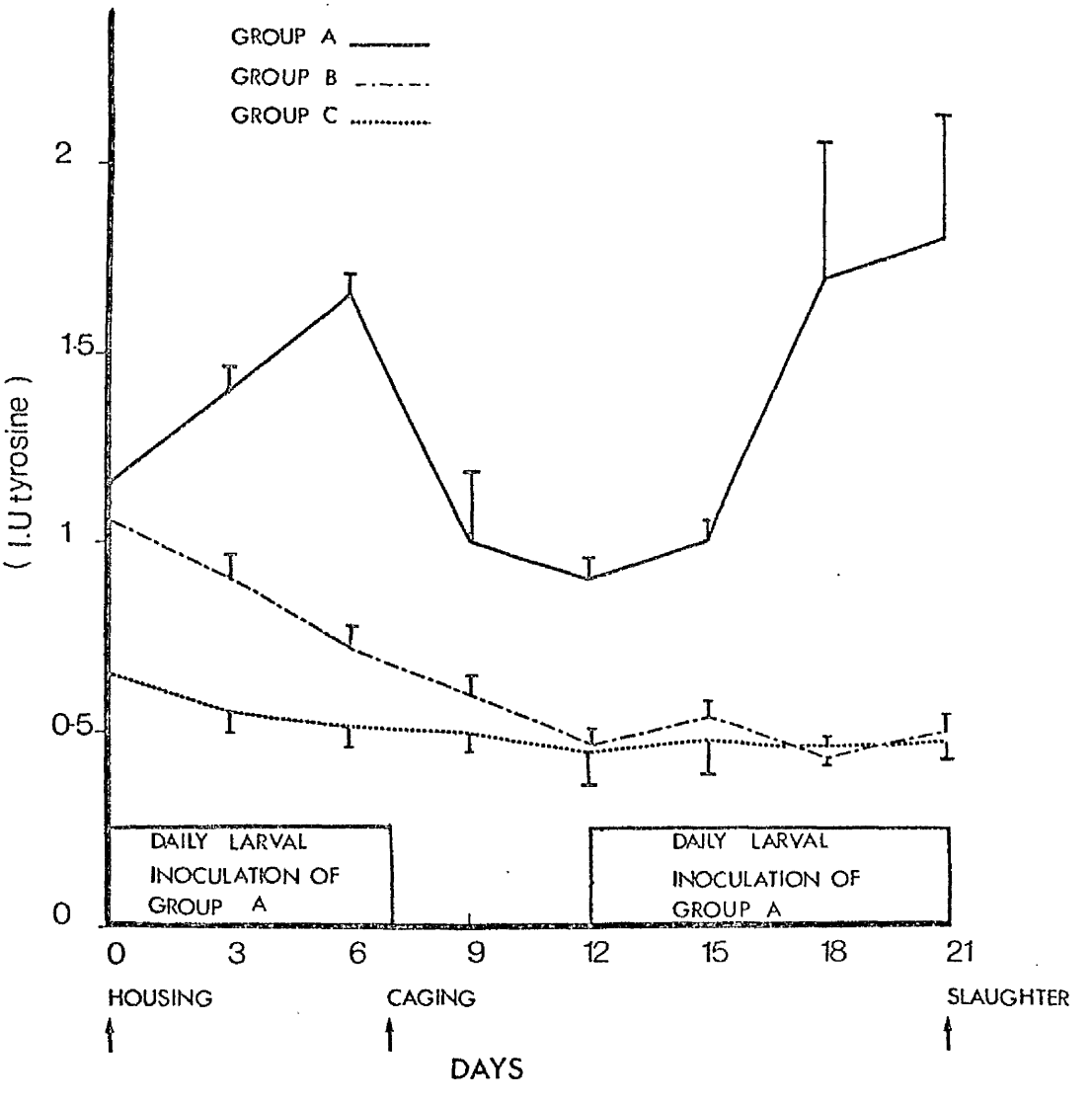


Figure 17. Mean bi-weekly plasma pepsinogen levels (Iu tyrosine) of the ewes in Group A (infected and challenged), Group B (infected and unchallenged) and Group C (uninfected and unchallenged).

values, ie. 0.7 i.u. were recorded from group C throughout the experiment.

A mild hypoalbuminaemia was detected in the ewes of both groups A and B relative to those of group C. The mean values from groups A, B and C were 30.5, 31.5 and 34.5 g/l respectively.

No significant differences were detected in the haematocrit values between the sheep (range 26%-34%).

Albumin catabolism.

It is apparent from Figure 18 that there were significant increases in albumin catabolism of adult ewes following exposure to larval challenge (groupA- compared with both non-challenged control groups B and C. The hypercatabolism in group A. demonstrated by both a rise in F (CA) and a reduction in the apparent half-life of plasma ^{125}I-albumin.

Plasma loss into the gut.

Initially the faecal plasma clearance values were similar in the three groups of animals (Fig. 19) but, following larval challenge, there was a significant increase in the loss of plasma into the gastro-intestinal tracts of the ewes in group A.

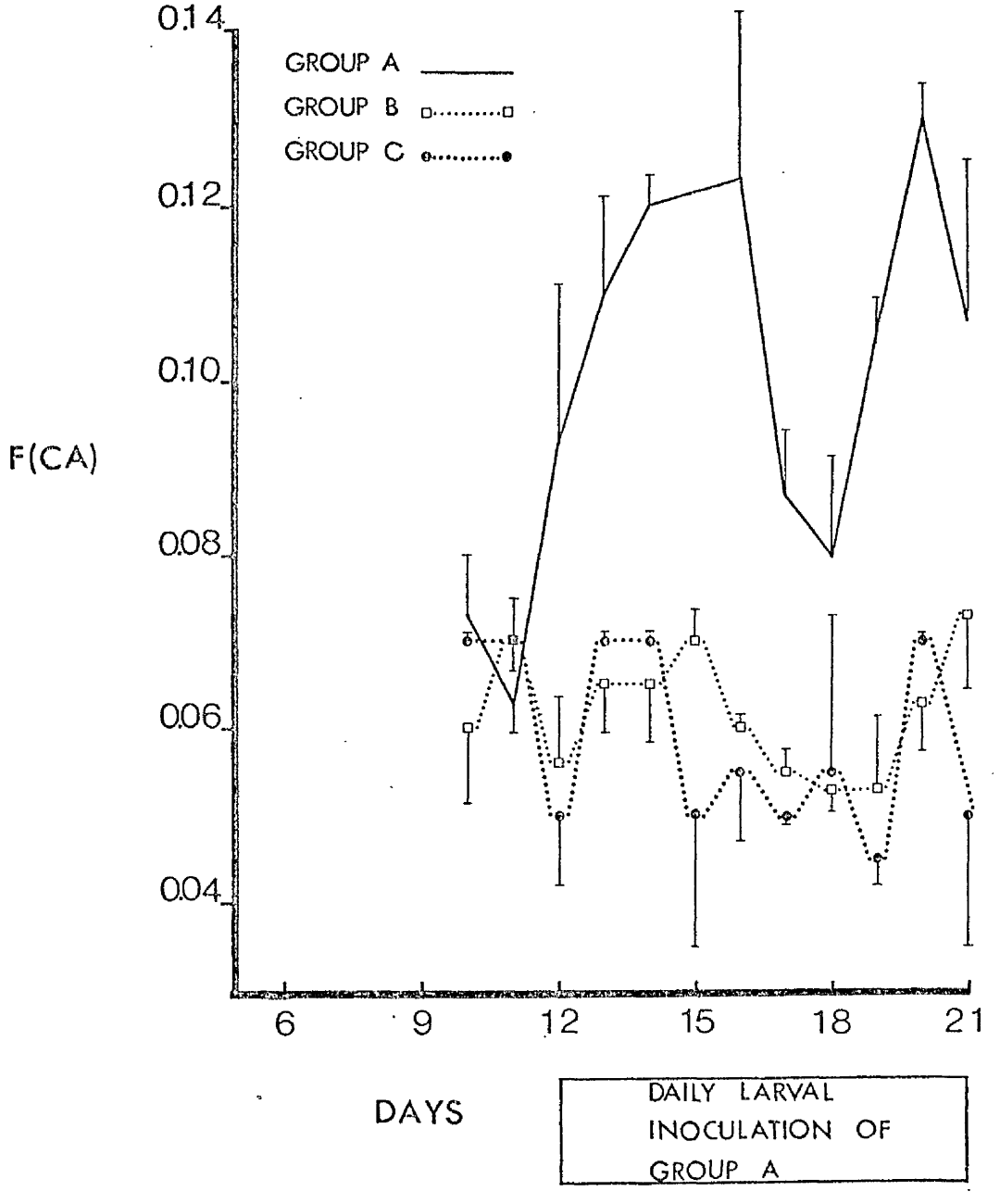


Figure 18. Albumin catabolism of the ewes in Group A (infected and challenged), Group B (infected and unchallenged) and Group C (uninfected and unchallenged)

