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RUMINANT PARASITIC GASTROENTERITIS: SOME OBSERVATIONS  
ON EPIDEMIOLOGY AND CONTROL

by

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

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# DECLARATION

The following publication was based on part of the work contained in this thesis:

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## SUMMARY

The studies described in this thesis were carried out to assess the advantages and disadvantages of two well recognised means of gastrointestinal parasite control in cattle i.e. anthelmintic prophylaxis and grazing management. In addition studies were initiated to examine the role of Nematodirus battus in outbreaks of parasitic helminthiasis in calves.

Traditional parasitological techniques were used for most of these studies but several innovations and modifications were introduced and where these proved successful their detailed description is given. Examples of successful modifications included replacement of the sieve in the standard Baermann apparatus with a disposable support system which greatly reduced the possibility of contamination and the use of a small hand operated washing machine to facilitate the washing of herbage in the pasture larval recovery technique.

A comparison of two first season strategic anthelmintic control programmes using ivermectin at 3, 8 and 13 weeks post turnout and a morantel bolus administered immediately prior to turnout highlighted a potentially harmful aspect of such prophylactic treatments in that substantial numbers of inhibited fourth stage Ostertagia larvae were acquired by first year strategically treated animals in their second grazing season when compared with non-strategically treated controls. The plasma pepsinogen profiles of the cattle in the second year of this



study were of particular interest. An initial increase in all three groups shortly after turnout was followed by a steady reduction in pepsinogen concentration in the control animals. This compared with a gradual increase in pepsinogen levels in the strategically treated animals until at housing similar group means, slightly higher than normal for this age of animal, were observed. These results suggest that plasma pepsinogen concentrations would be of little value in determining the level of inhibited Ostertagia in yearling cattle as, for example, in the pre-type II situation.

The use of a grazing strategy which involved the annual alternation of cattle and sheep over a four year period did not give an acceptable level of parasite control. Despite being very successful in the second year and giving some indication of continued benefit in the third grazing season, by the end of the fourth year the worm burdens of the calves grazed on alternated paddocks were similar to those from calves grazing a permanent calf paddock. The results of this study emphasise the need for continuous monitoring of the effectiveness of any two year alternate grazing strategies.

Another potential problem arose in respect of alternating cattle and sheep to control bovine ostertagiasis in that there was an apparent increase in infectivity and pathogenicity of Nematodirus battus in calves. This was observed in a field study where an untreated control group of first season calves showed

clinical signs of nematodiriasis with evidence of patent infections and subsequent pasture contamination. In two experimental investigations into the role of N. battus in clinical bovine nematodiriasis however, the establishment of patent experimental infections of N. battus in calves using a bovine derived strain of this species proved to be very difficult. Although patency, as demonstrated by positive faecal egg counts, was minimal in both monospecific and combined infections with O. ostertagi and C. oncophora, there was some gross and histopathological evidence of infection. Adult and larval stages of N. battus were detected in raised lesions on the mucosal surface of the small intestine while flattening of the villi with aggregates of lymphoid cells, eosinophils and macrophages were especially marked in these nodular regions. The reasons for the poor establishment of N. battus in these experimental studies remains obscure and is worthy of further investigation.

The findings of the studies described in this thesis emphasise the danger of presuming the success of even well established means of helminth control.

## GENERAL INTRODUCTION

Parasitism is one of three types of symbiotic relationships which occur in the animal kingdom. It can be distinguished from the other two, which are mutualism, where both host and symbiont depend on and are beneficial to each other, and commensalism where one partner is dependent on the other but does neither harm nor good, by the fact that in the case of parasitism the symbiont (or parasite) is both physiologically dependent on the host for its habitat and sustenance but at the same time can be harmful to it. Parasitology embodies the study of the relationships which exist between the parasite and both its physical and biological environments.

The physical environment and requirements of the free-living stages of parasites are varied but for most there must be protection against desiccation and unfavourable temperatures. This is achieved in many ways such as encystment, egg shell thickness or in the case of the trichostrongylids by the retention of the cast cuticle of the second stage larva. Food from the micro environment may or may not be a requirement.

The biological environment within the host consists of several specific microhabitats generally contained within the gastrointestinal and pulmonary tracts. In most instances of endoparasitism it is necessary for the various parasitic stages of the life cycle to pass through several of these microhabitats before finally coming to rest in the final or predilection site.

Most parasitic roundworms of economic importance belong to the largest sub-order of nematodes the Strongylata (Railliet and Henry, 1913). The sub-order Strongylata can be further divided

into three superfamilies of which two i.e. the Strongyloidea and Trichostrongyloidea (Cram, 1927) contain the majority of parasites most frequently responsible for outbreaks of disease in ruminants; these include gastrointestinal helminths such as Haemonchus, Ostertagia, Cooperia, Trichostrongylus and Nematodirus.

With the exception of some members of the Metastrongyloidea, which make use of terrestrial molluscs as intermediate hosts, the Strongylata are characterised by a direct life cycle involving a single host.

It is a generally held view that parasites of this sub-order originated from free-living Rhabditida (Skrjabin, 1941; Chitwood and Chitwood, 1950). The progression from the free-living state to one of parasitism probably followed a period of adaptation from low oxygen requirement e.g. in the soil, to the similar relatively anaerobic environment of the gastrointestinal tract. From the gastrointestinal tract it is likely that some of these "pioneer parasites" moved away from the intestine towards a more aerobic environment in the vascular system and the respiratory tract while development of others remained restricted to the wall of the intestine or the lumen of gastrointestinal glands (Skrjabin, 1941).

Historically, parasitism has been recorded since the early Roman times. A literature review of the latter half of the nineteenth century reveals that while there are numerous publications dealing with many diverse aspects of helminthiasis

the bulk of these tend to be concerned with tapeworms, particularly Echinococcus, and roundworms such as Ascaris. The zoonotic aspects of infection with these parasites no doubt influenced their prominence in the literature. Other genera, however, were also described. In 1879 Cobbold reported the finding of "remarkably numerous nematode parasites in herbivorous animals" and in 1873 the same author listed those found in the ox. Included are some of the species known today although most of these were described within the "catch all" genus Strongylus. For example Strongylus filicollis (Rudolphi, 1802), S. axei (Cobbold, 1864), S. ostertagi (Stiles, 1892), S. colubriformis (Giles, 1892), S. circumcinctus (Stadelman, 1894) and S. oncophora (Railliet, 1898). This trend persisted until Looss established Trichostrongylus as an independent genus in 1905 and in 1907 Ransom established the genera Ostertagia, Cooperia and Nematodirus. A flourish of taxonomic activity enabled Travassos (1921) to list 15 species of the genus Trichostrongylus, 13 species of Nematodirus, ten species of Ostertagia, six species of Cooperia and five species of Haemonchus.

Around the same period there was a renewed interest in the adverse effects of helminth parasites on grazing animals. There were a number of early clinical descriptions of parasitic disease (Gardener, 1911; Ackert and Muldoon, 1920) and since then parasitic gastroenteritis in both cattle and sheep has been reported from all parts of the United Kingdom and Europe. Recent reviews by Michel (1969; 1976), Armour (1980) and Armour and Ogbourne (1982) discuss many of the factors involved in the

epidemiology and control of ruminant parasitism.

The two essential components of a parasitic relationship to be considered are the host and the parasite.

#### Host

A "good" host/parasite relationship is one in which both the host and the parasite species can survive with minimal adverse effects on each other. In order to be successful the evolution of parasitism must aim toward an improvement in adaptation between the parasite and its host. In this context it should be remembered that parasites which kill their hosts are biological failures and it is generally the case that under optimal conditions most hosts can tolerate moderate burdens of parasites. Although it has been suggested that the control of parasitism may be harmful by delaying the development of acquired immunity to helminths in grazing animals (Gibson, 1973; Donald, Axelsen, Morley, Waller and Donnelly, 1979), there is no doubt that the host animals derive no benefit from the association and the ideal situation would be one in which parasites could be eliminated.

Ruminant parasitism is found worldwide but in Western Europe it is economically most important in cattle and sheep. Several host factors can influence the establishment of helminths in ruminants. For example young animals are usually most readily infected (Herlich, 1960; Viljoen, 1969; Gordon, 1973) and breed and sex differences can affect establishment rates (Dobson, 1964; Bawden, 1969b). Other host factors which may result in altered susceptibility to infection include a change in nutritional level

(Bawden, 1969a), presence of other parasites (Reinecke, 1974) and changes in host specificity of the parasite (Southcott and Barger, 1975). Management systems can also affect parasitism, for example dairy calves tend to have heavier worm burdens than their beef counterparts due to their earlier dependence on grass.

Within the host, Michel (1969a) maintains that worm burdens, which are related to the number of larvae ingested, are not built up gradually over a long period and that the loss and replacement of the parasite population means that those present at any given time have developed recently. It is also suggested that the number of eggs produced by a population of worms is limited by a self regulating mechanism irrespective of how many worms are present in the population (Michel, 1969b; 1969c).

#### The Parasites

The genera most commonly involved in parasitic gastroenteritis (PGE) of ruminants include Haemonchus, Ostertagia and Trichostrongylus (axei) in the abomasum and Cooperia, Trichostrongylus and Nematodirus in the small intestine. Of these Ostertagia and Cooperia are the genera most widely incriminated in bovine PGE while both Haemonchus and Ostertagia, plus Trichostrongylus and Nematodirus are regularly found in outbreaks of PGE in sheep.

In general the life cycle of Ostertagia can be taken as typical of the other genera. Two phases are recognised. Firstly there is a free-living or pre-parasitic phase from egg deposition to the development of the infective larval stage. This is followed by a parasitic phase which occurs within the host. The



prepatent period, i.e. the time taken from infection to egg laying by mature adult parasites, is generally between 18 and 21 days.

#### Pre-parasitic or Free-living Phase

Fertilised eggs laid by adult females are characteristically thin shelled oval structures and are in the early stages of segmentation when excreted in the faeces.

Egg embryonation and subsequent larval development is mainly dependent on temperature and humidity. Temperature requirements for egg development and hatching have been widely studied, often in great detail, but as Michel (1969) points out these may prove unimportant in practice. Support for this view is evident from the very variable results obtained for H. contortus e.g. Ransom (1906) reported a minimum hatching temperature of  $4.4^{\circ}\text{C} - 7.8^{\circ}\text{C}$  but Dinaburg (1944) maintained that no development would occur below a temperature of  $18^{\circ}\text{C}$  while more recent work by Silverman and Campbell (1958) suggested an optimum temperature of  $7.2^{\circ}\text{C}$ . A range of minimum hatching temperatures for various species was determined by Crofton (1965) including H. contortus ( $9^{\circ}\text{C}$ ), O. circumcincta ( $4^{\circ}\text{C}$ ), T. axei and T. vitrinus ( $8^{\circ}\text{C} - 9^{\circ}\text{C}$ ), and C. curticei and C. oncophora ( $16^{\circ}\text{C}$ ) but he suggests that these values might be altered by other parameters such as moisture and oxygen tension.

In addition Crofton, Whitlock and Glazer (1965) showed that different strains of one species can have differing critical development requirements e.g. an increase of  $4^{\circ}\text{C} - 5^{\circ}\text{C}$  was

required for hatching H. contortus eggs from Kentucky, U.S.A. compared with eggs obtained from Bristol, U.K. Similar results were obtained in a comparison of the eggs of O. circumcincta from the same sources (Crofton and Whitlock, 1965).

It is important that a distinction is made between the minimum temperature for development, the temperature at which development proceeds most quickly and the optimum temperature which results in the maximum production of infective larvae.

Belle (1959) also demonstrated an interaction between temperature and moisture with egg development proceeding slowly at low humidity levels when in conjunction with a low temperature. Moisture is essential to the survival of eggs but interestingly, as shown by Silverman and Campbell (1958), eggs failed to develop in waterlogged faeces a fact which they attributed to a lack of oxygen. Also, desiccation is only fatal to eggs which have failed to reach the embryonated stage; when embryonated they can survive for some months (Silverman and Campbell, 1958). It has been shown that hatching of such eggs can take place very quickly in response to moisture availability (Veglia, 1915; Furman, 1944a).

With such a range of factors influencing egg and larval development the comment (Michel, 1969) that "undue attention was paid to extremes rather than the mode" could be relevant.

After hatching, first stage larvae ( $L_1$ ) are very active and during feeding their intestinal cells become packed with food granules. Soon the larvae become quiescent and during a period of lethargus the separation of the cuticle from the underlying

epidermis occurs. This is quickly followed by ecdysis or moulting to the second larval stage ( $L_2$ ). A similar pattern of activity and lethargus precedes the second moult resulting in the final pre-parasitic stage, the third stage larva ( $L_3$ ) but in the case of most of the trichostrongyloids the cuticle of the  $L_2$  is retained and the  $L_3$  are thus totally 'ensheathed'. Although this sheath is protective it prevents feeding and  $L_3$  are wholly dependent on the stored nutrients obtained during the first and second larval feeding periods.

Third stage larvae can be identified to genus, and often to species, level morphometrically (Keith, 1953; Dunn, 1969).

#### The Ecology of Infective Trichostrongyloid Larvae

The  $L_3$  is one of the most important stages in the life cycle of any trichostrongyloid parasite. Ultimately it is on this stage that the propagation of the species depends since no further development takes place until ingestion by a suitable host. Thus they must be available, viable and present in sufficient numbers to ensure adequate establishment of a mature population to continue the life cycle. Many obstacles to such a successful conclusion are to be found and the ecology of these pre-parasitic stages has long been a subject of study, controversy and conjecture.

Despite the considerable mortality of eggs and early larval stages, large numbers of infective larvae can be found in many environmental situations and Crofton (1963) described four types of microhabitat for free-living larval stages i.e. soil, root-

mat, herbage and host faeces. In the latter Durie (1961) in Australia found that while there was sufficient moisture in a bovine dung pat to allow development of trichostrongyle eggs to L<sub>3</sub> the same did not hold true for sheep faecal pellets where an external source of moisture was required to complete development.

In the U.K. with its variable climatic conditions the effects of climate on larval survival have been studied by Taylor (1938; 1939), Crofton (1949; 1952) and many others. A general consensus is that the average survival period of most trichostrongyloid larvae is in terms of months, rather than weeks or days, and that a lifespan of at least six months is by no means unusual.

Trichostrongyloid L<sub>3</sub> are more resistant than the other free-living larval stages to desiccation (Furmann, 1944a; Donald, 1968) and this is usually attributed to the protection afforded by the retained cuticle of the L<sub>2</sub>. Surprisingly, however, D. viviparus L<sub>3</sub> are not more resistant than other trichostrongyloid larvae despite their retention of both pre-infective larval cuticles (Rose, 1956).

It is widely accepted that moisture and temperature are the two main environmental factors involved in larval survival and a temperature range of 10°C to 18°C combined with a 90% humidity is considered optimal. Although high temperatures and humidity will encourage faster development it should be remembered that the optima for larval growth and larval survival are different. Also climatic factors have a greater influence on pre-infective than on infective larval stages and it is possible that while recently

deposited eggs may be unable to hatch or develop, an existing third larval population can survive (Donald, 1968; Levine and Andersen, 1973). For example Shorb (1943; 1944) working in Beltsville, U.S.A. found that eggs of H. contortus deposited in winter did not survive and argued that, although favourable temperatures occurred during May to September, H. contortus transmission was unlikely to occur in much of the USA due to the destruction of eggs and pre-infective larvae during the cold winters and hot dry summers. In California Furmann (1944b) also found that herbage contamination did not follow egg deposition in summer although existing populations of third stage larvae survived.