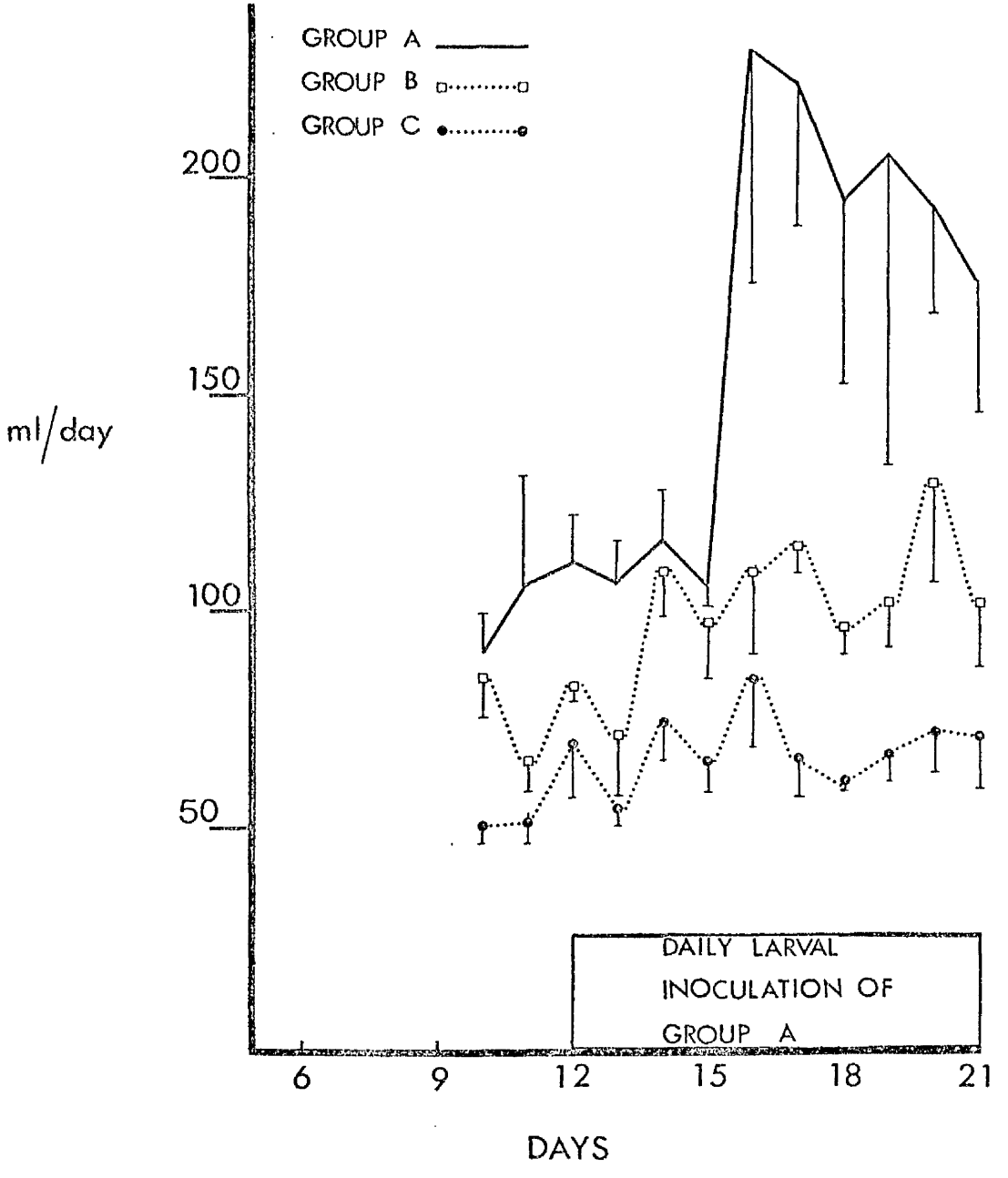


Figure 19. Losses of plasma into the gut of the ewes in Group A (infected and challenged), Group B (infected and unchallenged) and Group C (uninfected and unchallenged).

DISCUSSION.

The ewes in this study had previously grazed on permanent sheep pasture known to be contaminated with mixed trichostrongyle L_3 and since their lambs had been recently weaned, the immunity of these ewes should have been restored to a high level (O'Sullivan & Donald, 1970). Their resistance was confirmed by the very low worm burdens in the unchallenged group B ewes at slaughter and the low numbers established in group A following challenge (3%). Furthermore these worm numbers are likely to be a true indication of the burdens established since no loss of worms is likely to occur within 21 days of infection (Thomas and Waller, 1979).

However, despite the low worm burdens, it is evident that the larval challenge was associated with severe pathophysiological disturbances ; in particular, elevated plasma pepsinogen levels, altered albumin catabolism and increased losses of plasma protein into the gastro-intestinal tract.

Pepsinogen values were initially low in all the ewes, particularly in group C which had received regular anthelmintic therapy at pasture. Following the first series of daily larval inoculations to the group A ewes, their pepsinogen values increased but after cessation of larval challenge, the levels fell only to rise again during the second larval challenge. The maximum values of 1.7 and 1.8 i.u. although undoubtedly well above the normal values of uninfected sheep, are slightly lower than the

2.5 i.u. recorded by Anderson (1973) for immune ewes under natural challenge or 2.6 i.u. recorded for grazing lambs by Thomas and Waller, (1975). Throughout the experimental period, pepsinogen values of the non-challenged group were within the normal range for ovine pepsinogen levels of 0.7 i.u. recorded by Armour, et al, (1966).

From the radioisotopic studies, it is apparent that larval challenge was associated with an alteration in both albumin catabolism and plasma protein losses into the gastro-intestinal tract. In the case of the former, the initial values of all 3 groups were similar and clearly comparable to those previously recorded in non-parasitised sheep (Holmes and MacLean, 1971). However, following larval challenge of group A there was a marked rise in the fractional catabolic rates of albumin to double those of the unchallenged animals (Fig. 18). The hypercatabolism observed in group A was closely correlated with changes in the loss of plasma protein into the gastro-intestinal tract. As in the case of albumin catabolism, the initial values in all three groups of sheep were broadly similar and comparable to those previously reported (Holmes and MacLean, 1971 ; Symons, et al, 1981). However, after challenge plasma losses into the gastro-intestinal tract of the group A ewes rose dramatically to more than double (Fig. 18).

Several aspects of these results are noteworthy. First, there is a close correlation in the timing of the metabolic disturbances outlined above and the administration of trichostrongyle

infective larvae. Secondly, the values recorded for albumin catabolism and plasma leak following challenge of group A ewes were very similar to those previously recorded in naive lambs under challenge with O. circumcincta (Holmes and MacLean, 1971).

Thirdly, the level of the disturbances in albumin metabolism and plasma leak recorded in group A have previously been associated with a loss of production in naive sheep subjected to prolonged infection with several gastro-intestinal helminths (Holmes and MacLean, 1971 ; Symons, et al, 1981 ; Dargie and Berry, 1979)

Furthermore, it has recently been demonstrated that these production losses occur despite significant re-absorption of blood proteins which takes place further down the gastro-intestinal tract even in heavy parasitic infections of the abomasum (Rowe, et al, 1982).

The aetiology of the enteric plasma losses in primary infections of naive sheep has been attributed to physical changes in the gastro-intestinal tract induced by the migratory and feeding habits of maturing larvae and adult parasites. These changes permit a leak of macro-molecules from the tissue and plasma into the lumen of the gut. However, in the present experiment in which very few adult parasites were detected, it is unlikely that the elevated enteric plasma losses or the high plasma pepsinogen values had a similar aetiology. Rather, they are more likely to be associated with an increased permeability of the abomasal mucosa

created by an immune reaction induced by the ingested larvae. A possible basis for this lesion may be the development of hypersensitive state in the mucosa, as previously demonstrated by the enteric leak of plasma proteins during the self-cure reaction of rats infected with the nematode Nippostrongylus brasiliensis (Barth, Jarrett and Urquhart, 1966). More recently, a similar hypersensitive reaction in the abomasal mucosa has been proposed as the basis of elevated plasma pepsinogen values observed in adult grazing ruminants (Anderson, 1973 ; Armour, Bairden, Duncan, Jennings and Parkins, 1979).

Whatever the aetiology, it is most important to realise that metabolic changes can occur in immune ewes under larval challenge, although these changes cannot be detected by faecal egg counts alone but require the measurement of plasma pepsinogen levels and pasture larval numbers.

GENERAL DISCUSSION AND CONCLUSIONS.

The pathophysiological studies in Section 2 of this thesis were designed to investigate, first, whether a loss of plasma proteins occurred into the gut of the lambs under natural challenge with gastro-intestinal nematodes and secondly, if a similar loss occurred in immune ewes under a similar but experimental challenge.

In the lambs, a significant quantitative difference of protein loss into the gut was demonstrated between lambs receiving a heavy natural challenge and those undergoing a light challenge. These differences while significant would have been greater if the infected lambs could have been compared to helminth-naive lambs as has been done under experimental conditions by Holmes and MacLean (1971).

However, it is important that such measurements be undertaken in a natural environment where the larval challenge experienced by the lambs was from a mixed trichostrongyle infection, particularly as Steel, et al, (1982) have recently demonstrated that the pathophysiological disturbances in sheep are greater when both abomasal and intestinal parasites are present, as in the current investigation. Good agreement was also achieved between the level of plasma loss into the gut and the magnitude of the larval challenge, the loss being greater in July than in August and significantly less in October when the challenge was much lower.

What this loss means in economic terms is very difficult to gauge since there is undoubtedly some re-absorption of the 'leaked' protein in the large intestine. However, it appears that re-absorbed protein is not readily assimilated into the tissues but is in a form which is lost via the urine. Clearly a diversion of existing protein reserves must occur and indeed increased muscle protein synthesis has been demonstrated by Symons, et al, (1974). All of this requires energy and this must have a cost in terms of body fat and protein deposition as shown by Sykes and Coop (1976).

Whatever the cost in economic terms and some mathematical assessment of this is required the current work has demonstrated for the first time that the changes observed under experimental conditions also occur in naturally grazing lambs.

In the second experiment the theory proposed by Anderson (1973)that the development of a solid immunity to gastro-intestinal nematodes is preceded by a hypersensitive state which results in a highly permeable mucosa when these 'immune' animals are under larval challenge has been proved for the first time. As in Experiment 1 it was not possible to quantitate these losses in an economic sense but they must be considerable in terms of wool production and ewe body condition. They are probably of less importance during lactation as the ewe suffers a marked drop in immunity to parasites at this time, although this situation needs investigation. For example, in nematodiriasis the clinical picture was one of unaffected ewes and severely diarrhoeic lambs ;

but are ewes really unaffected and could they be suffering enteric losses of plasma proteins ?

Finally, the excellent temperal correlation between an elevation of plasma pepsinogens and the changes in albumin catabolism and plasma protein loss suggests that levels of this enzyme could be used as a marker for these pathophysiological changes.

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APPENDICES

Appendix 1. Technique for plasma pepsinogen estimation.

Reaction.

Plasma is incubated with bovine serum albumin (BSA) at pH 2 for 24 hours and the phenolic amino acids liberated (tyrosine like) are estimated using Folin-Ciocalteau Reaction. Corrections are made for the normal (ie. non incubated) content of tyrosine-like substances and also for the release of these substances from BSA when incubated alone.

Reagents.

2% Bovine Serum Albumin (BSA)

2N HCl

4% Trichloracetic Acid (TCA)

N/4 Caustic Soda

Folin-Ciocalteau's Reagent (diluted 1+2 with water)

Stock Standard Tyrosine. 1.812g tyrosine in 1000ml N/10 HCl (10 u mols/ml).

Working Standards 10ml Stock Standard diluted to 1000ml (2.0ml contains 0.2 u mols) and 20ml diluted to 1000ml (2.0ml contains 0.4 u mols)

Procedure

Plasma Tests 2.5ml plasma and 10ml 2% BSA. Adjusted to pH 2 with 2 N HCl (Approx 0.5ml) and water added to make total volume 15ml (2.0ml H_2O)

BSA Blank 2.5ml water and 10ml 2% BSA. Adjusted to pH 2 with 2N HCl (Approx 0.35ml) and water added to make volume to 15ml (2.15ml H_2O).

1. 6.0ml aliquots of tests were pipetted into universals and incubated at 37°C for 24 hours.

2. 6.0ml aliquots of BSA blanks were pipetted into another set of universals and the protein precipitated with 10ml of 4% TCA.

3. Precipitated blanks were allowed to stand for ten minutes and then filtered through a No. 44 Whatman filter paper.

4. Tests were precipitated after incubation with 10ml 4% TCA then processed as in (3) above.

5. 2ml of all filtrates were pipetted into suitably labelled flasks containing 20ml N/4 NaOH.

6. Flasks containing 2ml of each working standard were set up with 20ml N/4 NaOH ie. 2 u mols and 4 u mols tyrosine.

7. A reagent blank containing 2ml H_2O with 20ml N/10 NaOH was set up.

8. 3.0ml diluted Folin and Ciocalteau's reagent was added to all flasks.

9. After standing for 30 minutes the blue colour was read in a spectrophotometer at a wavelength of 680 mu.

Calculation of Results.

1. The reagent blank was subtracted from all readings.

2. From tyrosine standards the factor for conversion of all spectrophotometer readings to ù mols tyrosine was calculated and all readings converted to u mols tyrosine.

3. If incubated BSA and plasma = A
and non-incubated BSA and plasma = B
then A-B = total release of tyrosine on incubation.

4. If incubated BSA alone = C
and non-incubated BSA alone = D
then C-D = release of tyrosine from BSA substrate due to incubation alone, ie. NO PEPSINOGEN.

5. Therefore, (A-B) - (C-D) = tyrosine in u mols released on incubation of the equivalent of 0.125ml serum for 24 hours with substrate.

6. The amount of tyrosine in u mols released per 1000ml plasma per minute = International Units or x 1000 = milli Units tyrosine. (u mol (5) x 5.56.

APPENDIX 2.

Individual bi-weekly Eimeria species counts (oocyst/gram x 10^3) of Permanent Lambs grazing in 1980.