Crofton (1965), in an interesting reversal of the use of the set temperature-variable development theme adopted by most workers, compared the temperatures required for hatching within a standard period of 24 hours (which in practice would imply a fairly rapid development to infectivity) and found that this was achieved by different sheep parasite species as follows: H. contortus - 23°C, O. circumcincta - 20°C, C. curticei - 25°C, T. axei and T. vitrinus - 28°C. Using this data it could be suggested that in temperate areas O. circumcincta had a greater potential for the development of larger populations of infective larvae than Trichostrongylus species which is indeed the case. On the other hand the similarity in temperature requirements of Ostertagia and Haemonchus suggest that there should be little difference in distribution of these two genera in the U.K., which

is not the case, Haemonchus being more prevalent in the southern U.K.

Larval migration from faeces to herbage which has been referred to as translation (Michel and Parfitt, 1956) has been studied in some detail. Rose (1962) demonstrated the dependence of translation on rainfall and showed that in the absence of wet conditions the L<sub>3</sub> remain congregated within or adjacent to the faeces. Several workers have assessed the effect of different environmental factors on larval responses. For example increased activity was linked to an increase in temperature (Sturrock, 1965) and diminishing light intensity (Rogers, 1940) and there was an overall increase in larval recovery from herbage in the morning and evening than at any other time in the day (Rogers, 1940; Rees, 1950). Negative geotaxis was once thought to be of great importance in larval migration on to blades of grass but this movement of infective larvae on to herbage is now considered to be more in the nature of a "random walk" as described by Crofton (1954a).

Results of research activity into larval climatic requirements led to the introduction of bioclimatographs (Gordon, 1948) which were used to predict the probable geographical prevalence of parasite species. For example due to its higher temperature requirements Haemonchus could be expected to be more prevalent in areas of summer rainfall compared with Ostertagia which was more likely to be found where cooler weather conditions prevailed. Subsequently, Levine (1963) used climatic data to calculate potential periods of transmission and development

although his term "potential transmission period" and the use of narrowly defined periods of possible development and transmission has been criticised (Michel, 1969d). For some time forecasts of probable peak challenge periods have been available to assist in the control of nematodiriasis and fascioliasis (Ollerenshaw and Smith, 1969) while more recently a mathematical model for forecasting bovine ostertagiasis has been formulated (Gettinby, Bairden, Armour and Benitez-Usher, 1979).

#### The Epidemiology of Bovine PGE

In the U.K. the work of Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart (1965b), Michel (1969) and Armour (1974) have contributed greatly to current epidemiological knowledge of the pattern of availability of the free-living third larval stages of trichostrongyloids. However, information on the epidemiology and control of nematode parasitism is always accumulating, particularly with regard to further knowledge of larval survival and transmission and in the development of treatment and control strategies.

Although diverse conditions may influence the epidemiology of bovine parasitic gastroenteritis in Britain the key factors involved can be summarised as follows:

(i) Bovine PGE occurs mainly in the western half of the United Kingdom and is generally seen to be a disease of dairy or dairy X cattle.

(ii) Overwintered larval stages on pasture initiate the infection in calves turned out in early spring. Cycling of this infection results in pasture contamination from late May onwards.

(iii) Egg hatching and larval development although initially slow, speed up during the early summer and in consequence large numbers of L<sub>3</sub> become available during July - September.

(iv) The ingestion of sufficient numbers of these L<sub>3</sub> results in disease characterised by diarrhoea and weight loss. Although generally known as Type I ostertagiasis because of the contribution by Ostertagia species there is no doubt that other parasite genera can be present notably Cooperia (Borgsteede and Hendriks, 1979; Coop, Sykes and Angus, 1979; Henriksen, 1981) and occasionally Trichostrongylus (Ross and Woodley, 1968).

(v) After September, larval development and availability of L<sub>3</sub> on pasture decreases. Also the phenomenon of inhibition or hypobiosis occurs and fewer ingested larvae mature to the adult stage in the current grazing season.

(vi) Sufficient larvae overwinter on pasture until the following spring to be cycled by susceptible calves.

Several studies in the U.K. have demonstrated that the annual pattern of bovine trichostrongyloid larvae on herbage is consistent, with low levels being found in the spring which, when cycled by susceptible calves, give rise to high larval populations in autumn (Anderson et al, 1965b; Michel, 1969).

This seasonality of pasture contamination has been demonstrated in other parts of Europe by Burger, Eckert, Wetzel and Michael (1966), Malczewski (1970), Kloosterman (1971),



Raynaud, Laudren and Jolivet (1971). In the temperate areas of North America (Williams and Knox, 1976) and the eastern maritime regions of Canada (Smith, 1973) a similar pattern of contamination was observed. Comparable findings were demonstrated by Anderson (1971) and Smeal, Robinson and Fraser (1980) in the winter rainfall conditions of Australia. With regard to Ostertagia in the U.K. Michel (1969d) observed that herbage larval numbers followed a seasonal pattern with low levels being recovered in April and May. After early season grazing and egg deposition by calves three weeks later, an increase in numbers of larvae on herbage was rarely detected until late July or August. This was explained by the theory that eggs deposited over a long period of unsuitable conditions would develop rapidly over a short period when climatic conditions became more favourable. The resulting third stage pasture larval populations could then survive for the remainder of the season (Rose, 1961; Michel, 1967b). Climatic factors were seen to influence this pattern e.g. very dry summer and autumn conditions tended to delay the synchronous "flush" of larvae on pasture and it has been suggested that this was due to an inability of the larvae to leave the faecal pats in the absence of sufficient moisture (Rose, 1961). Michel (1969b) goes on to suggest that a large proportion of the larvae which persist on pasture throughout the winter are derived from eggs deposited before the middle of July. He is of the opinion that the disease producing generation of larvae, the first progeny of the cycled

overwintered stages, appears on herbage in July/August and that the second and possible third generations are of no great importance in terms of outbreaks of disease. Armour (1980) refers to a similar situation where an outbreak of helminth disease is caused by an "increase in the infecting mass" usually occurring seasonally and after at least one parasitic generation: he also links this to a combination of other factors including larval development, dissemination and survival plus animal management practices, stocking density and the immune status of the host. In the same paper, Armour goes on to suggest that in an already infected environment, in addition to an increase in infecting mass as described above, an alteration in susceptibility of the population either in existing stock or by the introduction of new stock, can lead to disease.

A number of studies in the U.K. have shown that while most trichostrongyle larvae can survive overwinter the residual infection in late spring is usually small (Crofton, 1952; Rose, 1965). This contrasts with observations in Scandinavian countries where sufficient larvae are often present at the start of the grazing period to cause clinical disease in calves (Tharaldsen, 1970; Nilsson and Sorelius, 1973).

As trichostrongyloid larvae move only a short distance from faeces (Furman, 1944b; Dinaburg, 1944; Rose, 1961; 1963; Sturrock, 1965) and animals have a tendency to avoid grazing near faeces (coprophobia) the question remained as to how ruminants so readily become infected with large numbers of worms. It has been shown, however, that although sheep will avoid areas contaminated

with fresh faeces they do not seem to object to grazing near faeces which is 5 - 10 days old (Crofton, 1958a). Also, although cattle will avoid recognisably large faecal masses, they will be less selective when the faeces has disintegrated or been spread over a wide area (Michel, 1969e) : the phrase "ring of repugnance" was used by Bastiman (quoted by Rose and Girling, 1976) for the area bordering bovine faecal pats which although lush in herbage is shunned by grazing cattle.

While rainfall is considered to be the major cause of larval migration from faeces other factors have been shown to be involved in larval dissemination. For example in piggeries, psychodid dung flies can transport larvae of Oesophagostomum spp from one area to another (Jacobs, Tod, Dunn and Walker, 1968) and Robinson (1962) described a novel method of translation of D. viviparus from faeces, larvae being "shot off" from the sporangia of the common bovine faecal fungus Pilobolus. Also in this context Gronvold (1979) and Oakley (1981) demonstrated the presence of infective trichostrongyloid larvae in earthworms.

As the temperature falls in the autumn, many of the L<sub>3</sub> ingested through September to late November do not reach maturity in three weeks but become inhibited in the gut mucosa at the early fourth larval stage (EL<sub>4</sub>) with, in the case of O. ostertagi, up to 80% of such larvae becoming arrested in development (Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart, 1965a; Armour, Jennings and Urquhart, 1969; Michel, 1974). It has been clearly shown (Ross, 1965; Anderson et al,

1965a; Armour, 1970; Smith, 1973) that in Western Europe and in North America larval inhibition coincides with the exposure of L<sub>3</sub> to falling temperatures but the underlying mechanism is still unknown. Armour and Bruce (1974) demonstrated temperature dependence experimentally by chilling larvae at 4°C for a variety of periods. Interestingly they found that, while up to six weeks chilling resulted in a high percentage of inhibition, if the cold treatment was continued the hypobiotic potential of the larvae decreased proportionally. In other areas e.g. Northern Nigeria (Hart, 1964) and South America (Furlong, Lovisi de Abreu and Verneque, 1985; Fiel, Steffan, Ambrustolo and Biondani, 1985), larval inhibition appears to be related to an increase in environmental temperature while in Australia the phenomenon is associated with dry, arid conditions (Hotson, 1967; Anderson, 1971).

#### Parasitic Phase

On ingestion of L<sub>3</sub> the sheath, or retained L<sub>2</sub> cuticle, is cast. This is the turning point in the transition from the pre-parasitic to the parasitic mode. Two to three days later the third stage larvae enter the abomasal glands. Here the two final moults take place and it is in the mucosal glands that most of the pre-patent phase of the life cycle takes place (Threlkeld, 1946; Rose, 1969).

Detailed studies of the sequential development of the lesions associated with Type I ostertagiasis were carried out by Ritchie, Anderson, Armour, Jarrett, Jennings and Urquhart (1966), Jennings, Armour, Lawson and Roberts (1966) and Murray, Jennings

and Armour (1970). These authors divide the parasitic larval development into two phases. The first of these follows immediately after infection when the cellular changes are confined to parasitised glands. The second phase occurs from around 16 days onwards when young adult parasites emerge on to the mucosal surface: In this phase a marked loss of cellular integrity develops and this spreads to the surrounding non-parasitised mucosa. Hyperplasia is a common sequel and is thought to be due to a response by the surrounding glands to maintain epithelial continuity.

Of the many cell types involved the impairment of the parietal, or acid producing cells, is probably of most significance. The loss of acid producing capacity causes a marked rise in abomasal pH which in turn impairs digestion with secondary bacterial complications adding to the problem.

Finally the loss of integrity of the mucosal epithelium allows leakage of macromolecules to and from the circulation resulting in an elevation of plasma pepsinogen together with a decrease in albumin blood levels leading to hypoalbuminaemia. In a recent study it was suggested that in response to the presence of adult parasites, the zymogen cells also secrete increased amounts of pepsin directly into the circulation (McKellar, Duncan, Armour and McWilliam, 1986).

Infections with inhibition prone larvae acquired late in the year are generally asymptomatic and this is sometimes known as the pre-Type II phase of ostertagiasis. Maturation of these EL<sub>4</sub>

takes place during winter and spring and when large numbers resume development synchronously, then clinical Type II disease ensues.

#### Diagnosis of Bovine PGE

Probably the best known and certainly one of the simplest diagnostic techniques used for parasitic infections is the detection of parasite eggs or larvae in the faeces. Faecal egg output is often used as a "yardstick" of infection but Spedding (1952b) found no correlation between ovine faecal egg counts and nematode worm burdens. Also Mayhew (1940) and Kelley (1955) suggested that dietary factors could influence the numbers of eggs present in faeces at any given time. Both of these authors, however, based their findings on the fact that increased hay consumption was linked to a decrease in faecal egg count without taking any account of faecal consistency. More recently Maciel (1984), showed that egg counts varied both between different aliquots from the same sample and between samples taken at different times during the day suggesting that faecal egg count data should never be taken alone as evidence of parasitic disease. The deficiencies of faecal egg counts for the quantitative diagnosis of trichostrongyloid infections in calves were previously emphasised by Brunsdon (1971) and confirmed earlier reports of the difficulty in interpreting such data in calves (Rubin, 1967; Michel, 1968).

In general the significance or otherwise of a faecal egg count depends on many factors such as host immune status, faecal consistency and parasite species. For example high fecundity is

a characteristic of some parasite genera including Haemonchus and Cooperia while in others such as T. axei, egg output is extremely low. Also time of the year may be important as with Ostertagia which is prone to inhibition: in this case a low faecal egg count during the pre-Type II phase in autumn/winter cannot be taken as indicative of a low level of parasitism.

Serological examination has also been employed to detect parasitism. The estimation of plasma pepsinogen levels has been widely used as an indicator of abomasal damage in ostertagiasis (Armour, Jennings, Murray and Selman, 1973; Ford, 1976) although its usefulness as a diagnostic test has been questioned in a study conducted over a six month period in which similar high pepsinogen values were observed in calves which had been treated with an anthelmintic to remove their adult worm population and in untreated controls (Michel, Lancaster, Hong and Berrett, 1978). This is perhaps not surprising since it was noted earlier that "plasma pepsinogen levels do not decline as quickly as the rapid restoration of normal gastric function after worms are lost or removed might lead one to expect" (Jennings, Armour, Kirkpatrick and Murray, 1967; Brunson, 1969).

More recently attention has been focused on serum or plasma gastrin as an indicator of abomasal dysfunction due to parasitism (McKellar, 1984; Entrocasso, McKellar, Parkins, Bairden, Armour and Kloosterman, 1985) and several workers have attempted to develop an Enzyme Linked Immunosorbent Assay (ELISA) for the diagnosis of parasitism (Keus, Kloosterman and Van den Brink,

1981). Although such a test may be of use in the detection of previous or current lungworm infection in cattle, it would appear that gastrointestinal parasites have many common antigens which make their specific diagnosis difficult.

#### Control of Bovine PGE

Recognition of an epidemiological pattern underlying outbreaks of disease has led to the development of several strategies for the control of ruminant trichostrongylosis (Michel, 1966; 1976; Gordon, 1973; Brunsdon, 1980; Morley and Donald, 1980; Armour and Ogbourne, 1982; Jorgensen, 1983; Nansen, 1986). It is clear from these that parasite control may be achieved in one of three ways i.e. (1) by the use of anthelmintics, (2) by grazing management or (3) by a combination of both.

##### (1) Control by anthelmintic prophylaxis

Since the introduction in 1962 of perhaps the first efficient broad spectrum anthelmintic, thiabendazole (Thibenzole, Merck, Sharp and Dohme) antiparasitic drugs have been widely used. These have included a number of useful drugs such as morantel tartrate, tetramisole and levamisole (Armour et al, 1973). More recently the new benzimidazoles with a wider spectrum of activity e.g. fenbendazole, oxfendazole and albendazole and the non-benzimidazole, ivermectin, have become available.

Control measures using anthelmintics are generally aimed at reducing herbage infectivity e.g. by early season prophylactic treatments (Pott, Jones and Cornwell, 1974; Armour, 1978; Herd



and Heider, 1980; Jorgensen, 1983) but they are also used therapeutically to combat the effects of high levels of contamination e.g. by late season tactical drenching to alleviate helminthiasis (Cornwell, Jones and Pott, 1971; Nansen, Jorgensen, Henriksen and Foldager, 1988a). To be successful any anthelmintic drenching programme should be carefully planned and must always take account of factors such as local weather conditions, previous and current pasture management systems, grazing and aftermath availability and type of stock involved. The choice of anthelmintic compound in practice, however, is often made on factors such as cost without thought of efficacy or development of resistance (Arundel, 1985).