Month	Week	LAMB NUMBER											
		Y42	Y44	Y45	Y46	FR72	FR78	FR88	FR89	FR90	FR92	FR93	FR94
April	1-2	0	0	0	0	0	0	0	0	0	0	0	0
	3	10	0	0	50	50	0	0	0	0	0	20	0
		103	n.s	n.s	500	800	n.s	n.s	n.s	2	n.s	43.7	0
	4	704.4	202.8	98.1	2320	2232	4252	2232	n.s	131	15	132	1932
May	5	326.6	241.2	105	187.8	144	293.4	27	1570	50.4	48.6	n.s	144
		98.4	383.4	48.6	327.6	447.4	169.2	81	378	989	77.4	33.2	54
	6	180	842.4	124.2	225.6	279	120.6	458.4	n.s	385.2	35.4	162	84.6
		70.2	1114	106.2	162.2	694.8	95.4	n.s	169.2	363.6	n.s	n.s	61.2
	7	430.2	549	110	383.4	1298	95.4	246.6	540	118.8	111.6	426	322.2
		224.5	334.6	109.6	136.8	304.4	89.7	154.5	n.s	125	95.8	119	132.5
	8	122.3	292.6	n.s	75.2	644.5	34.9	114	76	104	n.s	92.6	169.6
		85.1	112.5	n.s	132.2	226.9	n.s	107.1	n.s	235.6	99.5	66.1	533.9

APPENDIX 2 (continued)

Month	Week	LAMB NUMBER											
		Y42	Y44	Y45	Y46	FR72	FR78	FR88	FR89	FR90	FR92	FR93	FR94
June	9	85.1	83.6	n.s	125.4	416.5	41.8	148.9	96.5	30.5	98	59.8	n.s
		71.5	94.2	58	148.2	200.6	58.5	151.2	153.5	157.3	n.s	17.9	469
	10	76	66.1	138.3	134.5	223.5	16	146.1	27.3	219.2	93.5	41.8	459
		76	23.5	86.3	122.5	127.8	1.5	42.5	76	150.5	61.5	21.3	267.5
	11	55.6	31.6	93.5	50.9	63	1.5	n.s	28.1	89.6	56.2	62.3	n.s
		49.4	34.5	143.7	18.3	n.s	1.5	n.s	63	70.7	76.7	10.6	34.1
	12	32.7	17.5	64.6	n.s	28.8	0.5	13.7	45.6	49.5	32.7	15.9	19
		3	n.s	n.s	9.9	23.5	7	6	58	52.2	23.5	9.9	17.5
	13	n.s	15.2	n.s	10.6	6.8	1.5	4.5	6.8	50.1	20.5	40	22.4
		2.2	3.8	3.8	3	10.6	0.7	7	4.5	12.2	34.2	48.6	18.2
July	14	12.5	2.5	15.9	8.3	12.4	0	8.3	5.3	30.4	16.7	6.8	15
		7.6	7.6	2.6	6.8	3	0	n.s	n.s	n.s	9.8	3.8	6.8
	15	2.3	3	n.s	2.3	18.6	2.2	3.8	n.s	39	15.5	3.8	3.2
		7.6	4.9	n.s	1.1	21.6	0	7.6	16.7	12	12	4.1	12.9
	16	6.4	2.7	22.1	5.4	9.1	0	2.2	8.3	n.s	3.8	1.9	10.4
		*	0.7	9.1	*	15.4	0	*	3.2	n.s	0	6.8	1.1
	17	-	1.1	18.2	-	13.8	0.1	-	13.8	65	10.6	1.9	6.8
		-	0.3	4.1	-	23.5	0.3	-	1.5	23.5	7.6	3.2	4.9

* Animal Slaugthered

n.s. No Sample

APPENDIX 3

Individual bi-weekly Nematodirus battus faecal egg counts (eggs/gram) of permanent grazing lambs in 1980

Month	Week	LAMB NUMBER											
		Y42	Y44	Y45	Y46	FR72	FR78	FR88	FR89	FR90	FR92	FR93	FR94
April	1-4	0	0	0	0	0	0	0	0	0	0	0	0
May	5	0	300	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	20	0	0	0	0
	6	0	600	0	0	0	0	0	0	0	0	600	0
		0	0	0	0	0	0	0	0	0	0	n.s	0
	7	0	300	0	0	0	0	300	300	300	0	0	300
		40	0	40	0	120	0	40	n.s	80	0	120	40
	8	0	120	n.s	80	120	0	40	80	80	n.s	80	0
		40	40	n.s	120	0	n.s	80	n.s	120	40	200	240
June	9	0	0	n.s	0	40	40	40	280	0	40	80	n.s
		120	40	80	0	0	80	80	0	120	0	80	40
	10	120	160	240	200	80	0	40	120	320	40	360	280
		360	40	40	600	40	160	280	320	520	480	280	280
	11	200	0	520	1000	80	80	n.s	280	240	600	640	n.s
		680	40	1280	1000	n.s	320	n.s	680	840	720	200	560
	12	1000	680	720	n.s	80	120	720	440	640	640	520	400
		1800	n.s	n.s	1720	280	240	1080	480	40	360	240	40
	13	n.s	0	n.s	1840	200	600	1160	360	160	80	120	240
		1640	0	1080	680	160	680	800	400	80	0	400	40

APPENDIX 3 (Continued)

Month	Week	LAMB NUMBER											
		Y42	Y44	Y45	Y46	FR72	FR78	FR88	FR89	FR90	FR92	FR93	FR94
July	14	2220	0	1560	1140	200	800	1140	220	80	n.s	20	60
		880	0	600	1360	360	340	940	0	0	0	380	40
	15	480	0	n.s	200	320	840	1060	n.s	0	0	120	300
		1040	60	n.s	1380	420	1120	720	140	10	0	0	240
	16	1020	0	0	2440	240	220	480	140	n.s	20	260	300
		*	0	0	*	380	400	*	120	n.s	0	n.s	120
	17	-	0	0	-	10	0	-	0	0	0	0	0
		-	0	0	-	0	0	-	0	0	0	0	0
August	18	-	0	0	-	0	0	-	0	0	0	0	0
		-	0	0	-	0	0	-	0	0	0	0	0
	19	-	0	0	-	0	0	-	0	0	0	0	0
		-	0	0	-	20	40	-	0	20	0	0	0
	20	-	n.s	20	-	20	40	-	0	0	20	n.s	0
		-	0	n.s	-	0	160	-	0	40	0	0	0
	21	-	0	**	-	n.s	0	n.s	120	20	20	20	20
		-	0	0	-	250	0	-	0	40	0	0	0

APPENDIX 3 (Continued)

Month	Week	LAMB NUMBER											
		Y42	Y44	Y45	Y46	FR72	FR78	FR88	FR89	FR90	FR92	FR93	FR94
Sept.	22	-	40	-	-	0	300	-	n.s	80	60	0	20
		-	0	-	-	0	0	-	40	60	40	0	0
	23	-	0	-	-	0	0	-	0	60	0	0	0
		-	0	-	-	0	0	-	0	0	0	0	0
	24	-	0	-	-	0	0	-	0	0	0	0	0
		-	0	-	-	0	0	-	0	0	0	0	0
	25	-	0	-	-	0	0	-	0	0	0	0	0
		-	0	-	-	0	0	-	0	10	0	0	0
Oct.	26	-	0	-	-	0	0	-	0	0	0	0	0
		-	0	-	-	10	0	-	20	100	10	0	0
	27	-	0	-	-	0	0	-	30	40	30	0	20
		-	0	-	-	20	0	-	10	70	0	30	0

n.s = no sample

* = slaughtered

** = died

APPENDIX 4

Individual fortnightly plasma pepsinogen levels (i.u. tryosine) of grazing ewes in 1980.

Month	Week	NUMBER OF EWE							
		P61	P62	P66	P67	P71	P72	P74	P75
April	1	0.5	0.6	0.6	0.8	0.5	0.9	0.9	0.5
	3	0.6	0.7	0.6	0.9	0.6	1.1	0.9	0.8
May	5	0.9	0.8	0.9	0.8	1.0	0.6	0.6	0.9
	7	1.7	1.3	0.8	0.9	1.1	1.0	1.9	1.0
June	9	1.2	2.7	1.0	1.8	2.4	2.0	1.0	1.5
	11	1.9	2.3	3.0	2.3	3.0	3.0	1.2	1.8
July	13	1.2	2.3	2.0	2.0	2.1	3.0	1.0	2.1

APPENDIX 4

Individual fortnightly plasma pepsinogen levels (. i.u. tryosine) of permanent grazing lambs in 1980

Month	Week	LAMB NUMBER											
		Y42	Y44	Y45	Y46	FR72	FR78	FR88	FR89	FR90	FR92	FR93	FR94
June	9	0.8	0.9	1.2	1.6	0.7	0.5	0.9	1.9	1.8	0.6	1.7	1.9
	11	1.0	1.2	2.1	2.0	0.9	0.6	1.1	2.3	1.4	1.0	1.5	2.0
July	13	1.2	1.3	2.4	3.0	0.9	0.5	1.0	2.1	2.0	1.0	1.6	2.0
	15	* -	1.0	1.0	* -	1.1	0.4	-	3.2	1.2	1.5	1.4	1.3
August	17	-	1.1	1.0	-	0.9	0.4	-	1.8	1.6	1.3	1.2	1.4
	19	-	1.0	1.8 **	-	1.3	1.1	-	3.0	1.8	1.2	1.7	1.6
Sept.	21	-	1.0	-	-	2.5	1.4	-	3.8	2.6	1.2	1.8	2.4
	23	-	0.5	-	-	0.6	0.4	-	1.2	0.8	0.5	0.8	0.8
Oct.	25	-	0.6	-	-	0.8	0.5	-	1.2	0.9	0.7	0.8	0.9
	27	-	0.9	-	-	0.6	0.7	-	1.5	0.9	0.6	1.0	1.2

* Animal slaughtered
** Animal died

APPENDIX 5

Individual bi-weekly trichostrongyle faecal egg counts (eggs/gram) of grazing ewes in 1980

Month	Week	EWE NUMBER							
		P61	P62	P66	P67	P71	P72	P74	P75
April	1	600	50	1250	0	150	650	0	0
		100	0	350	50	100	n.s	50	0
	2	50	0	0	0	50	550	0	0
		0	0	n.s	100	50	0	0	0
	3	0	0	0	0	0	50	50	50
		50	0	0	250	0	50	0	50
	4	n.s	0	n.s	850	0	200	0	n.s
		50	100	650	0	250	0	600	0
May	5	100	150	50	n.s	0	250	100	50
		200	50	50	1400	n.s	100	100	100
	6	300	250	300	950	100	n.s	100	100
		700	150	350	400	0	100	250	100
	7	620	180	280	n.s	90	n.s	260	70
		1270	420	310	n.s	70	250	80	100
	8	240	240	230	70	n.s	360	130	150
		250	560	140	n.s	50	410	80	220
June	9	0	250	40	60	50	140	90	110
		n.s	500	30	230	80	130	30	250
	10	30	n.s	60	420	170	220	40	80
		90	330	10	580	80	240	40	70
	11	40	160	20	710	100	140	60	190
		40	160	10	180	n.s	280	20	180
	12	20	n.s	40	200	10	n.s	0	120
		60	280	10	210	100	40	10	150
July	13	40	240	0	n.s	110	n.s	20	110
		20	420	20	50	70	20	0	110
	14	10	n.s	10	100	100	n.s	0	n.s
		0	n.s	20	140	160	360	0	120

APPENDIX 5

Individual bi-weekly trichostrongyle faecal egg counts (Eggs/gram) of permanent grazing lambs in 1980

Month	Week	LAMB NUMBER											
		Y42	Y44	Y45	Y46	FR72	FR78	FR88	FR89	FR90	FR92	FR93	FR94
April	1-4	0	0	0	0	0	0	0	0	0	0	0	0
May	5	0	600	0	0	0	0	0	600	0	0	0	0
		0	600	300	0	300	0	0	600	0	0	300	0
	6	0	300	300	600	0	0	300	n.s	300	0	300	600
		300	0	600	600	0	0	0	0	300	0	0	0
	7	0	600	300	300	300	0	600	300	600	0	600	300
		200	60	280	440	120	0	480	n.s	720	400	720	320
	8	160	1200	n.s	320	580	0	160	840	440	n.s	520	800
		480	880	n.s	280	520	n.s	640	n.s	720	280	360	1000
June	9	320	160	n.s	840	1000	40	840	1440	640	240	160	n.s
		320	360	320	720	840	160	1720	1840	240	n.s	120	1080
	10	720	320	640	800	240	40	1640	480	840	760	40	1320
		480	480	440	680	720	160	1320	1200	400	640	160	560
	11	440	960	520	640	440	160	n.s	920	360	400	200	n.s
		760	760	1480	640	n.s	0	n.s	1000	240	880	280	80
	12	840	560	480	n.s	560	80	360	480	240	600	600	200
		1040	n.s	n.s	760	920	80	1360	400	600	840	1120	760
	13	n.s	640	n.s	840	1080	160	2160	960	160	200	1360	440
		1920	160	280	280	200	280	3000	120	200	80	1160	1280

APPENDIX 5 (Continued)

Month	Week	LAMB NUMBER											
		Y42	Y44	Y45	Y46	FR72	FR78	FR88	FR89	FR90	FR92	FR93	FR94
July	14	700	140	480	520	600	240	680	420	180	80	720	480
		240	80	80	540	240	120	680	n.s	n.s	n.s	1260	600
	15	160	300	n.s	1120	760	880	620	n.s	0	120	440	480
		800	160	n.s	580	380	560	1000	520	10	820	900	760
	16	260	200	20	740	140	840	680	660	n.s	160	600	520
		*	160	0	*	780	900	*	320	n.s	460	n.s	1000
	17	-	0	0	-	0	0	-	10	0	0	0	0
		-	20	0	-	0	0	-	0	0	0	0	0
August	18	-	0	0	-	0	0	-	0	0	0	0	0
		-	0	0	-	0	0	-	0	0	0	0	20
	19	-	0	0	-	0	0	-	0	0	0	0	0
		-	0	60	-	0	160	-	0	20	0	0	0
	20	-	n.s	360	-	0	860	-	20	20	20	n.s	20
		-	n.s	**	-	140	1360	-	40	200	80	60	220
	21	-	180	-	-	180	440	-	n.s	n.s	100	40	160
		-	160	-	-	200	6060	-	700	320	260	500	640

APPENDIX 5 (Continued)

Month	Week	LAMB NUMBER											
		Y42	Y44	Y45	Y46	FR72	FR78	FR88	FR89	FR90	FR92	FR93	FR94
Sept.	22	-	120	-	-	380	4920	-	n.s	400	80	1120	1360
		-	200	-	-	340	0	-	900	600	400	1480	1300
	23	-	n.s	-	-	220	0	-	n.s	420	720	1280	1320
		-	0	-	-	0	0	-	0	0	0	0	0
	24	-	0	-	-	0	0	-	0	0	0	0	0
		-	0	-	-	0	0	-	0	0	0	0	0
	25	-	0	-	-	0	0	-	0	0	0	0	0
		-	10	-	-	10	0	-	0	0	0	0	0
Oct.	26	-	40	-	-	90	70	-	100	60	20	20	60
		-	50	-	-	80	250	-	270	150	200	20	30
	27	-	150	-	-	110	320	-	420	90	70	60	70
		-	240	-	-	130	590	-	920	170	720	n.s	580
	28	-	240	-	-	200	800	-	320	110	710	530	290
		-	210	-	-	470	1040	-	930	200	840	810	410

n.s = no sample

* = slaughtered

** = died

APPENDIX 6

Differential Count of larvae obtained following culture of faeces from 1980 Permanent Ewes and expressed as percentages.

Month	Week	O.circumincta	H.contortus	Trichostrongylus spp	C.ovina
April	1	59	0	8	33
	2	45	2	16	39
		87	0	22	1
	3	55	0	13	32
		70	0	30	0
	4	75	9	13	3
		74	8	14	4
May					
	5	70	11	18	1
		56	12	22	10
	6	52	23	11	13
		44	13	12	31
	7	55	13	25	7
		25	17	9	50
	8	48	14	10	28
		24	23	4	49
June	9	18	27	2	53
		40	49	5	6
	10	45	40	5	10
		51	20	25	4
	11	15	44	1	40
		30	45	5	20
	12	25	27	9	39
		27	30	6	37
July	13	30	28	10	42
		39	13	21	37
	14	40	8	22	30
		33	5	5	57

APPENDIX 7

Differential Count of larvae obtained follwoing culture of faeces from 1980 Permanent Lambs and expressed as percentages.