It is of interest that although thiabendazole was extremely effective in the treatment of ruminant PGE, the paucity of epidemiological knowledge in the early 1960's limited its use in prophylactic schemes. It is clear, however, from data accumulated since that time, that in the U.K. in most years the pattern if not the magnitude of infective larval availability on pasture can be predicted. Successful anthelmintic dosing strategies have put this knowledge to use in interrupting the parasite life cycle thus reducing pasture contamination. For example, Cornwell, Jones and Pott (1973), using morantel tartrate (Banminth, Pfizer Ltd.) demonstrated that treatment of young calves on three occasions at monthly intervals from July to September resulted in a mean liveweight gain of 11 kg compared with untreated controls grazing the same area. Later Pott et al

(1974) using treatments concentrated in the early part of the season demonstrated an improved mean liveweight gain of 25 kg over untreated calves. This was achieved by grazing the treated and untreated animals separately. The result of this separation was to considerably reduce the number of infective L<sub>3</sub> available to the treated calves in the latter half of the season. In another study, Bairden (1980) allowed young calves to graze for the first three weeks of the season before treating them with thiabendazole. After a further three weeks at grass a second thiabendazole treatment was administered. This simple regime of treatment before or at patency (the prepatent period of most trichostrongyles is approximately three weeks) reduced faecal egg output, subsequent pasture larval numbers and thus worm burdens at post-mortem at the end of the grazing season.

While all of these systems have met with some success the control achieved was not absolute and in the three and six week treatment by Bairden (1980) for example, two of the twelve treated calves developed clinical PGE. As it is now known that some strains of Ostertagia species have a shorter prepatent period than 21 days, the three week interval between treatments undoubtedly allowed several days pasture contamination with parasite eggs to occur.

With the advent of fenbendazole (Panacur, Hoechst U.K. Ltd.) which was one of the first compounds to demonstrate a high level of efficacy against larval stages, including inhibited early fourth stage larvae (EL<sub>4</sub>) of O. ostertagi (Duwel, 1974; Duncan, Armour, Bairden, Jennings and Urquhart, 1976; Duncan, Armour and

Bairden, 1978) the efficacy of a three and six week treatment regime in calves was shown to be extremely effective in terms of parasite control (Bairden, 1980).

Ivermectin (Ivomec, Merck, Sharp and Dohme, Ltd.), an antiparasitic compound derived from the bacterium Streptomyces avermitilis (Campbell, Fisher, Stapley, Schonberg and Jacob, 1983), has been shown to have a residual effect against  $L_3$  ingested for up to 14 days after treatment; this has led to the recommendation of a three, eight and 13 week dosing regime for all season control of gastrointestinal parasites and lungworms. In this regime it is claimed that for up to 18 weeks after turnout no pasture contamination will take place and thus any significant build up of herbage larval populations will be prevented during the grazing season. Several studies have confirmed the efficacy and residual activity of ivermectin against a variety of helminth parasites (Armour, Bairden and Preston, 1980; Barth, 1983; Bremner, Berrie and Hotson, 1983; Armour, Bairden, Batty, Davison and Ross, 1985; Armour, Bairden, Pirie and Ryan, 1987). However, included in the broad spectrum of activity of ivermectin is an effect against a wide range of non-parasitic invertebrates which has led to several investigations into the adverse environmental effects of drug excreted in ruminant faeces after treatment. In particular excreted ivermectin can interfere with the natural breakdown and disposal of faecal pats by dung beetles etc. (Wall and Strong, 1987; 1988). Other studies, however, in the U.K. and Germany

have found little or no interference with such processes although it is agreed that the main organism involved in the breakdown of faeces in these areas is the common earthworm against which ivermectin has no effect (McKeand, Bairden and Ibarra-Silva, 1988; Jacobs, Pilkington, Fisher and Fox, 1988; Schaper and Liebisch, 1991).

A major drawback of any regime of strategic treatments is the necessity to gather and handle stock which is often inconvenient and time consuming. Several interesting anthelmintic formulations and different administration routes have been devised with a view to reducing the labour or expense involved. Examples include the incorporation of anthelmintics in cattle feed blocks (McBeath, Best, Preston and Thompson, 1977) or water delivery systems (Downey, O'Shea and Spillane, 1974; Downey and O'Shea, 1985) and spot-on and pour-on preparations for transcutaneous administration. Such in-feed, water and topical preparations appear to have distinct practical advantages over the more traditional oral or injectable methods of administration but they also have problems in terms of restrictions in use e.g. suitable weather conditions for pour-on application are necessary as treatment during wet weather is not recommended.

Perhaps of greater interest is the concept of dosing with anthelmintic devices programmed to deliver calculated amounts of drug over extended periods. Several of these, mainly in the form of gut-dwelling boluses, are now available and can be categorised broadly as either 'sustained release', where the compound diffuses from the device at a given rate, or 'pulse release'

where a therapeutic dose of anthelmintic is delivered over a given time sequence.

Although much pioneering work on intraruminal devices was carried out in sheep by Mason and Laby (1978) and an oxfendazole slow release capsule in cattle (Anderson and Laby, 1979) the first commercially available anthelmintic bolus for cattle (Paratect, Pfizer Ltd.) was marketed in 1982. This was a sustained release type bolus and contained the anthelmintic morantel tartrate. Administered at turnout, the bolus is active for a period of 90 days and thus prevents the build up of infection on the pasture by early season cycling of infection (Armour, Bairden, Duncan, Jones and Bliss 1981). However morantel tartrate is not a highly efficient anthelmintic and this may be an advantage in that it allows the acquisition of immunity.

The use of boluses has been criticised for several reasons. It has been suggested, for example, that the continued use of the same anthelmintic compound over an entire grazing season will lead to the development of parasite resistance. Other criticisms include the practical difficulties encountered in abattoirs where the presence of steel remnants or positional weights from boluses can interfere with offal processing. A new type of long acting device has been formulated which overcomes some of these problems in that it consists of a rectangular biodegradable laminate which, after administration in the form of a cylinder, unrolls in the rumen or reticulum.

## (2) Control by grazing management

Helminth control using grazing management generally involves the integrated grazing of cattle and sheep in mixed and alternate grazing systems (Rutter, 1975; Barger and Southcott, 1975; Brunsdon, 1980; Morely and Donald, 1980; Ministry of Agriculture Fisheries and Food, 1980). Alternate or mixed grazing systems depend for their success on the host specificity of the parasite species involved. For example the cattle and sheep species of Ostertagia, O. ostertagi and O. circumcincta respectively, do not readily infect the alternate host and when they do, they rarely develop to patency. Thus when cattle are put to graze on a former sheep paddock although they will ingest the larvae derived from the previous ovine contamination, little or no further contamination of the area will take place. In the following year when sheep are turned out on these paddocks very few, if any, infective larvae should be available. If a three year rotational system including forage or other crops is used this can be an effective method of parasite control. A three year alternation of cattle, sheep and crops is often advocated (Thomas, 1982).

Other forms of management include zero grazing, practised in parts of Europe, where first year calves are kept indoors and fed cut herbage for part or all of the grazing season. In Denmark and the Netherlands a late turnout in June was credited for significantly reduced worm burdens in young calves (Borgsteede, 1977; Foldager, Sejresen, Brolund Larsen, Nansen, Jorgensen, Hansen and Henriksen, 1981; Nansen, Jorgensen, Henriksen and Foldager, 1987). The leader/follower or "dilution" system

described by Leaver (1970) is based on the rotational grazing of calves on permanent pasture followed by older heifers or cows. In their short grazing period in each area the calves will only crop the upper, leafy part of the herbage thus avoiding the concentration of larvae in the lower parts which the following older animals can ingest with impunity due to their acquired resistance. Nagle, Brophy, Caffrey and O'Nuallain (1980) assessed the additional effect of an anthelmintic treatment of young calves in such a leader/follower system and recorded a significantly better performance from treated animals compared with untreated controls.

In the first of two studies Barger and Southcott (1975) demonstrated the effectiveness of host alternation on the decontamination of cattle pastures by stocking them with sheep for two months. In the second more detailed study (Southcott and Barger, 1975) an attempt was made to clean up both cattle and sheep paddocks by set stocking with the alternate host. After six, 12 or 24 weeks stocking with the alternate host, susceptible calves or lambs were grazed and necropsied for worm burden estimation. They concluded that the use of cattle to significantly reduce herbage larval levels was successful over the three periods chosen but found that the maximum 24 week period was necessary for sheep to completely "clean up" former cattle paddocks. This result was at variance with that obtained in their first study when a period of only eight weeks resulted in a reasonable level of decontamination.

In Northern Ireland improved lamb weight gains were attributed to the presence of fewer parasites in sheep due to mixed grazing with cattle (Conway, 1968) and increased productivity, associated with low worm burdens in sheep, was also demonstrated in a mixed grazing system by Arundel and Hamilton (1975). Interestingly Conway (1968) indicated that cattle and sheep grazing together showed improved production per unit area compared with set-stocked single host species. This he argued was due to the efficient pattern of sheep grazing, as they were willing to eat herbage rejected by cattle. Unfortunately no indication of the parasitological status of these animals was given. Finally in Norway, Helle (1981) compared alternate and mixed grazing of cattle and sheep over a five year period and concluded that in terms of parasite control and liveweight gains the alternate grazing system was superior.

A disadvantage of alternate grazing systems is that not all ruminant gastrointestinal nematodes are host specific, an example being T. axei which can be found in both cattle and sheep. This species can also infect other hosts including goats, horses and pigs. Obviously in the case of alternation or mixing of these host species there would be limited control of T. axei and indeed it has been suggested that the passage of T. axei through the alternate host tends to enhance its establishment in the other host species (Ross and Purcell, 1969; Bairden, 1980). It may be that while in such a situation the control of ostertagiasis can be achieved but the problem caused by T. axei may worsen. It is interesting in this context that the only outbreak of naturally



occurring trichostrongylosis ascribed to T. axei in Britain occurred in Northern Ireland where integrated grazing of sheep and cattle is not uncommon (Ross and Woodley, 1968). Another facet of host species integration is parasite adaptation and there are several reports of cross transmission of some cattle parasites to sheep and vice versa (Herlich and Stewart, 1954; Smith and Archibald, 1965; Arundel and Hamilton, 1975; Bisset, 1980; Helle, 1981). Alternate grazing with cattle and sheep has also been shown by Eysker and Jansen (1982) to encourage the establishment of bovine species such as C. oncophora and N. helvetianus in lambs grazed on a previous cattle paddock. No significant increase in O. ostertagi in these animals was noted. Al Saqur, Armour, Bairden, Dunn and Jennings (1980), Bissett (1980) and Al Saqur, Bairden and Armour (1982) also incriminated O. leptospicularis, normally found in sheep and deer, as a species contributing to bovine ostertagiasis. More recently it has been shown that an increase in the level of Nematodirus battus infection occurred in calves which were set stocked on former sheep grazing (Bairden and Armour, 1987; Armour, Bairden, Dalgleish, Ibarra-Silva and Salman, 1988; Coop, Jackson and Jackson, 1991). Over a four year alternation of sheep and cattle this trend continued and finally resulted in clinical nematodiriasis in calves. It is interesting that a failure to control nematodiriasis by mixed grazing of cattle and sheep was noted almost 30 years ago in Northern Ireland (Baxter, 1959).

### (3) Control by a combination of anthelmintic treatment and grazing management

Of the third means of control, i.e. a combination of stock management and anthelmintic treatment the most widely used is that known as the "Dose and Move" system for cattle which was developed at Weybridge (Michel, 1966). This very successful means of helminth control is widely practised in the U.K. and elsewhere and is again based on a knowledge of the annual pattern of availability of infective larvae on herbage. In this system, calves turned out to graze in the spring are treated in early July and moved to a previously ungrazed area, usually an aftermath grazing. In theory by this time any overwintered larvae will have succumbed and relatively clean calves are being turned on to relatively clean grazing. Sometimes referred to as an evasive strategy, the dose and move system has also proven successful in Denmark (Henriksen, Jorgensen, Nansen, Sejrsen, Larsen and Klausen, 1976b; Foldager et al, 1981). However, Le Jambre (1978) suggested one possible drawback to the dose and move system in that the new generation of worms after the move to clean pasture will consist entirely of the progeny of any parasites which have survived treatment and may select for anthelmintic resistance. Donald (1983) however, points out that selection for resistance will depend on many factors including the frequency of treatment and the "cleanliness" and subsequent grazing history of the pasture.

The dose and move system has not always been successful however and in an experimental situation clinical ostertagiasis has occurred in calves treated and moved to aftermath (Bairden, Parkins and Armour, 1979). Soil was suggested as a possible refuge and subsequent source of the infective larvae and in later studies infective trichostrongyle larvae were consistently recovered from soil samples in paddocks regularly grazed by calves (Bairden, 1980; Al Saqur et al, 1982).

## **MATERIALS AND METHODS**

## INTRODUCTION

Although a research technique is often modified to suit a particular requirement or situation such alterations are not always detailed. In the following description all techniques are described exactly as carried out and where pertinent a comment on the plus or minus aspects of a particular change is given.

### 1. Animals

The calves used in the experimental studies were mainly of the Friesian breed although occasionally Ayrshire or Hereford calves had to be included. All animals were reared under conditions which precluded infection with helminths and to facilitate handling the male calves were castrated.

Scottish Blackface sheep were used throughout the field studies and were obtained locally. As parasite-naive animals were not readily available the ewes and lambs were treated where necessary prior to or during grazing. The lambs were docked and castrated in accordance with normal farm procedure.

Calf weights were determined using a standard cattle weigh crush.

Supplementary feeding consisting of 1 kg to 3 kg of hay per calf per day was given towards the end of the grazing season i.e. late September/early October when necessary.

### 2. Grazing

The plots used in this study were situated within the grounds of Glasgow University Veterinary Hospital and had been grazed regularly by ruminants for a number of years. The area of each grazing plot was standardised at 0.33 hectares and

separation of adjacent plots was achieved by means of a double wire mesh fence with 1.5 metres between fences.

### 3. Clinical Examination

The animals were observed daily and examined each week when their condition was assessed on the basis of appearance, appetite and manifestations of disease such as diarrhoea and weight loss.

### 4. Parasitological Procedures

#### (a) Faecal analysis

A modification of the McMaster flotation technique devised by Gordon and Whitlock (1939) was used to detect the presence of trichostrongyle eggs in faeces. In this technique three grams of faeces were homogenised with 42 ml water and the resultant suspension passed through a coarse mesh sieve of aperture size 250 microns (Endecotts Ltd., Morden Factory Estate, London) which, while retaining the larger particles of debris, allowed the passage of nematode eggs (size range 70 - 165 microns). After thorough mixing of the filtrate 15 ml were transferred to a flat-bottomed centrifuge tube and centrifuged at 2,000 revolutions per minute (rpm) for two minutes. The supernatant was then discarded and the remaining faecal mass broken up by rotary agitation (Whirlmixer, Scientific Industries Ltd.). The tube was then filled to its former level with saturated sodium chloride solution and after inverting six times a volume of the suspension sufficient to fill both chambers was quickly transferred by pipette to a McMaster slide (Gelman Hawksley Ltd., Harrowden, Northampton). The numbers of eggs

under both etched areas of the slide were counted and the result multiplied by 50 to give the number of eggs per gram of faeces according to the following calculation:

3 grams of faeces in 42 ml give 1 gram in 15 ml.

Volume under one square equals 0.15 ml.

No. of eggs seen in one square  $\times 100$  = No. of eggs/gram.

No. of eggs seen in two squares  $\times 50$  = No. of eggs/gram.