Month	Week	O. circumcincta	H. contortus	Trichostrongylus spp	C. ovina
May	5	0	0	0	0
		63	0	37	0
	6	79	1	20	0
		56	6	38	0
	7	50	10	40	0
		54	6	40	0
	8	59	14	27	0
		64	15	21	0
June	9	67	23	15	0
		70	18	11	0
	10	32	23	13	32
		23	17	7	53
	11	35	23	17	25
		21	18	5	56
	12	7	21	2	70
		20	9	1	70
July	13	13	10	2	75
		31	20	2	46
	14	37	22	3	38
		18	30	2	50
	15	33	5	5	57
		50	15	3	32
	16	0	0	0	0
		0	0	0	0

APPENDIX 7 (Continued)

Month	Week	O. circumcincta	H. contortus	Trichostrongylus spp	C. ovina
August	17	0	0	0	0
		0	0	0	0
	18	80	5	15	0
		54	33	13	0
	19	45	23	32	0
		39	48	12	1
	20	30	50	15	5
		40	35	17	8
Sept.	21	33	43	8	16
		0	0	0	0
	22	0	0	0	0
		0	0	0	0
	23	0	0	0	0
		0	0	0	0
	24	75	7	18	0
		90	5	5	0
Oct.	25	76	16	8	0
		74	6	20	0
	26	43	40	12	5
		42	23	18	17
	27	40	40	10	10
		40	40	15	5

APPENDIX 8

Weekly counts of *Trichostrongyle* spp and *Chabertia ovina* L3 recovered from pasture in 1980

Month	Week	O. circumcincta	H. contortus	Trichostrongylus spp	N . battus	C. ovina
April	1	2222	0	2000	0	202
	2	2247	1348	1573	0	887
	3	2500	0	833	769	277
	4	2366	0	363	181	181
May	5	250	250	250	0	0
	6	1250	250	750	0	250
	7	236	0	236	0	0
	8	263	0	0	2894	0
June	9	0	0	0	7143	0
	10	8333	0	1666	7777	0
	11	12000	285	5428	3142	285
	12	22400	2000	16000	4800	0
	13	18000	1226	12408	2449	612
July	14	11717	808	8282	606	0
	15	6776	1014	2808	0	434
	16	645	3548	161	322	0
	17	5000	1764	3529	0	0
August	18	754	754	188	188	0
	19	1730	384	1346	192	0
	20	111	111	0	0	0
	21	435	145	145	145	0
Sept.	22	175	0	350	350	0
	23	212	212	0	0	0
	24	200	0	200	400	200
	25	204	204	612	408	0
Oct	26	400	200	0	600	0
	27	262	0	0	262	0

APPENDIX 9

Individual worm burdens of tracer lambs grazing in 1980.

August necropsy	LAMB NUMBER		
	Y64	Y65	890
Ostertagia spp			
♂	100	2600	1700
♀	0	3200	600
L5	300	2100	1600
DL4	300	2200	5400
EL4	0	0	200
Total	700	10100	9500 (2)
		Mean 6766	
H. contortus			
♂	100	300	200
♀	0	200	0
L5	0	1200	200
DL4	0	600	800
EL4	0	0	0
Total	100	2300	1200
		Mean 1200	
Trichostrongylus spp			
♂	0	800	1400
♀	0	900	800
L5	0	300	1400
DL4	0	200	0
EL4	0	0	0
Total	0	2200	3600
		Mean 1933	

APPENDIX 9 (Continued)

	LAMB NUMBER		
	Y64	Y65	890
N. battus			
♂	0	0	1400
♀	0	0	200
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	0	0	1600
		Mean 533	
N. filicollis			
♂	0	0	0
♀	0	0	200
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	0	0	200
		Mean 66	
Totals	800	14,600	16,100
		Mean 10,500	

APPENDIX 9 (continued)

September necropsy	LAMB NUMBER		
	926	941	943
Ostertagia spp			
♂	1000	800	1300
♀	1000	800	1300
L5	500	100	100
DL4	500	100	0
EL4	500	200	100
Total	3500	2000	2800
	(14)	(10)	(3.5)
		Mean 2766	
H. contortus			
♂	400	100	400
♀	500	100	300
L5	200	0	0
DL4	0	0	0
EL4	0	0	0
Total	1100	200	700
		Mean 666	
T. axei			
♂	0	0	0
♀	0	0	100
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	0	0	100
		Mean 33	

APPENDIX 9 (continued)

	LAMB NUMBER		
	926	941	943
Trichostrongylus spp			
♂	0	200	0
♀	0	800	0
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	0	1000	0
		Mean 333	
N. battus			
♂	1000	400	400
♀	800	600	600
L5	100	100	800
DL4	200	800	1400
EL4	0	0	0
Total	2100	1900	3200
		Mean 2400	
N. filicollis			
♂	0	0	0
♀	100	0	0
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	100	0	0
		Mean 33	
C. ovina			
Adult	13	5	15
		Mean 11	
T. ovis			
Adult	0	1	2
		Mean 1	
Totals	6813	5106	6817
		Mean 6,245	

APPENDIX 9 (continued)

Early October necropsy.	LAMB NUMBER		
	893	923	931
Ostertagia spp			
♂	800	1500	800
♀	700	900	500
L5	0	0	100
DL4	200	300	100
EL4	700	600	700
Total	2400	3300	2200
	(29)	(18)	(32)
	Mean 2633		
H. contortus			
♂	500	400	500
♀	300	400	200
L5	0	0	100
DL4	0	0	0
EL4	0	0	0
Total	800	800	800
	Mean 800		
T. vitrinus			
♂	0	300	0
♀	0	100	0
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	0	400	0
	Mean 133		

APPENDIX 9 (continued)

	LAMB NUMBER		
	893	923	931
N. battus			
♂	1000	0	1000
♀	600	0	1200
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	1600	0	2200
	Mean 1266		
N. filicollis			
♂	0	0	0
♀	200	0	0
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	200	0	0
	Mean 66		
Ch. ovina			
Adult	0	0	0
Tr. ovis			
Adult	0	0	0
Totals	5000	4500	5200

Mean 4,900

APPENDIX 9 (continued)

Late October necropsy.	LAMB NUMBER		
	821	965	N.T
Ostertagia spp			
♂	0	100	200
♀	100	200	400
L5	100	0	0
DL4	100	0	100
EL4	1500	500	200
Total	1800 (83)	800 (62.5)	800 (25)
	Mean 1133		
H. contortus			
♂	400	600	200
♀	100	400	400
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	500	1000	600
	Mean 700		
T. vitrinus			
♂	100	0	300
♀	0	0	100
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	100	0	400
	Mean 166		

APPENDIX 9 (continued)

	LAMB NUMBER		
	821	965	N.T
N. battus			
♂	200	0	0
♀	100	0	200
L5	300	200	600
DL4	0	200	200
EL4	0	0	0
Total	600	400	1000
	Mean 666		
N. filicollis			
♂	0	0	0
♀	0	0	0
L5	0	200	0
DL4	0	0	0
EL4	0	0	0
Total	0	200	0
	Mean 66		
Ch. ovina			
Adult	0	0	0
Tr. ovis			
Adult	2	10	35
	Mean 15.5		
Totals	3002	2410	2835
	Mean 2,749		

APPENDIX 9 (continued)

November necropsy	LAMB NUMBER		
	Y43	Y62	Y63
Ostertagia spp			
♂	0	0	100
♀	100	200	0
L5	0	0	0
DL4	0	0	0
EL4	0	500	200
Total	100	700 (71)	300 (66)
		Mean 366	
H. contortus			
♂	0	100	0
♀	0	100	0
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	0	200	0
		Mean 66	
Trichostrongylus spp			
♂	0	100	0
♀	0	0	0
L5	0	0	0
DL4	0	0	0
EL4	0	0	0
Total	0	100	0
		Mean 33	

APPENDIX 9 (Continued)

	LAMB NUMBER		
	Y43	Y62	Y63
N. battus.			
o	0	0	100
♀	200	600	0
L5	600	1000	300
DL4	200	400	300
EL4	600	200	0
Total	1600	3000	700
		Mean 1766	
N. filicollis.			
o	200	0	100
♀	600	400	0
L5	200	400	100
DL4	0	0	0
EL4	0	0	0
Total	800	800	200
		Mean 666	
Total	2500	4800	1200

Mean 2,833

APPENDIX 10.