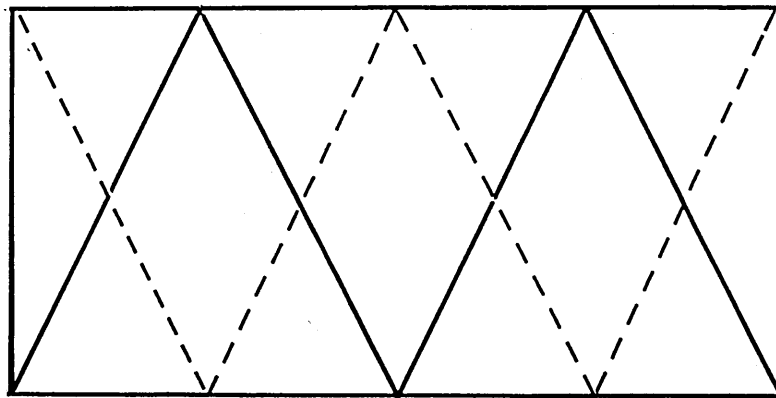
When the above technique failed to detect eggs a more sensitive method was employed. In this saturated sodium chloride was added to the tube containing the faecal suspension, in saturated salt, until a positive meniscus formed. After a further two minutes centrifugation at 2,000 rpm a coverslip was then added and the tube allowed to stand for two to three minutes. The coverglass was then removed vertically and transferred to a microslide. All eggs present were counted and the result expressed as eggs/gram of faeces.

This method differs from the differential flotation technique described in the Ministry of Agriculture Fisheries and Food Reference Book 418 (1986) in that the coverglass is added after the final centrifugation. This is of great advantage as, unless a specially adapted tube carrier such as those used in the now obsolete Clayton Lane centrifuge is available, a large proportion of coverglasses are lost during this second spinning with a resultant loss of sample material and the added burden of centrifuge bowl cleaning. One "spin off" of this technique was that an extremely clean preparation of eggs was obtained, a fact which has been made further use of in other areas of study in the

department.

(b) Analysis of herbage for the presence of  
trichostrongyle  $L_3$

Pasture samples were collected by crossing the experimental plot as shown in the following diagram.



Route 1 ———

Route 2 - - - -

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 basically similar to that used by Parfitt (1955). The similarity between our method and that referred to was true for the first part of the technique i.e. when the samples were washed in water but even at this stage there was a slight difference in



technique. The polythene bag containing the grass was weighed then put into a small hand operated washing machine (Easy Pressure Washer, Classic Supplies Ltd., Leeds). Six litres of lukewarm water were added, the bag knotted at the top and the machine closed then turned through two hundred revolutions. Before removing from the machine a small incision was made in one corner of the bag and the washings allowed to pass through a coarse mesh sieve (aperture size 2 mm) into a bucket. The bag containing the washed herbage was then removed and as much fluid recovered from it as possible by squeezing. The grass was then taken from the bag, spread on a tray and dried in an incubator at 70°C. When thoroughly dry the herbage was again weighed and this dry weight used in the final calculation of numbers of larvae per kilogram dried herbage ( $L_3$ /kdh). The washings contained in the bucket were filtered through a 38 micron sieve and the material retained by the sieve washed directly from the sieve through a coarse filter paper (Whatmans Grade 113, 18.5 cm) using a Buchner funnel and vacuum pump. A single milk filter (Maxa Milk Filters, A. McCaskie Ltd., Stirling) was put on top, and the whole inverted and placed on a Baermann funnel. After standing for at least six hours, 10 ml of fluid were withdrawn and the larvae in 1 ml differentiated and counted.

Several points are worth noting about the above methodology. Aspects in its favour included the following:-

(a) the herbage remained in the collection bag until the final drying stage. This avoided loss of material during washing, and reduced any mess connected with transfer of wet grass from

buckets; it also facilitated the recovery of washings as fluid could be very efficiently "wrung" from the bag.

(b) the coarse paper used during filtration to collect the larval/debris mixture from the washings permitted virtually no larval loss which can occur when wide mesh filters e.g. milk filters, are used.

(c) the use of a milk filter as a supporting "sieve" on the Baermann funnel made this part of the system disposable a distinct plus point in terms of preventing sample contamination.

(d) finally in place of the usual spring or screw operated tubing clips, the use of roller clamps (similar to those used on surgical drips) enabled the quick and efficient withdrawal of the relatively small final volume of 10 ml fluid.

The main point for criticism must be in the sieving of the herbage washings through a 38 micron sieve. This was necessary to reduce the volume (approximately 6 litres) to enable it to be passed through a paper filter. Apart from the introduction of another step in the procedure, the aperture size of this sieve made it extremely difficult to clean properly and indeed even after several washes, larvae could still be found intertwined in its mesh. To minimise possible contamination a ten minute boiling stage for used sieves was introduced and while not necessarily removing the larvae this at least reduced the chance of their recovery during subsequent Baermannisation.

The criteria for larval identification were those detailed in the Ministry of Agriculture Fisheries and Food Reference

Book 418 (1986) and used by Keith (1953) i.e. body length, prolongation of the second larval sheath beyond the tail of the third stage larva and the presence of refractile structures.

(c) Post-mortem worm burdens

Prior to post-mortem, the calves were held indoors for at least seven days which facilitated the distinction of arrested and developing fourth stage larvae. They were then killed using a captive bolt pistol and immediately exsanguinated. The entire gastrointestinal tract was removed from the body cavity after ligaturing the pyloric sphincter/duodenal junction to prevent mixing of the abomasal and small intestinal contents. In practical terms the ligature between abomasum and duodenum is probably unnecessary since differentiation of adult genera whatever their position in the gut is not difficult. The large intestine and its contents were discarded as L<sub>3</sub> of Oesophagostomum radiatum were absent from all the pasture samples and Trichuris species eggs from all of the faecal samples examined.

After opening, the abomasal and small intestinal contents were washed separately into buckets and the volume made up to a standard four litres (except where an unusually large amount of material was present when a greater volume of water was required). Duplicate samples of 200 ml were withdrawn and formalised for subsequent examination. The remaining contents were then passed through gauze squares and suspended in warm water for three hours. Parasites which migrated through the gauze into the warm water were collected and formalised for

subsequent detailed examination and speciation. The abomasal mucosa was then scraped off and digested in a pepsin/hydrochloric acid mixture for six hours at 42°C to recover the larval stages present within the mucosal glands. The digested mixture was again made up to four litres and 200 ml samples withdrawn as before.

Prior to counting, 2 ml of iodine solution was added to the 200 ml sample. Four ml aliquots were then transferred to a lined petri plate and 1 to 2 ml sodium thiosulphate added. While this effectively decolourised the background, the parasites retained the stain. The trichostrongyles present in 10 x 4 ml aliquots were counted and classified as adult male or female, developing fourth or fifth larval stages or early fourth stage larvae (EL<sub>4</sub>) depending on bursal or vulvar development, the presence of a sheath projection and size respectively. Any EL<sub>4</sub> present were considered as being arrested in development since this stage is reached a few days after ingestion and the animals had been retained free from further infection for a minimum of seven days prior to necropsy.

One adaptation of this technique was to omit the formaldehyde preservation stage by adding 2 to 3 ml of iodine solution to the sample at the time of necropsy thus fixing and staining the material at the same time. This proved to be very successful in terms of fixation although the intestinal material generally required an additional 1 to 2 ml iodine. The staining aspect was less successful due to the intense colouration of the

iodine which after a few days made morphological detail more obscure.

(d) Culture of O. ostertagi and C. oncophora infective larvae

Faeces from animals with monospecific infections of O. ostertagi or C. oncophora were collected either directly from the rectum or using a harness and bag system. Sufficient granular vermiculite was added until the firm consistency required to allow preparation of fist-sized faecal balls was achieved. These were then incubated at 24°C in disposable plastic cartons at 24°C for fourteen days after which the infective larvae were recovered by Baermannisation.

(e) Culture of N. battus infective larvae

N. battus eggs were obtained from calves which had acquired infection naturally whilst grazing permanent pasture which, from 1982-1985 had been used in an alternate grazing system with cattle and sheep and from 1986 to 1988 had been grazed by calves alone. Faeces was collected directly from the rectum and homogenised. The resulting suspension was passed through a sieve (aperture size 250 microns) and, following a further cleaning by centrifugation in saturated NaCl, passed through a sieve (aperture size 38 microns) to collect the eggs. These were transferred to petri dishes and cultured at 22°C for three weeks in 1 to 2 cm of water. The larvated eggs were hatched by either shaking with glass beads or by cracking between glass plates. Following Baermannisation the larvae were counted and dispensed into the appropriate doses.

(f) Preparation and administration of infective larval inocula

Freshly harvested infective larvae were used. The numbers of L<sub>3</sub> in 40 x 0.025 ml aliquots were determined and the volume containing the appropriate larval numbers dispensed into narrow necked bottles. Larvae were given orally, the bottle rinsed with clean water and the washings also administered.

5. Blood Examination

Plasma pepsinogen estimation

Increased plasma pepsinogen levels have been shown to be related to the degree of abomasal malfunction caused by the development and maturation of O. ostertagi within the mucosal glands (Ritchie et al, 1966; Jennings et al, 1966). The monitoring of pepsinogen levels as an aid to the diagnosis of bovine ostertagiasis was first suggested by Anderson et al (1965b) and has since been widely used for this purpose (Armour, 1974; Ford, 1976) although Michel et al (1978) have criticised its usefulness. Blood samples for pepsinogen estimation were taken directly from the jugular vein into heparinised vacutainer tubes (Becton-Dickinson Ltd., York House, Empire Way, Wembley) and centrifuged at 3,000 rpm for 30 minutes. The plasma obtained was then processed to determine the level of pepsinogen present the technique used being that described by Edwards, Jepson and Wood (1960) in which the plasma is incubated with bovine serum albumin (BSA) at pH 2 for 24 hours and the phenolic amino acids liberated estimated using the Folin-Ciocalteu reaction. Corrections are made for the normal (i.e. non-incubated) content

of tyrosine substances and also for the release of these substances from BSA when incubated alone. Plasma pepsinogen values have been expressed as milli-units (mU) of tyrosine to the nearest 100.

#### 6. Histopathological Techniques

Prior to washing for worm recovery, tissue samples were taken from the duodenum, jejunum and ileum and processed as described by Armour et al (1988).

#### 7. Statistical Analysis

Where appropriate statistical analysis was carried out using Animal Designs 1, V 1.21 5/6 (Data International Services, Glasgow).

Comparisons between two groups were undertaken using the two sample t-test with unequal variances. Comparisons between three and more groups were analysed using one way analysis of variance followed by the Newman Keuls Multiple Range test. In the case of counts e.g. worm burdens and eggs per gram, the logarithmic transformation was used before analysis.

## **CHAPTER ONE**

# **A FIELD STUDY ON THE EFFICACY OF A MORANTEL SUSTAINED RELEASE TRILAMINATE BOLUS IN THE CONTROL OF BOVINE GASTROINTESTINAL PARASITISM**



## INTRODUCTION

Although anthelmintics were originally employed therapeutically to treat outbreaks of disease, over the last 20 to 30 years they have been increasingly used strategically or prophylactically on the basis of epidemiological knowledge. For example in the 'Dose and Move' system (Michel, 1966) animals, after anthelmintic treatment, were moved from an area where there would be a predictable increase in pasture infection to relatively "clean" aftermath grazing while early season treatments as described by Pott et al (1974) and Armour (1978), were used to prevent contamination of grazing with nematode eggs which could lead to a subsequent increase in pasture infectivity in a set-stocked situation. With increasing labour costs and mechanisation resulting in fewer personnel being employed on farms, gathering for treatment in spring and early summer presented an inconvenience at a busy time in the farming calendar. This was largely overcome with the development of slow or pulse release devices which were administered to animals prior to turnout thus allowing them to be set-stocked, often on grazing far away from the farm facilities, with no further requirement for handling to administer anthelmintics. The first of these, the morantel slow release bolus (Paratect, Pfizer Ltd.) was a new concept in that it provided sustained anthelmintic delivery over two to three months: this became available commercially in 1982. Recently a new delivery system for the same drug, designated the morantel slow release trilaminate bolus (Paratect-Flex, Pfizer Ltd.), has been developed. Compared with the cylindrical metal

tube of the original Paratect bolus, which was designed to stay in the rumen by its weight, the new device is retained due to its shape. Administered as a rolled up cylinder, the outer covering dissolves on contact with the rumenal fluid and allows the trilaminate to unroll. Being biodegradable, no bolus residues should be present at slaughter or during meat processing thus overcoming one of the criticisms levelled at the steel bolus.

Ivermectin (Ivomec, Merck, Sharp and Dohme Ltd.), which appeared commercially in 1982 was the first broad spectrum endectocide which also had persistent activity for two to three weeks against reinfection with bovine lungworm and gastrointestinal parasites (Armour et al, 1985; 1987). This led to the development of a novel programme of strategic treatments at 3, 8 and 13 weeks after turnout, where grazing calves were protected for a total period of 15 weeks. This regime was based on a three week parasite prepatent period: the first treatment at three weeks removed the initial infection acquired after turnout and protected for a further two weeks. The next treatment at eight weeks removed any worms acquired from reinfection and similarly protected animals from reinfection for two weeks. The third treatment at 13 weeks meant that no eggs should be produced until week 18 of grazing. This effectively prevented the normal pattern of early season infection, pasture contamination with nematode eggs and mid to late summer increase in pasture infectivity. Although more labour intensive than the bolus system, an added attraction was that ivermectin had not

only a broad spectrum of anthelmintic activity but was also effective against a number of common external parasites.

In this study the efficacy and convenience of an MSRT Flex Bolus administered at turnout was assessed and compared with ivermectin prophylactic treatments at 3, 8 and 13 weeks post-turnout in the control of gastrointestinal parasites in large groups of cattle on a farm in southwest Scotland. In addition a proportion of animals from each group was grazed for a second season to assess their immune status to subsequent exposure to gastrointestinal parasites.

#### EXPERIMENTAL DESIGN

The experimental design is summarised in Table 1. The farm used was situated on the west coast of Scotland at Campbeltown, Kintyre. The grazing area consisted of 30 acres which was subdivided into three paddocks each of ten acres. There had been no previous history of clinical parasitic disease in this area until 1987 when, after drainage and reseedling, a severe outbreak of ostertagiasis occurred in set-stocked calves resulting in the death of several animals.

The study was carried out over a two year period. In the first year, 1988, 84 calves of mixed breed and sex were randomised on Day 0 (5th May) into three groups each of 28 animals. All animals had been previously vaccinated with a commercial bovine lungworm vaccine (Dictol, Pitman Moore Ltd.). At turnout no treatment was administered to the Group 1 cattle (allocated to the ivermectin treatment group) or the control

**Table 1. Experimental Design**

**FIRST GRAZING SEASON      1988**

<b>Paddock 1</b>	<b>Paddock 2</b>	<b>Paddock 3</b>
<b>GRAZED BY 28 IVERMECTIN TREATED CALVES</b>	<b>GRAZED BY 28 CONTROL CALVES</b>	<b>GRAZED BY 28 MSRT FLEX BOLUS CALVES</b>

**2 TRACER CALVES INTRODUCED TO EACH Paddock  
IN MAY AND OCTOBER**

**SECOND GRAZING SEASON      1989**

	<b>Half of Paddock 2</b>	
	<b>GRAZED BY 4 EX-MSRT 4 EX-CONTROL 4 EX-IVERMECTIN CALVES</b>	

**2 TRACER CALVES INTRODUCED  
IN MAY AND OCTOBER**

animals of Group 2. Three hours prior to turnout the calves allocated to Group 3 were given an MSRT Flex Bolus. Scheduled prophylactic treatments with ivermectin at a dose rate of 200 mcg/kg were administered by subcutaneous injection to the calves of Group 1 on Days 21, 56 and 91 post-turnout.

The parameters measured were monthly bodyweights, serum pepsinogen levels and faecal egg counts and worm burdens of selected calves at necropsy. In addition, levels of infective trichostrongyle larvae on pasture were assessed by examining monthly grass samples and by introducing two tracer calves into each paddock at the beginning and end of grazing. The tracer calves were allowed to graze for fourteen days before being housed and necropsied two weeks later.

Four animals from each group were kept overwinter and grazed for a second season: these calves were treated with ivermectin at housing. In the second year of the study the four animals retained from each group were grazed together on half of the former control paddock from May to October. Again the animals were weighed, bled and faecal sampled monthly when pasture samples were also taken. At the end of the second grazing season the cattle were necropsied three weeks post-housing and their worm burdens established. Tracer calves again grazed the experimental area for two weeks in May and October before being housed for two weeks prior to necropsy.

## RESULTS

The results from each year are presented separately.

### YEAR 1 - 1988

#### Clinical

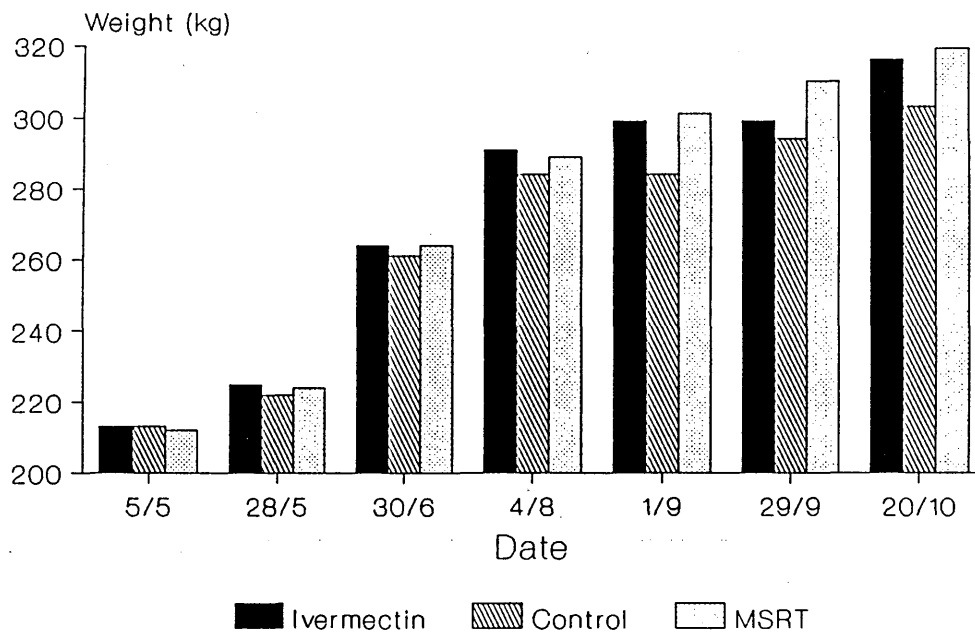
Apart from sporadic coughing during the first few weeks, no clinical signs were observed from any of the principal cattle in the early part of the grazing season. From mid-August however, the control calves in Group 2 became diarrhoeic and this combined with weight loss and high faecal worm egg counts necessitated therapeutic anthelmintic treatment of these animals on Day 119 (1/9/88). Two animals from this group and two from each of the other groups were removed and necropsied prior to this treatment with fenbendazole at 7.5 mg/kg bodyweight (Panacur, Hoechst U.K. Ltd.).

Group mean bodyweights are shown in Figure 1 with individual data being recorded in Appendix A. From initial mean bodyweights of 212 kg - 213 kg the animals of Groups 1, 2 and 3 had, until the removal of six replicates from each group in mid-September due to pressure on grazing, gained 86, 71 and 92 kg respectively. A further eight replicate animals were taken from the grazing on Day 154 (6/10/88) and at the final weighing on Day 168 (20/10/88) mean bodyweights of 316 kg (Group 1), 303 kg (Group 2) and 319 kg (Group 3) were recorded. There was no significant weight difference between groups.

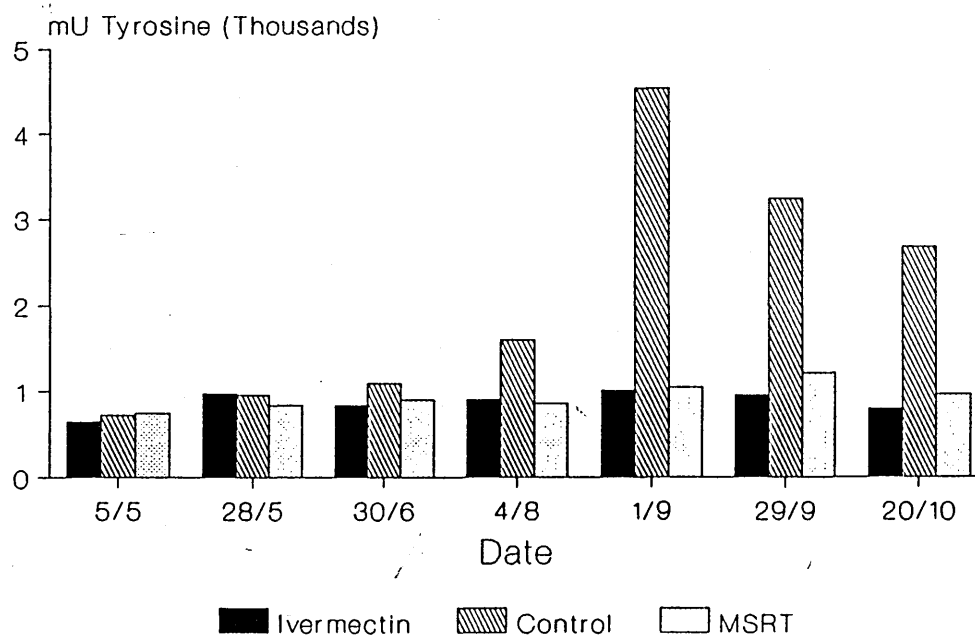
#### Biochemical

Figure 2 illustrates the mean pepsinogen levels of the principal calves; individual data is given in Appendix B.

**FIGURE 1 GROUP MEAN BODYWEIGHTS  
1988**



**FIGURE 2 GROUP MEAN PEPSINOGEN LEVELS  
1988**



The pepsinogen levels of Groups 1 and 3 remained at normal levels throughout the experimental period with mean maxima of 1,010 mU (Group 1) and 1,216 mU (Group 3) being recorded on Days 119 (1/9/88) and 147 (29/9/88) respectively. In contrast pepsinogen values of the Group 2 control cattle had increased to a mean maximum value of 4,539 mU by Day 119 (1/9/88) and despite therapeutic anthelmintic treatment an average level of 2,676 mU was recorded at housing from these animals. Both the ivermectin and MSRT treated groups had significantly lower pepsinogen levels ( $p < 0.05$ ) than the control cattle.

#### **Faecal Egg Counts**

Group mean faecal egg counts are shown in Figure 3 and detailed in Appendix C. No positive samples were recorded on Day 0 and on Day 23 (28/5/88) only the calves of Group 1 showed a low count i.e. a mean of two eggs/gram (epg). Throughout the season low numbers of eggs were excreted by the animals of both Groups 1 and 3 with egg counts of 105 epg (Group 1) and 80 epg (Group 3) being recorded at the end of the grazing period.

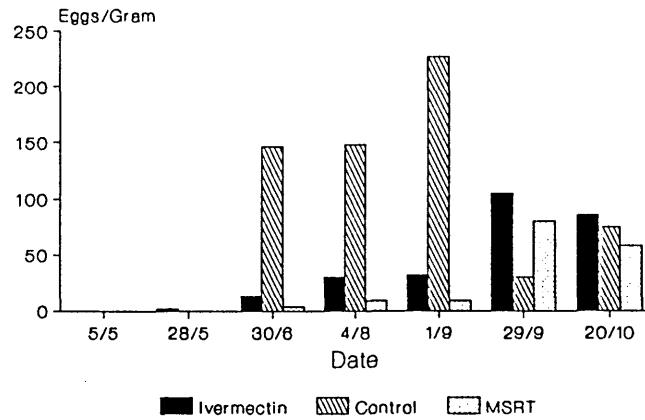
Egg counts from the control calves were consistently higher, although not significantly different, to those of Groups 1 and 3 up to the time of salvage anthelmintic treatment and ranged from an average 146 to 227 epg.

#### **Pasture Larval Counts**

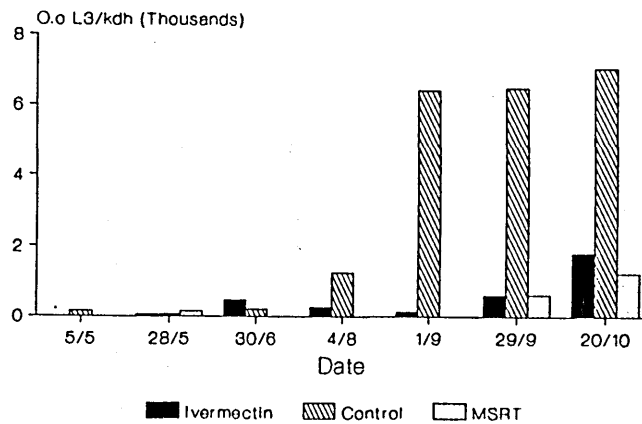
Figure 4 illustrates the Ostertagia  $L_3$  recoveries per kilogram dried herbage ( $L_3$ /kdh) from the three grazing areas; these are detailed in Appendix D. Prior to the commencement of



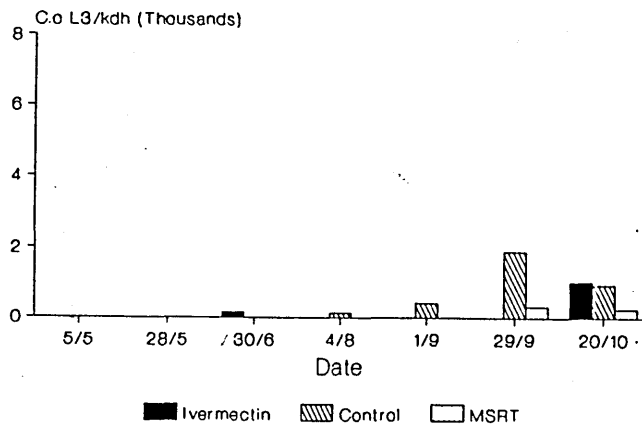
**FIGURE 3 GROUP MEAN FAECAL EGG COUNTS  
1988**



**FIGURE 4 O.ostertagi LARVAL RECOVERIES  
1988**



**FIGURE 5 C.oncophora LARVAL RECOVERIES  
1988**



grazing, Ostertagia L<sub>3</sub> were recovered from all three areas. Thereafter until Day 119 (1/9/88) low numbers of Ostertagia L<sub>3</sub> (<500 L<sub>3</sub>/kdh) were detected on the paddock grazed by the Group 1 (ivermectin) animals while in the Group 3 (MSRT) paddock Ostertagia L<sub>3</sub> were detected on two occasions when recoveries of 142 and 606 L<sub>3</sub>/kdh were noted. By the end of the trial period higher levels of Ostertagia L<sub>3</sub> were noted on these areas when recoveries of 1,795 and 1,220 L<sub>3</sub>/kdh respectively were recorded. In contrast the pattern of Ostertagia L<sub>3</sub> on herbage from the control area showed an increase from early August onwards reaching a maximum recovery of 7,073 L<sub>3</sub>/kdh at the time of housing. Ostertagia L<sub>3</sub> numbers were only significantly different ( $p < 0.05$ ) between the control and MSRT calf paddocks.

Cooperia L<sub>3</sub> recoveries are shown in Figure 5 and detailed in Appendix D. Very few Cooperia larvae were detected throughout the grazing season in any of the three paddocks. Higher maximum levels were found in the Group 2 (1,892 L<sub>3</sub>/kdh) and Group 1 (1,026 L<sub>3</sub>/kdh) grazing areas compared with a maximum recovery of 244 L<sub>3</sub>/kdh from the Group 3 paddock. No significant differences in Cooperia L<sub>3</sub> recoveries were noted between groups.

#### **Worm Burdens**

Tracer worm burden data are shown in Table 2 with the necropsy data from the principal calves being given in Table 3.

Low numbers of Ostertagia, ranging from 600 - 1,900, were recovered from the spring tracer calves and all worms present were adult. Cooperia burdens were also low and ranged from 0 - 2,800. These results contrasted with the maximum Ostertagia

Table 2  
Spring Tracer Worm Burdens - 1988

Paddock 1						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
T1A	1,000	0	1,000	0	0	0
T1B	700	0	700	0	0	0
Paddock 2						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
T2A	600	0	600	400	0	400
T2B	1,400	0	1,400	200	0	200
Paddock 3						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
T3A	800	0	800	0	0	0
T3B	1,900	0	1,900	1,400	1,400	2,800

Autumn Tracer Worm Burdens

Paddock 1						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
Y28	300	14,500	14,800	0	300	300
Y184	100	18,400	18,500	0	0	0
Paddock 2						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
B70	200	193,000	193,200	500	500	1,000
B73	700	97,000	97,700	0	0	0
Paddock 3						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
G4	100	17,500	17,600	0	100	100
G196	200	11,000	11,200	0	200	200

Table 3  
Principal Calf Worm Burdens - 1988

Animals necropsied at time of anthelmintic treatment (Day 119)

Paddock 1						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
Y52	0	0	0	700	200	900
Y53	5,200	400	5,600	1,000	400	1,400
Paddock 2						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
B82	59,000	34,800	93,800	4,000	0	4,000
B83	43,800	35,200	79,000	2,000	0	2,000
Paddock 3						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
G22	6,400	2,400	8,800	0	0	0
G23	3,000	400	3,400	0	0	0

At end of study

Paddock 1						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
Y49	700	9,000	9,700	500	0	500
Y51	0	1,400	1,400	2,800	0	2,800
Paddock 2						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
B79	200	414,000	414,200	6,400	500	6,900
B81	6,800	126,000	132,800	2,000	0	2,000
Paddock 3						
No.	Adult	<u>O. ostertagi</u>	Total	Adult	<u>C. oncophora</u>	Total
		L <sub>4</sub>			L <sub>4</sub>	
G19	4,100	13,000	17,100	2,100	0	2,100
G21	0	9,600	9,600	0	0	0

burdens of 18,500, 193,200 and 17,600 from the autumn tracers grazed on Paddocks 1, 2 and 3 respectively. Very low Cooperia burdens were again recorded in all of the tracer calves. The majority of both Ostertagia and Cooperia recovered from the autumn tracer calves were at the arrested fourth larval stage.

In the principal calves necropsied at the time of therapeutic anthelmintic treatment large numbers of Ostertagia spp were present in the Group 2 control cattle (maximum 93,800) compared with relatively low worm burdens in the treated Groups 1 and 3 (maximum 5,600 and 8,800 respectively). The population structure at this time showed an over 40% incidence of inhibition. Low to moderate numbers of Cooperia spp were present only in the animals of Groups 1 and 2: no Cooperia spp were recovered from the bolused calves of Group 3.

At the end of the first grazing period in October very high control burdens of Ostertagia spp were observed in the animals of Group 2 with a maximum of 414,200 being recorded compared with low to moderate recoveries of Ostertagia from the calves of Groups 1 and 3 (maximum burdens of 9,700 and 17,100 respectively). At this time up to 99% of the Ostertagia population was at the arrested fourth larval stage. Low to moderate numbers of Cooperia were again present in most of the calves.

## YEAR 2 - 1989

### Clinical

No clinical signs were observed in any of the animals

throughout the second grazing season.

Group mean bodyweights are illustrated in Figure 6 and detailed in Appendix E. From initial mean bodyweights of 285, 300 and 286 kg for Groups 1, 2 and 3 mean weight gains of 125, 136 and 133 kg respectively were recorded at the end of the grazing period. No significant difference in liveweight gains was recorded between groups.