Composition of Lamb supplementary diet

Unmollassed sugar beet pulp	90.00 kg
Siftings (barley chaff)	30.00 kg
Granstock	3.50 kg
Di-calcium phosphate	0.50 kg
Sheep trace elements	0.25 kg

APPENDIX 11 (continued)

Individual bi-weekly Eimeria species counts (oocyst/gram) of Ewes grazing in 1981.

Month	Week	EWE NUMBER						
		363	791	754	663	P33	515	382
April	1	0	0	0	0	100	250	50
		50	0	50	0	50	100	0
May	2	100	350	150	50	150	100	0
		0	300	100	300	250	0	200
	3	250	200	0	0	150	200	200
		n.s	100	150	50	150	n.s	n.s
	4	200	50	800	200	800	50	150
		450	50	100	450	n.s	50	5250
	5	1000	100	250	100	0	0	4200
		0	0	150	300	100	150	550
June	6	150	150	200	150	100	200	150
		0	150	100	250	250	200	250
	7	100	50	100	150	100	100	200
		0	0	0	50	0	300	0
	8	n.s	150	600	50	0	150	0
		100	100	0	200	0	200	0
	9	0	50	50	250	250	50	200
		0	50	50	250	50	800	n.s

n.s. = No Sample

APPENDIX 11

Individual bi-weekly Eimeria species counts (oocyst/gram x 10^3) of Permanent Lambs 1981.

Month	Week	LAMB NUMBER										
		Y13	Y14	Y16	Y17	Y22	Y3	Y7	Y8	Y9	Y10	Y19
April	1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
May	2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
	3	0 30	0 6.6	0 19.6	0 n.s	0 n.s	0 n.s	0 n.s	0 90	0 65	0 7.8	0 0.6
	4	64 3.8	n.s 68	710 116.7	17.2 1.8	n.s 1188.3	3 136	31 50.7	78.1 52.8	0.9 18.6	0.3 16.6	n.s 24.3
	5	1.2 34	114.6 122.4	50.4 57.6	293.6 500	45 32.7	42.3 37.5	n.s. 252	51.1 n.s.	n.s. 37.5	30.9 806.4	102.6 89.1
June	6	73.2 59.1	85 66	25.8 51.6	76.5 154	29.4 18	25 49.8	54.6 45.6	141 117	78 134	129.6 51.2	108 60
	7	25.2 n.s	105.6 37.4	205.8 89.2	137.4 76	3 6	407.6 9.9	51 115	39.6 81.6	128.4 55.8	185.4 n.s.	103.8 70.2
	8	18.8 39.6	28.2 77.4	20.4 21.2	3 127.8	9.5 19.8	62.4 77.4	42.6 36	n.s 54	180 120.4	152.4 54	118.8 95.4
	9	7.2 19.8	22.2 36.4	31 84	31.8 39.6	17.6 10.8	36 59.4	4.8 78	4.2 2.2	77 25	109 94.2	48.6 36.6

APPENDIX 11 (continued)

Month	Week	LAMB NUMBER										
		Y13	Y14	Y16	Y17	Y22	Y3	Y7	Y8	Y9	Y10	Y19
July	10	6	31.8	30	102	12	19.2	6	21	10.8	31.8	87.6
		6.6	12.6	72.6	18	*	*	9	6	n.s	*	78.6
	11	3	15	11.4	21.6	-	-	6	15	12	-	40.8
		4	3.6	24.6	41.4	-	-	4.8	27	6	-	31.8
	12	3	9.6	9	n.s	-	-	12	18	12	-	14.6
		12	3	18.5	18	-	-	84	25.8	3	-	10.7
	13	6	1.2	6	27	-	-	1.2	6.6	3	-	1.8
		0.6	3	9	11.5	-	-	1.2	0.6	1.2	-	3.6
	14	0.6	3	2.4	7.2	-	-	1.8	5.6	1.8	-	4.8
		9	1.2	6	2.4	-	-	2.4	1.2	7.8	-	3

APPENDIX 12

Individual bi-weekly Nematodirus battus faecal egg counts (egge/gram) of Permanent grazing Lambs in 1981.

Month	Week	LAMB NUMBER										
		Y13	Y14	Y16	Y17	Y22	Y3	Y7	Y8	Y9	Y10	Y19
April	1	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0
May	2	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0
		0	0	n.s	n.s	0	0	0	n.s	0	0	n.s
	4	700	n.s	500	0	n.s	150	250	450	200	500	100
		0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	50	0	0	0
		0	0	0	0	0	0	0	0	0	0	0
June	6	150	50	0	0	300	0	0	650	250	200	0
		200	100	350	450	550	950	0	900	1440	650	350
	7	n.s	400	1050	300	550	950	0	900	1100	n.s	200
		800	200	950	900	400	950	0	n.s	2400	0	200
	8	950	600	1200	600	750	1100	0	450	3000	0	150
		350	400	1150	50	1350	1150	0	50	1450	100	0
	9	350	300	750	0	450	700	0	50	750	0	0
		350	250	250	0	350	150	100	50	200	50	100

APPENDIX 12 (continued)

Month	Week	LAMB NUMBER										
		Y13	Y14	Y16	Y17	Y22	Y3	Y7	Y8	Y9	Y10	Y19
July	10	0	150	700	0	*	*	0	0	n.s	*	0
		100	50	450	0	-	-	0	0	100	-	0
	11	100	100	200	0	-	-	0	0	200	-	0
		100	150	300	n.s	-	-	0	0	300	-	0
	12	0	50	200	0	-	-	0	0	250	-	0
		0	150	300	0	-	-	0	0	0	-	0
	13	50	250	350	0	-	-	0	0	200	-	0
		0	100	0	0	-	-	0	0	0	-	0
August	14	50	450	200	0	-	-	0	0	150	-	0
		0	100	0	0	-	-	0	0	0	-	0
	15	0	0	0	0	-	-	0	0	0	-	0
		100	300	0	0	-	-	0	0	100	-	0
	16	0	100	0	0	-	-	0	0	50	-	0
		0	100	0	0	-	-	0	0	0	-	0
	17	0	0	0	0	-	-	0	0	0	-	0
		0	50	0	0	-	-	0	0	0	-	0
	18	0	50	0	0	-	-	0	0	0	-	0
		0	0	0	0	-	-	0	0	0	-	0

September and October samples all negative * Animal Slaughtered

APPENDIX 13

Individual fortnightly P.C.V. % of Ewes grazing in 1981.

Month	Week	EWE NUMBER						
		663	754	P40	363	515	382	P33
April	1	25	25	28	29	32	31	27
May	3	24	20	22	28	23	26	21
	5	28	26	28.5	35	22.5	30	27.5
June	7	34	26	30	35	28	32	29
	9	33	28	30	27	35	30	33

APPENDIX 13

Individual fortnightly(P.C.V. %) of Permanent Lambs grazing in 1981.

Month	Week	LAMB NUMBER										
		Y13	Y14	Y16	Y17	Y22	Y3	Y7	Y8	Y9	Y10	Y19
June	7	35	35	34	30	28.5	35	36	31	25	31.5	33
	9	35	31	31	34	26	37	31	33	34	35	31
July	11	35	35	38	33	*	*	36	32	34	*	34
	13	34	33.5	34.5	32	-	-	34.5	32	32.5	-	34.5
August	15	30	32.5	31	30	-	-	36	29	31	-	31
	17	34	35	33	33	-	-	36	32	34	-	30
Sept.	19	31	28	30	28	-	-	35	30	30	-	31
	21	31	29	30	30	-	-	35	30	24	-	28
October	23	32	33	29	28	-	-	26	33	30	-	32
	25	32	33	30	30	-	-	34	32	30	-	30

* Animal Slaughtered

APPENDIX 14

Individual fortnightly plasma pepsinogen levels (i.u. tyrosine) of Permanent Lambs grazing in 1981.