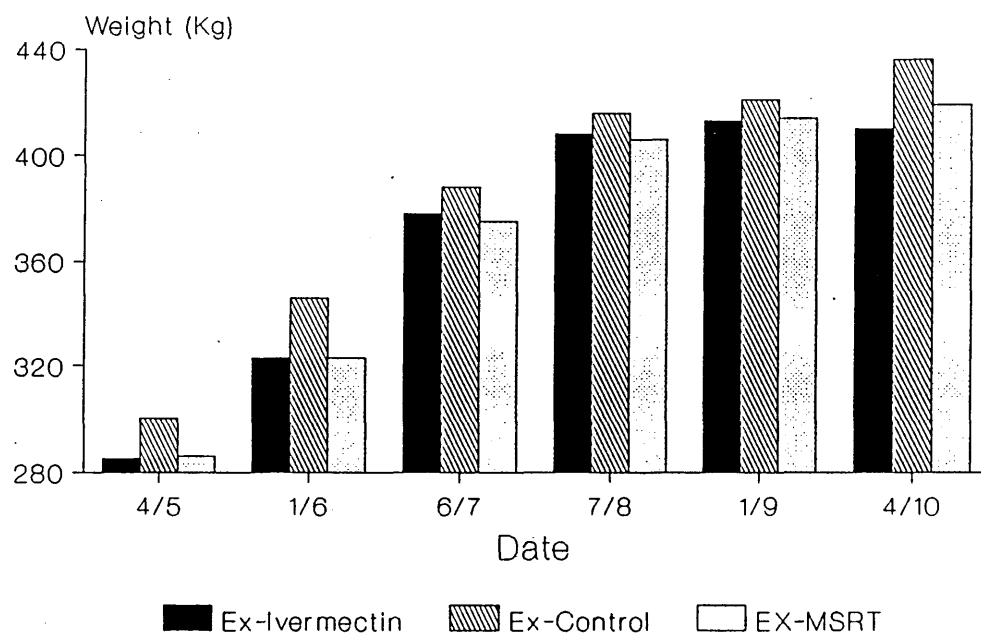
#### Biochemical

Figure 7 illustrates the mean monthly pepsinogen levels with this data being detailed in Appendix F. At the start of grazing low pepsinogen levels were recorded from the animals of both previously treated groups (763 mU - Group 1 and 894 mU - Group 3) compared with a relatively high figure of 2,138 mU from the ex-control cattle. The rise in pepsinogen levels seen in all animals in the first month of grazing continued in those of the Group 1 and Group 3 cattle but levelled off in the ex-controls. Final values of 1,960, 2,196 and 2,289 mU Tyrosine were recorded from Groups 1, 2 and 3 respectively. The difference in pepsinogen levels between the ex-control cattle and those of both the ivermectin and MSRT treated calves was significant ( $p < 0.05$ ).

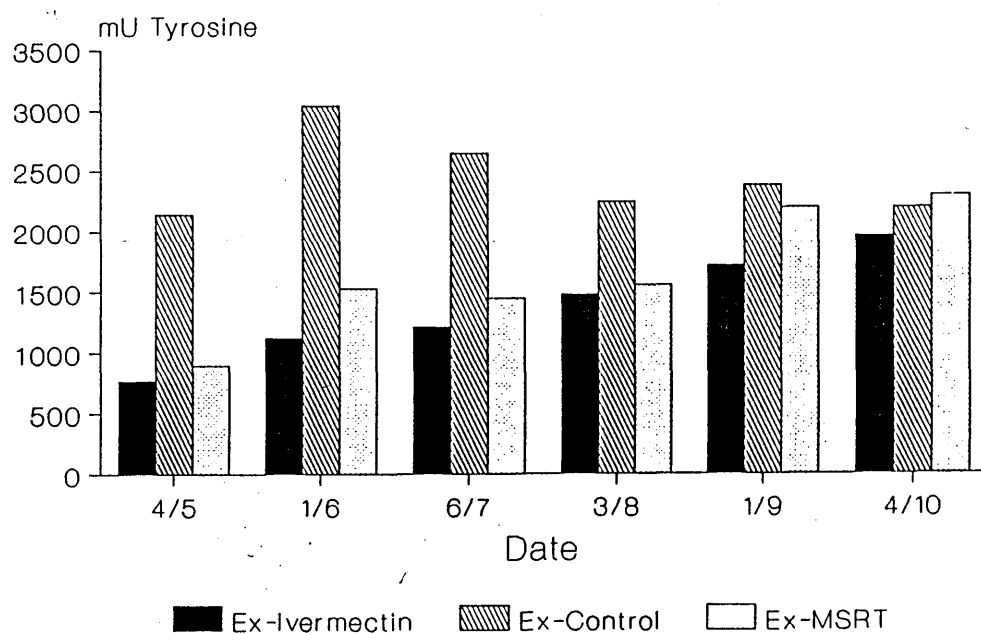
#### Faecal Egg Counts

Group mean faecal egg counts are shown in Figure 8 and given in Appendix G. Very low egg counts were observed throughout the grazing period with mean maximum values of 117, 50 and 63 epg being noted for Groups 1, 2 and 3 respectively with no significant differences being observed.

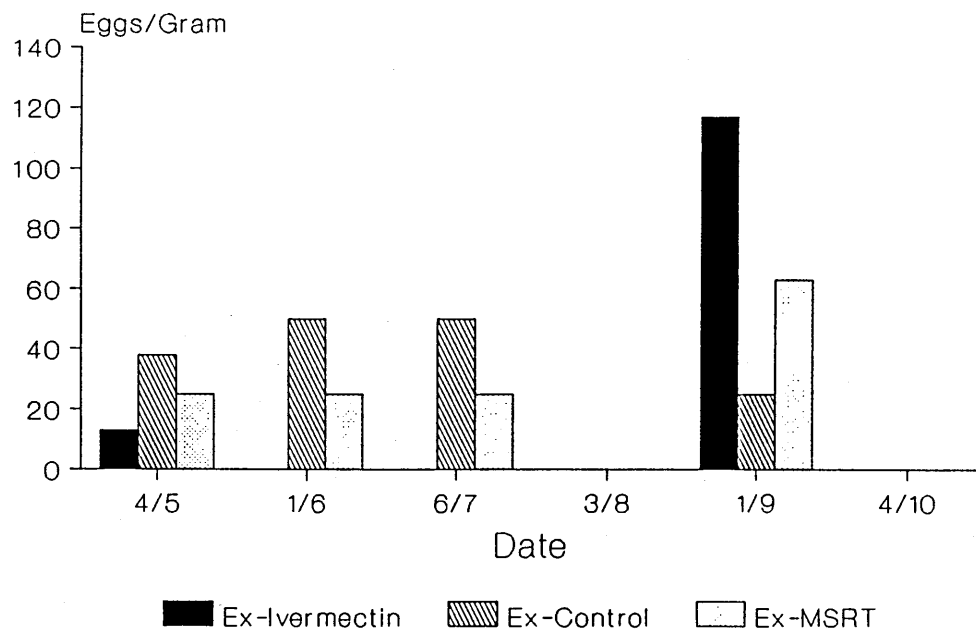
**FIGURE 6 GROUP MEAN BODYWEIGHTS  
1989**



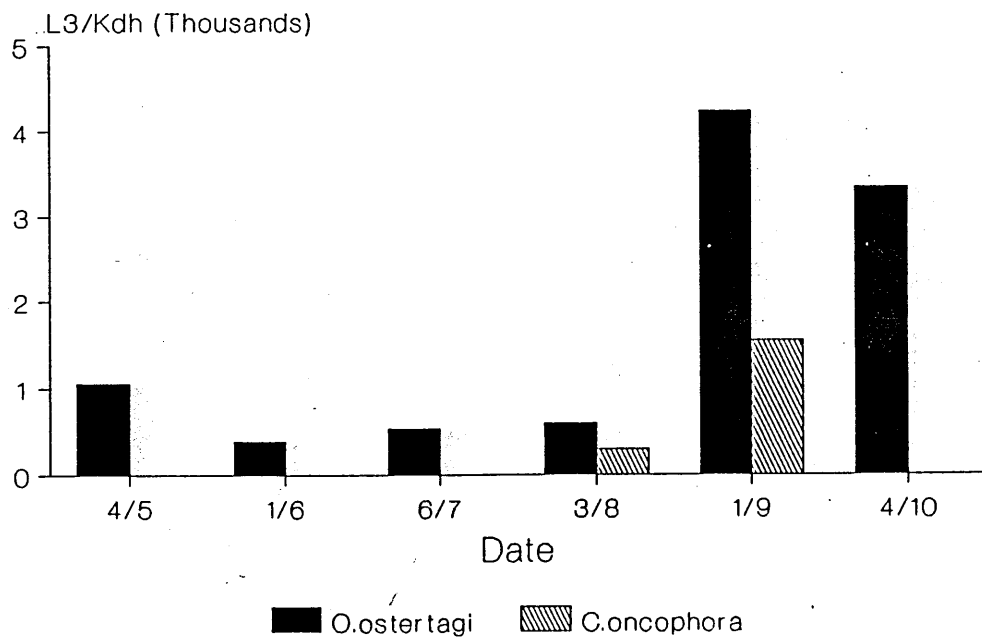
**FIGURE 7 GROUP MEAN PEPSINOGEN LEVELS  
1989**



**FIGURE 8 GROUP MEAN FAECAL EGG COUNTS  
1989**



**FIGURE 9 O.ostertagi and C.oncophora  
LARVAL RECOVERIES 1989**





### Pasture Larval Counts

Recoveries of Ostertagia and Cooperia infective third stage larvae are shown in Figure 9 and recorded in Appendix H. Ostertagia L<sub>3</sub> levels ranged from 385 to 4,230 L<sub>3</sub>/kdh while Cooperia L<sub>3</sub> were only detected twice, i.e. in August and September (maximum 1,438 L<sub>3</sub>/kdh).

### Worm Burdens

Tracer worm burdens are given in Table 4. Very low worm burdens were detected in the spring tracer calves with a maximum of 700 Ostertagia and 300 Cooperia being found. A much higher recovery of Ostertagia was obtained from the autumn tracer animals with a maximum of 15,200 being recorded. No Cooperia species were recovered from the autumn tracers.

Table 5 details worm burdens of the principal animals. In these high Ostertagia burdens were present in the animals which had been treated with ivermectin or given an MSRT bolus in their first grazing season with mean total recoveries of 230,100 and 156,950 being recorded. This compared with a group mean recovery of 39,875 from the ex-control yearlings. In each group 96 - 98% of the parasites present were at the inhibited fourth larval stage. No other parasite genera were present.

### CONCLUSIONS

One of the interesting features of this study was the source of the infective larvae responsible for the outbreak of PGE in 1987. This was unexpected considering there had been no previous history of helminthiasis on this area, however there are several

Table 4

## Tracer Worm Burdens - 1989

## Spring Tracer Worm Burdens

No.	Adult	<u>O. ostertagi</u>		Total	Adult	<u>C. oncophora</u>		Total
			<u>L<sub>4</sub></u>				<u>L<sub>4</sub></u>	
G45	400		100	500	300		0	300
G46	300		400	700	300		0	300

## Autumn Tracer Worm Burdens

No.	Adult	<u>O. ostertagi</u>		Total	Adult	<u>C. oncophora</u>		Total
			<u>L<sub>4</sub></u>				<u>L<sub>4</sub></u>	
Y160	5,200		8,800	14,000	0		0	0
Y161	6,000		9,200	15,200	0		0	0

Table 5  
Principal Worm Burdens - 1989

<hr/>			
Ex-Ivermectin		<u>O. ostertagi</u>	
No.	Adults	EL <sub>4</sub>	Total
<hr/>			
Y44	11,000	668,400	679,400
Y46	4,900	82,300	87,200
Y48	2,800	41,600	44,400
Y58	0	109,400	109,400
<hr/>			
Mean	4,675	225,425	230,100
SD	4,044	256,888	260,453
<hr/>			
Ex-Control			
No.	Adults	EL <sub>4</sub>	Total
<hr/>			
B72	3,200	34,800	38,000
B75	0	22,300	22,300
B76	1,800	69,400	71,200
B78	0	18,000	18,000
<hr/>			
Mean	1,250	38,625	39,875
SD	1,344	20,178	20,900
<hr/>			
Ex-MSRT			
G5	1,600	109,600	111,200
G15	1,300	256,200	257,500
G16	8,600	161,000	169,600
G27	800	88,700	89,500
<hr/>			
Mean	3,075	153,875	156,950
SD	3,203	64,671	65,025
<hr/>			

**Anthelmintic Prophylaxis Study**  
**Statistical analysis of principal worm burdens - 1989**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 16-Jul-91

Experiment title..... Principal O.o burdens 1989  
 Number of treatments... 3

	Description:	No. of obsvs.
Treatment 1	Ex-ivermectin	4
Treatment 2	Ex-control	4
Treatment 3	Ex-MSRT	4

Replicate	Treatments 1	2	3
1	679400.00	38000.00	111200.00
2	87200.00	22300.00	257500.00
3	44400.00	71200.00	169600.00
4	109400.00	18000.00	89500.00

**ONE WAY ANALYSIS OF VARIANCE :**

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	1.06	2.00	0.53	4.30
Experimental error	1.11	9.00	0.12	
Total	2.16	11.00		

Critical value for a 5% significance test is  $F_{.95(2,9)} = 4.26$

Since the calculated F-value is greater than or equal to the critical F-value, then the data suggests that treatment effects are different.

**NEWMAN KEULS MULTIPLE RANGE TEST :**

K	Means	Ranges	L.S.R.	Treatment	St. Error
	4.51			2	
2	5.11	3.20	0.56	1	0.175
3	5.16	3.95	0.69	3	

Treatment Comparison	Significantly Different	GEOMETRIC Treat.MEANS
3 V 2	No	1 130245.31
3 V 1	No	2 32282.12
1 V 2	Yes	3 144388.62

reports of parasitism on areas with a supposedly low level of pasture infectivity (Bairden et al, 1979; Bairden, Armour and McWilliam, 1985).

Prior to the beginning of the study pasture larval levels were low and by turnout larvae had virtually disappeared from the herbage samples. This together with the low worm burdens of the spring tracer calves indicated a small initial challenge. Despite this, significant faecal egg counts were consistently found in the control animals and the consequent contamination led to a marked increase in infective larvae on pasture in the autumn. Although positive faecal egg counts were recorded from both the ivermectin and MSRT treated groups of cattle from mid-season onwards, pasture larval levels on their paddocks did not rise markedly until the end of September.

The pepsinogen levels mirrored the infection pattern as indicated by faecal egg output and herbage larval counts. Also it was obvious from the fall in both pepsinogen values and faecal egg output at the beginning of September that the therapeutic anthelmintic treatment of the control cattle had been successful. The high level of pasture infectivity during the latter part of the grazing season was reflected in the high worm burdens of the principal control animals necropsied in October. The lack of herbage at this time possibly exacerbated the situation by forcing the calves to graze closer to faecal pats around which there would be a concentration of infective larvae.

In conclusion it was shown that, in terms of increased weight gains, lower levels of pasture contamination and reduced

worm burdens in first year grazing calves, the MSRT Flex Bolus and prophylactic treatments with ivermectin at 3, 8 and 13 weeks post-treatment were equally effective. However, the very high numbers of Ostertagia spp inhibited fourth stage larvae in both the previously treated MSRT and ivermectin animals which were grazed for a second season suggested that such treatment may have interfered with the development of immunity to gastrointestinal parasites.

## **CHAPTER TWO**

### **A STUDY ON THE CONTROL OF BOVINE PARASITIC GASTROENTERITIS BY PASTURE MANAGEMENT WITH PARTICULAR REFERENCE TO THE ALTERNATE GRAZING OF CATTLE AND SHEEP**

## INTRODUCTION

Grazing management systems to control bovine parasitic gastroenteritis, often referred to as integrated or clean grazing systems, have been widely advocated and include mixed grazing, alternate grazing and rotational grazing practices. In essence, two or more classes of host grazing together, alternately or in rotation form the basis of control. These may be of the same or different species with, in the latter case a cattle/sheep combination being the most common. When the same host species is involved then animals of different ages and immune status are used e.g. cows and calves.