Month	Week	LAMB NUMBER										
		Y13	Y14	Y16	Y17	Y22	Y3	Y7	Y8	Y9	Y10	Y19
June	7	0.5	0.7	0.4	0.9	0.5	0.7	0.5	0.4	0.5	0.4	0.6
	9	0.6	0.6	0.4	0.7	0.5	0.6	0.5	0.4	0.5	0.6	0.6
July	11	0.6	0.8	0.4	0.7	*	*	0.3	0.4	0.5	*	0.5
	13	1.0	0.9	0.7	1.4	-	-	0.6	0.6	0.7	-	0.5
August	15	1.1	0.9	0.6	0.6	-	-	0.8	1.1	1.3	-	0.6
	17	1.7	1.2	1.5	2.4	-	-	1.4	1.6	1.8	-	1.5
Sept.	19	2.0	1.6	2.0	3.9	-	-	1.8	1.8	2.3	-	1.8
	21	2.2	1.7	2.6	3.5	-	-	2.0	2.9	2.2	-	3.1
October	23	2.6	1.7	1.3	4.1	-	-	2.2	2.0	2.6	-	3.1
	25	1.5	1.5	0.8	4.2	-	-	2.2	1.4	1.1	-	1.8

* Animal Slaughtered

APPENDIX 15.

Individual bi-weekly trichostrongyle egg counts (epg) of Ewes grazing in 1981.

Month	Week	EWE NUMBER						
		663	754	P40	363	515	382	P33
April	1	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
May	2	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
	4	n.s	50	100	0	n.s	n.s	0
		50	250	150	0	150	0	0
	5	100	100	100	0	50	300	n.s
		350	300	0	200	0	350	0
June	6	0	550	200	0	50	100	100
		50	250	450	0	0	200	50
	7	50	200	250	0	100	400	50
		0	150	300	0	100	450	200
	8	50	250	650	0	300	400	150
		250	650	550	n.s	500	n.s	100
	9	0	300	700	50	150	300	250
		0	350	300	0	200	100	150

APPENDIX 16

Individual bi-weekly trichostrongyle Faecal egg counts (eggs/gram) of Permanent Lambs in 1981.

Month	Week	LAMB NUMBER										
		Y13	Y14	Y16	Y17	Y22	Y3	Y7	Y8	Y9	Y10	Y19
April	1	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0
May	2	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0
June	6	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	50	0
	7	n.s	0	50	100	50	0	0	150	0	n.s	0
		100	50	0	0	0	0	0	n.s	50	0	0
	8	50	0	0	0	0	0	0	50	0	0	0
		50	0	0	0	100	0	0	100	0	0	0
	9	50	50	100	0	150	0	0	150	0	0	0
		0	50	0	0	0	150	50	100	150	0	0

APPENDIX 16 (continued)

Month	Week	LAMB NUMBER										
		Y13	Y14	Y16	Y17	Y22	Y3	Y7	Y8	Y9	Y10	Y19
July	10	50	0	50	0	*	*	0	n.s	0	*	0
		50	0	50	0	-	-	50	150	200	-	50
	11	0	0	0	50	-	-	100	200	250	-	150
		150	0	100	n.s	-	-	100	200	450	-	0
	12	50	100	100	0	-	-	200	100	50	-	50
		0	50	400	100	-	-	0	250	400	-	0
	13	50	50	100	150	-	-	100	50	150	-	100
		0	0	50	250	-	-	300	50	250	-	200
August	14	50	0	150	200	-	-	150	300	300	-	500
		n.s	0	50	150	-	-	n.s	200	600	-	n.s
	15	0	150	100	150	-	-	400	300	250	-	700
		0	50	250	1000	-	-	200	200	100	-	450
	16	100	350	100	1550	-	-	100	150	0	-	0
		n.s	50	n.s	150	-	-	0	50	150	-	100
	17	0	200	1700	300	-	-	n.s	350	400	-	100
		50	750	n.s	250	-	-	100	500	0	-	50
	18	0	100	300	500	-	-	50	150	150	-	0
		300	200	600	0	-	-	0	250	0	-	0

APPENDIX 16 (continued)

Month	Week	LAMB NUMBER										
		Y13	Y14	Y16	Y17	Y22	Y3	Y7	Y8	Y9	Y10	Y19
Sept.	19	200	350	300	450	-	-	50	150	50	-	100
		50	550	1250	n.s	-	-	100	0	50	-	150
	20	150	400	500	n.s	-	-	50	350	n.s	-	50
		100	200	300	1850	-	-	0	50	600	-	0
	21	50	150	150	1350	-	-	250	100	650	-	50
		0	100	500	300	-	-	250	350	1650	-	100
	22	150	0	0	0	-	-	200	100	800	-	50
		100	100	750	150	-	-	150	100	0	-	50
Oct.	23	0	50	500	250	-	-	0	50	100	-	50
		50	150	250	250	-	-	300	0	0	-	150
	24	50	100	150	250	-	-	250	250	0	-	50
		100	50	50	350	-	-	200	50	0	-	50
	25	100	100	100	350	-	-	350	100	n.s	-	0
		50	100	400	50	-	-	0	250	50	-	100
	26	0	50	350	0	-	-	50	600	250	-	50

n.s. = No Sample

* = Slaughtered

APPENDIX 17

Differential counts of larvae obtained following culture of faeces from 1981 Permanent Lambs and expressed as percentages.

Month	Week	*Ostertagia* spp	*H. contortus*	*Trichostrongylus* spp
April	1	0	0	0
May	2-5	0	0	0
June	6	80	15	5
	7	66.5	11	23.5
	8	75	10	15
	9	77.5	15	7.5
July	10	89.5	7	3.5
	11	74	10	16
	12	82.5	7	10
	13	87	7	6
August	14	77	2.5	20.5
	15	74.5	6	19.5
	16	54.5	17.5	28
	17	67.5	10	22.5
	18	n.s	n.s	n.s

APPENDIX 17 (continued)

Month	Week	Ostertagia spp	H. contortus	Trichostrongylus spp
Sept.	19	100	0	0
	20	91.5	1	7.5
	21	87.5	1.5	11
	22	90	2.5	7.5
Oct.	23	75.5	9.5	15
	24	83	5	12
	25	70.5	11	18.5

APPENDIX 18

Weekly Number of L_3 recovered from pasture of trichostrongyle spp and Ch. ovina in 1981.

Month	Week	Ostertagia spp	H. contortus	Trichostrongylus spp	N. battus	Ch. ovina
April	1	200	200	200	2600	0
May	2	684	692	96	8521	0
	3	171	52	0	14112	0
	4	338	0	0	7980	0
	5	96	96	96	3655	0
June	6	201	0	0	6425	58
	7	204	0	0	3731	102
	8	365	153	0	885	0
	9	664	0	0	917	0
July	10	1054	140	140	193	0
	11	3807	153	489	229	0
	12	1180	173	84	270	0
	13	1830	136	194	105	0

APPENDIX 18 (continued)

Month	Week	Ostertagia spp	H. contortus	Trichostrongylus spp	N. battus	Ch. ovina
August	14	4045	110	337	0	0
	15	1356	345	186	211	0
	16	1652	0	199	151	0
	17	582	0	106	159	0
	18	174	0	0	0	0
Sept.	19	718	445	150	142	0
	20	937	45	358	204	0
	21	1670	248	313	657	0
	22	1230	185	646	332	136
Oct	23	951	0	370	636	75
	24	1766	288	334	577	0
	25	2150	580	1195	250	0
	26	950	100	1210	275	0