Several factors influence the success of integrated grazing systems and these include host specificity of the parasite and the duration of pasture resting, or alternate use, in relation to the persistence of parasite contamination. For example, studies using sheep (Southcott, Major and Barger, 1976) and sheep and cattle (Donald, Morely, Waller, Axelsen and Donnelly, 1978) show that infective larvae ( $L_3$ ) of gastrointestinal nematodes can survive on pastures for six months or more, the precise period being very much time and weather dependent. Other factors such as the continuing presence of  $L_3$  in faeces and soil can also influence the duration of this critical period (Kauzal, 1941; Gibson and Everett, 1967; 1972; Callinan, 1978a, 1979). The species of parasite is also important in determining the optimal resting period before a pasture can be classified as "safe". In temperate regions of Australia, for example, a three month period has been shown to be sufficient to significantly reduce the



levels of Trichostrongylus, but not Ostertagia, of sheep (Donald et al, 1978). Also species of some genera e.g. Ostertagia, show little or no cross infectivity between cattle and sheep compared with others e.g. some species of Cooperia and Trichostrongylus which can reproduce successfully in both hosts (Roberts, 1942; Porter, 1953; Rose, 1968). For example T. axei is almost equally infective for sheep and cattle and integrated grazing systems are therefore of little use in its control. Indeed this parasite may show enhanced infectivity in such situations (Kates and Turner, 1960) although an experimental study demonstrated a reduction in infectivity of T. axei for calves after several passages in sheep over a period of almost four years (Ross and Purcell, 1969). With the exception of T. axei therefore, it would seem that cross-infection of parasite species is not an important drawback to integrated grazing systems involving cattle and sheep.

Rotational grazing systems involving only cattle have been subjected to a number of criticisms and in some cases have proved to be less effective than set-stocking of cattle on pasture (Levine and Clark, 1961; Michel, 1969). It is suggested that in the set-stocked situation the sparser nature of the herbage results in the larval stages being denuded of cover and, being more exposed, liable to succumb more readily to the effects of adverse environmental conditions. In contrast the more luxuriant herbage cover in rotational systems affords protection and encourages larval survival. Two rotational systems are currently

recognised. In the first, young calves graze ahead of a similar number of older animals (Leaver, 1970) while in the second, sometimes known as the Ruakura system after its New Zealand origin (McKeekan, 1947), a smaller number of susceptible calves (approximately 1/20th of the grazing component) are grazed ahead of a group of adult cows (Michel, 1972). It has been suggested that the success of the latter method was due not only to the greater dilution of larvae on herbage but also in part to a more efficient utilisation of pasture.

Good control has also been achieved by methods which involve either grazing sheep and cattle together (Arundel and Hamilton, 1975) or alternate grazing with these two host species (Southcott and Barger, 1975; Rutter, 1975). Where suitable grazing land is at a premium the mixed system is favoured and indeed forms an integral part of many small farming units. It is, however, worth noting that the control obtained by Arundel and Hamilton (1975) was not achieved by mixed grazing alone as during the course of the study both the sheep and cattle were dosed with levamisole on several occasions.

In 1982 a study was initiated to assess the effects of an annual alternation of cattle and sheep on faecal egg output, subsequent pasture larval contamination and acquired worm burdens.

#### **EXPERIMENTAL DESIGN**

The experimental design is summarised in Table 6. The paddocks used in this study were located at the Faculty of

**Table 6. Experimental Design**

<b>HOST SPECIES</b>				
	<b>Paddock 1</b>	<b>Paddock 2</b>	<b>Paddock 3</b>	<b>Paddock 4</b>
<b>1982</b>	<b>Calves</b>	<b>Calves</b>	<b>Sheep</b>	<b>Sheep</b>
<b>1983</b>	<b>Calves</b>	<b>Sheep</b>	<b>Calves</b>	<b>Sheep</b>
<b>1984</b>	<b>Calves</b>	<b>Calves</b>	<b>Sheep</b>	<b>Sheep</b>
<b>1985</b>	<b>Calves</b>	<b>Sheep</b>	<b>Calves</b>	<b>Sheep</b>

Veterinary Medicine at Glasgow University and had been variously set-stocked with either cattle or sheep for a number of years previously. Throughout this period information on herbage larval populations had been accumulated.

Four paddocks, each of approximately 0.33 hectares, and bounded by either woods or non-stocked land were used and treated as follows:

- |           |  |
|-----------|--|
| Paddock 1 | Permanent cattle plot grazed by calves in all four years                           |
| Paddock 2 | Cattle/sheep plot grazed by cattle in 1982 and then alternated annually with sheep |
| Paddock 3 | Sheep/cattle plot grazed by sheep in 1982 and then alternated annually with cattle |
| Paddock 4 | Permanent sheep plot grazed by ewes and lambs in all four years                    |

The calves were set stocked from early May to late September/October, depending on weather conditions, while the sheep were usually turned out soon after lambing in late April. In the sheep paddocks the ewes and lambs were together until weaning in mid-July when the adults were removed.

Routine management practices such as castration and dehorning of calves were carried out prior to turnout, while the lambs were docked and castrated with rubber rings as soon after birth as possible. The ewes were dosed with fenbendazole at 7.5 mg/kg at turnout to minimise their contribution to pasture contamination.

The animals were observed daily, faecal and herbage samples

collected weekly and at fortnightly intervals blood samples were taken.

Where necessary supplementary feeding was given in the form of hay and/or concentrates and the paddocks were fertilised immediately prior to and mid-way through the grazing season.

## RESULTS

The results are presented mainly in relation to the bovine host although in the case of Ostertagia and Cooperia pasture larval recoveries, data from all four grazing areas are given.

### Serum Pepsinogen Levels 1982 - 1985

Group mean serum pepsinogen levels are presented in Figures 10a - 10c with individual data being given in Appendix I.

#### Paddock 1 - Calves on permanent cattle grazing (Figure 10a)

With the exception of 1984, when a mid-season drop in pepsinogen levels occurred, values in all four years followed the pattern seen in naturally infected calves with normal levels (mean range 693 - 1,349 mU Tyrosine) at the beginning of each grazing season rising to a mean peak range of 3,928 - 4,759 mU Tyrosine at housing.

#### Paddock 2 - Calves on alternate cattle and sheep grazing (Figure 10b)

In the first year (1982) from an initial level of 598 mU Tyrosine, and despite therapeutic anthelmintic treatment being given at Weeks 6 and 14, a very high mean maximum level of 6,602 mU Tyrosine was recorded in Week 20 from the calves grazing on Paddock 2. In the next year of stocking cattle on this area

Figure 10a. Group Mean Pepsinogen Levels  
Paddock 1 - Permanent Cattle Grazing

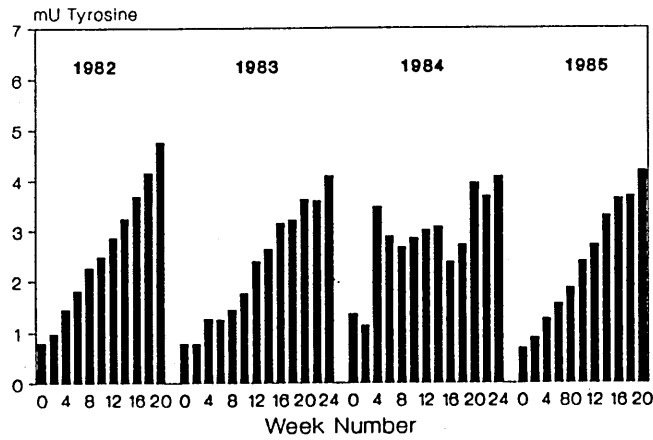


Figure 10b. Group Mean Pepsinogen Levels  
Paddock 2 - Cattle/Sheep Grazing

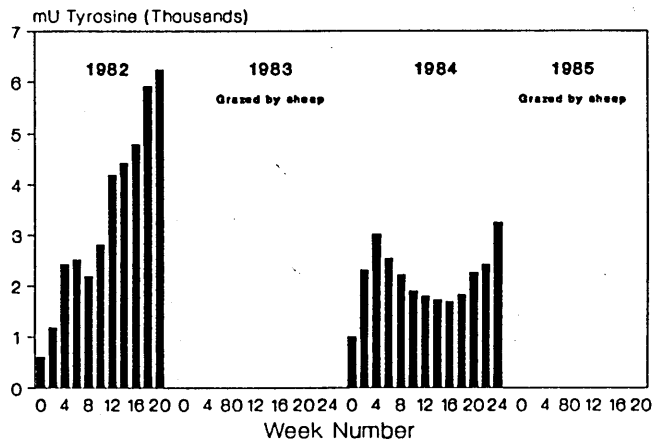
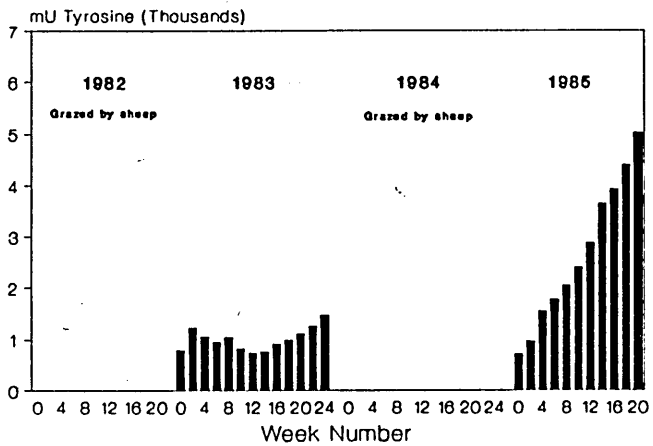


Figure 10c. Group Mean Pepsinogen Levels  
Paddock 3 - Sheep/Cattle Grazing



(1984) i.e. after it had been grazed by sheep in 1983, pepsinogen levels were relatively low with the mean maximum value of 3,245 mU Tyrosine being recorded at the end of the grazing period.

#### **Paddock 3 - Alternate sheep and cattle grazing (Figure 10c)**

In 1983, the year of the study during which calves were first grazed on this area, group mean pepsinogen levels were normal at the beginning of the season and remained so throughout the grazing period until housing when the mean maximum value of 1,464 mU Tyrosine, still within the normal range, was recorded. In the final year of the trial, the calves which grazed this area showed a sustained rise in pepsinogen levels with a group mean value of over 2,000 mU Tyrosine being recorded by Week 10. This trend continued until at housing a mean value of 4,924 mU Tyrosine was recorded. Statistically significant differences ( $p < 0.05$ ) were observed between the calves on the permanent compared with those on the alternate grazing in 1983 and 1984.

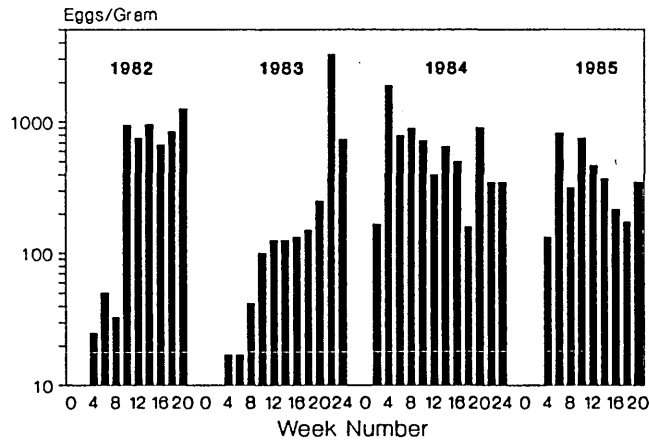
#### **Faecal Egg Counts 1982 - 1985**

Figures 11a - 11c illustrate group mean faecal egg counts while individual results are detailed in Appendix J.

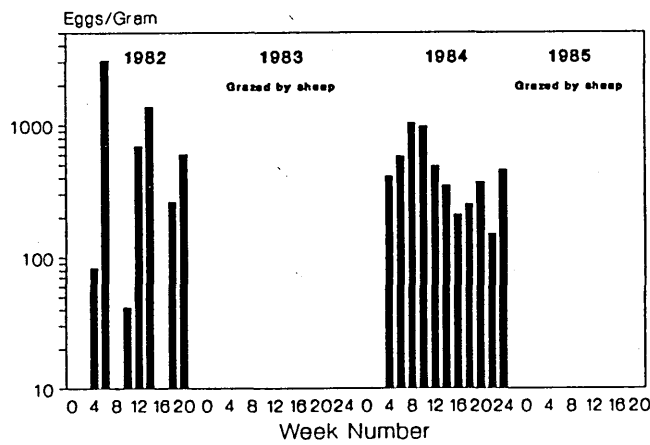
#### **Paddock 1 - Calves on permanent cattle grazing (Figure 11a)**

In 1982, the first year of the study, low numbers of eggs were detected in the set-stocked control calves from Weeks 4 to 8 with a rapid rise on Week 10 which was sustained and reached a mean maximum of 1,258 epg at the end of the grazing season in Week 20. A basically similar pattern with a more gradual rise in

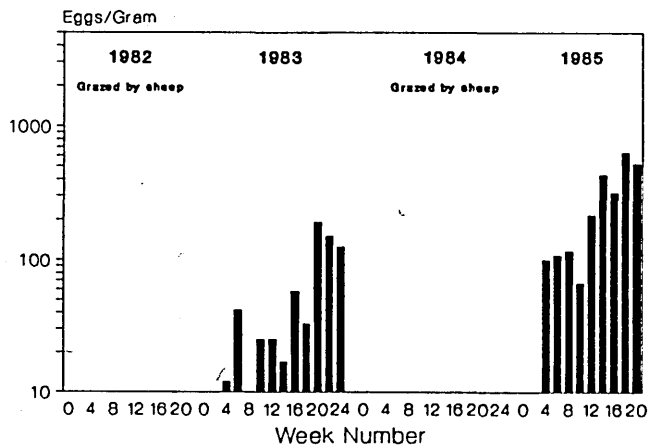
**Figure 11a. Group Mean Faecal Egg Counts**  
Paddock 1 - Permanent Cattle Grazing



**Figure 11b Group Mean Faecal Egg Counts**  
Paddock 2 - Cattle/Sheep Grazing



**Figure 11c Group Mean Faecal Egg Counts**  
Paddock 3 - Sheep/Cattle Grazing





egg output was observed from the calves grazing this area in 1983 although the maximum count was much higher at 3,267 epg in Week 22. In 1984, the peak count of 1,900 epg occurred in Week 4 and later mean egg counts, although substantial, did not rise above this level. Finally in 1985 moderate egg counts were recorded during most of the grazing period with a group mean maximum of 825 epg being observed in Week 6.

**Paddock 2 - Calves on alternate cattle and sheep grazing  
(Figure 11b)**

In 1982, with calves grazing on Paddock 2, a marked rise in egg output was evident in Week 6 with a group mean output of 3,075 epg being recorded. At this time a therapeutic anthelmintic treatment, using oxfendazole at 4.5 mgm/kg bodyweight, was considered necessary. Two weeks post-treatment no eggs were found in the faeces of this group but six weeks later in Week 14 there was a second rise in egg output to a mean of 1,380 epg when a further treatment with oxfendazole was given; a final mean count of 600 epg was recorded in Week 20. In 1984, when calves were again set-stocked in Paddock 2, a group mean faecal egg count of 408 epg was recorded in Week 4 rising to the group mean maximum of 1,033 epg in Week 8.

**Paddock 3 - Calves on alternate sheep and cattle grazing  
(Figure 11c)**

In year two (1983) egg counts from the calves grazing Paddock 3 i.e. that grazed previously by sheep, were markedly lower than those of the set-stocked control calves with a mean maximum of only 192 epg being seen in this group in Week 20.

Finally in 1985 the calves on Paddock 3 showed an early group mean of 100 epg (Week 4) which rose to a maximum of 750 epg in Week 18. There was no statistically significant difference in faecal egg counts from the calves on the permanent compared with those on the alternate grazing in any year.

#### Pasture Larval Counts 1982-1985

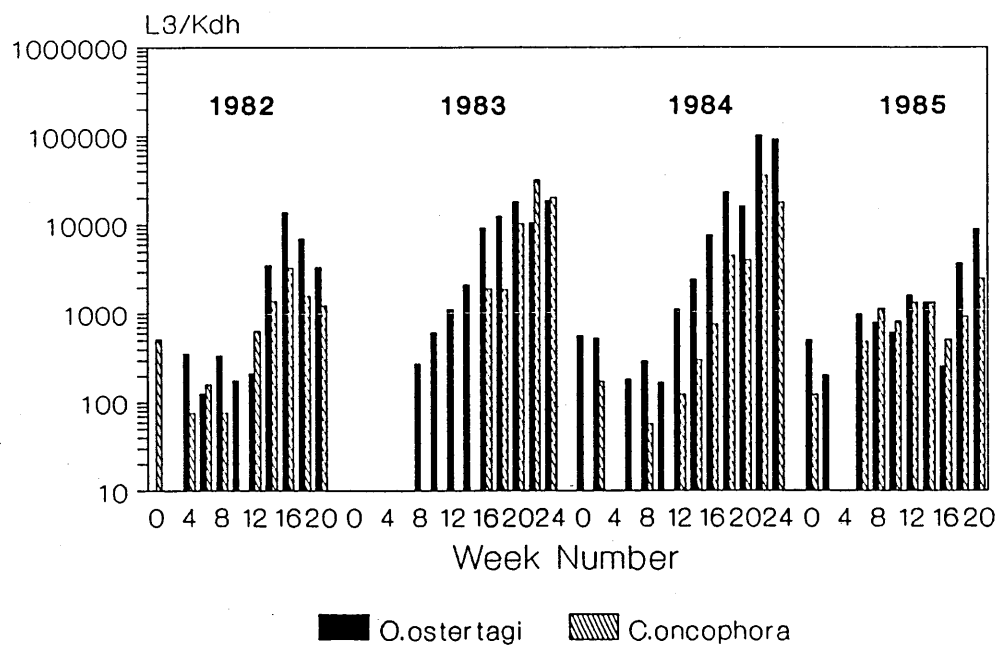
Ostertagia and Cooperia L<sub>3</sub> recoveries per kilogram dried herbage (L<sub>3</sub>/kdh) are shown in Figures 12a - 12d and detailed in Appendix K.

##### Paddock 1 - Permanent cattle grazing (Figure 12a)

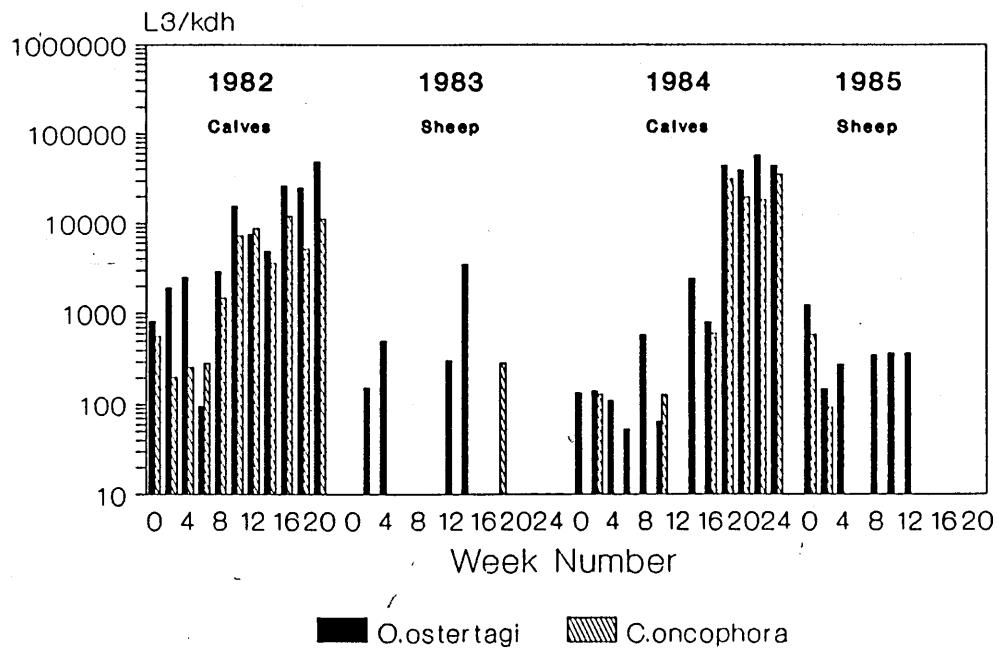
At the beginning of the study low numbers of Ostertagia L<sub>3</sub> (126 - 355 L<sub>3</sub>/kdh) were recovered from Weeks 4 to 12 after which a marked increase was observed reaching a maximum recovery of 13,664 in Week 16. In the second year Ostertagia L<sub>3</sub> were recovered from Week 8 onwards with high numbers being recorded at the end of the grazing period (maximum 18,592 L<sub>3</sub>/kdh). Even higher recoveries of L<sub>3</sub> were obtained during 1984 with the peak level of 102,222 occurring in Week 22. With the exception of Week 4 larvae were recovered from this area at every sampling. Finally in the last year of the study lower levels of Ostertagia larvae were recovered throughout the grazing period with a maximum of 8,896 L<sub>3</sub>/kdh in Week 20.

Cooperia larval recoveries were generally lower than Ostertagia and tended to increase towards the middle of the season. Peak recoveries of 3,282 (1982), 31,812 (1983), 35,555 (1984) and 2,542 (1985) were recorded.

**Figure 12a Pasture Larval Recoveries**  
**Paddock 1 - Permanent Cattle Grazing**



**Figure 12b Pasture Larval Recoveries**  
**Paddock 2 - Cattle/Sheep Grazing**



#### Paddock 2 - Alternate cattle and sheep grazing (Figure 12b)

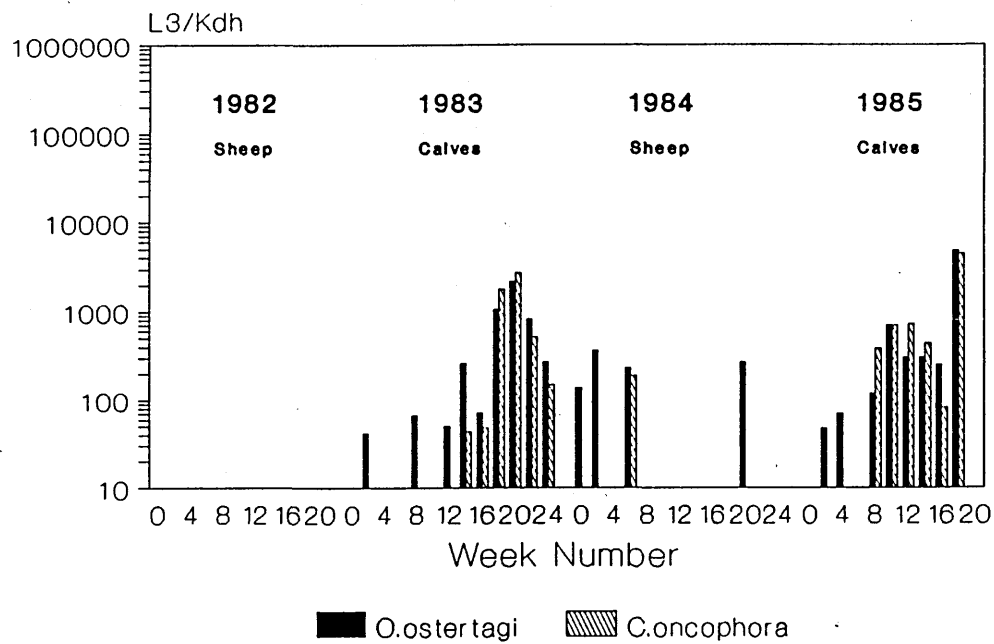
During the first year of the study (1982) when cattle were grazed on this area Ostertagia L<sub>3</sub> were consistently recovered, numbers ranging from 96 in Week 6 to 48,342 L<sub>3</sub>/kdh in Week 20. In the second grazing season of 1983, when the alternate ovine hosts were grazed, reduced numbers of Ostertagia L<sub>3</sub> were present with a maximum of only 3,487 L<sub>3</sub>/kdh in Week 14. In the following year (1984) when calves again grazed this area Ostertagia L<sub>3</sub> recoveries, although initially low (0 - 581 from Week 0 to Week 12), rose in the latter part of the season to 57,770 L<sub>3</sub>/kdh in Week 22. Finally in 1985 when sheep occupied Paddock 2 for the last year, Ostertagia L<sub>3</sub> numbers were very low with the highest recovery of 1,224 L<sub>3</sub>/kdh being recorded in the first week of grazing.

Cooperia L<sub>3</sub> were regularly detected in moderate to high numbers during the first year with a maximum recovery of 11,995 L<sub>3</sub>/kdh being noted in Week 16. In contrast larvae of this species were recovered only once during 1983 (290 L<sub>3</sub>/kdh, Week 18). In the last two years a high/low pattern was again repeated with maximum recoveries of 35,219 and 599 L<sub>3</sub>/kdh being recorded in 1984 and 1985 respectively.

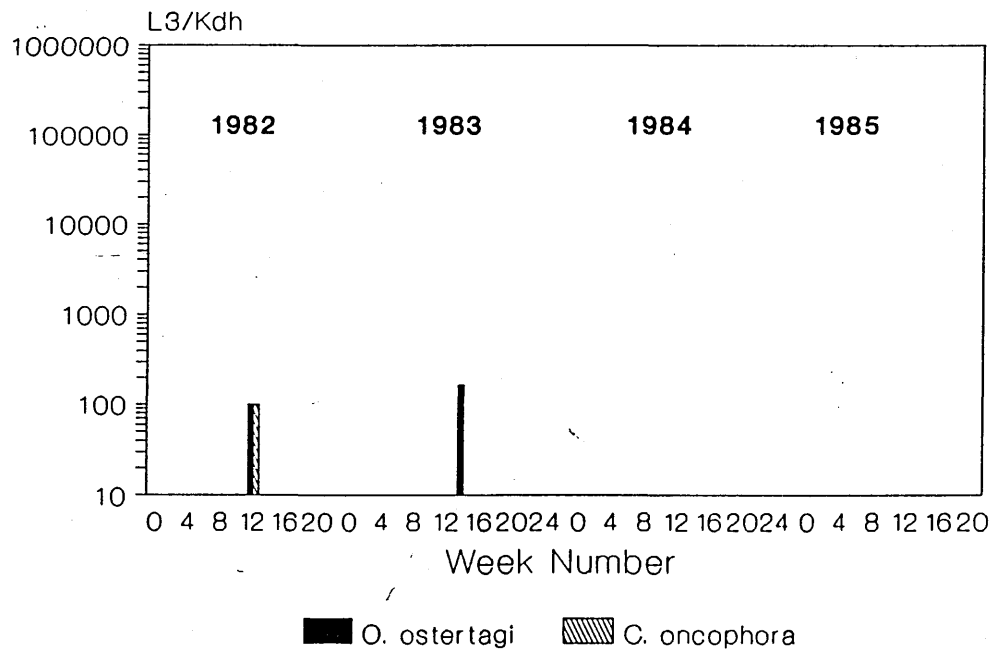
#### Paddock 3 - Alternate sheep and cattle grazing (Figure 12c)

During the first year of the study when set-stocked with sheep, no Ostertagia were recovered from this area. In 1983, when grazed by cattle, very low numbers of L<sub>3</sub> were present on the paddock until Week 20 when the maximum recovery of 2,203 L<sub>3</sub>/kdh was recorded. In the following year (1984) larvae were recovered

**Figure 12c Pasture Larval Recoveries**  
Paddock 3 - Sheep/Cattle Grazing



**Figure 12d Pasture Larval Recoveries**  
Paddock 4 - Permanent Sheep Grazing



on only a few occasions and although  $L_3$  numbers fluctuated they never rose above 270  $L_3$ /kdh during this sheep grazing period. In 1985 the highest recorded level of Ostertagia  $L_3$  in this plot over the four year period (4,872  $L_3$ /kdh) was observed in Week 18 during its final grazing by calves.

Overall negative or very low and irregular recoveries of Cooperia  $L_3$  were also obtained from this area with maximum recordings of 0 (1982), 2,763 (1983), 191 (1984) and 4,488 (1985) being noted.

A comparison of Ostertagia and Cooperia pasture larval recoveries show a statistical difference only in year 1 (1982) in which the designated alternate paddock, grazed by calves, had significantly higher levels ( $p < 0.05$ ) than that taken as permanent cattle grazing. When sheep grazed the alternated paddocks there were statistically fewer Ostertagia and Cooperia present in all four years.

#### Paddock 4 - Permanent sheep grazing (Figure 12d)

Ostertagia infective third stage larvae were only recovered from this area on two occasions i.e. Week 12 in 1982 (217  $L_3$ /kdh) and Week 14 in 1983 (166  $L_3$ /kdh) with Cooperia  $L_3$  being recovered once in Week 12 in 1982 (217  $L_3$ /kdh).

#### O. ostertagi and C. oncophora Worm Burdens 1982 - 1985

Table 7 details group mean worm burdens with individual data being given in Appendix L.

Table 7  
Group Mean Worm Burdens

Paddock 1 - Permanent Cattle Grazing

	Adult	<u>Ostertagia</u> L <sub>4</sub>	Total	Adult	<u>Cooperia</u> L <sub>4</sub>	Total
1982	46,850	17,300	64,150	1,933	583	2,517
1983	36,567	59,000	95,567	7,083	19,183	26,267
1984	8,850	38,950	47,800	2,700	8,700	11,400
1985	18,783	35,333	54,117	1,417	3,583	5,000

Paddock 2 - Alternate Cattle and Sheep Grazing

	Adult	<u>Ostertagia</u> L <sub>4</sub>	Total	Adult	<u>Cooperia</u> L <sub>4</sub>	Total
1982	54,817	66,317	121,133	11,283	6,817	18,100
1984	6,800	16,383	23,183	12,783	39,850	52,633

Paddock 3 - Alternate Sheep and Cattle Grazing

	Adult	<u>Ostertagia</u> L <sub>4</sub>	Total	Adult	<u>Cooperia</u> L <sub>4</sub>	Total
1983	9,250	19,200	28,450	4,950	13,633	18,583
1985	24,033	22,367	46,400	26,917	20,783	47,700

#### Paddock 1 - Calves on permanent cattle grazing

At the end of the first grazing season a group mean Ostertagia worm burden of 64,150 (range 23,600 - 84,000) and a group mean burden of 2,517 Cooperia (range 600 - 5,300) were recovered. After the second year group mean recoveries of 95,567 Ostertagia (range 41,700 - 137,800) and 26,267 Cooperia (range 2,400 - 63,800) were observed. In 1984 group means of 47,800 Ostertagia (range 14,200 - 89,900) and 11,400 Cooperia (range 400 - 42,800) were recorded and in the final year of the study the group mean worm burdens were 54,117 Ostertagia (range 19,800 - 83,300) and 5,000 Cooperia (range 1,200 - 12,900).

#### Paddock 2 - Calves on alternate cattle and sheep grazing

In 1982, at the end of the grazing period, high levels of Ostertagia were present at necropsy - group mean 121,133 (range 43,500 - 299,800) with a group mean of 18,100 for Cooperia (range 6,700 - 40,000). Two years later (1984) when cattle grazed this paddock again mean Ostertagia burdens of the calves were 23,183 (range 1,500 - 52,400) with a marked increase in the mean level of Cooperia to 52,633 (range 5,200 - 242,900). No significant difference in Ostertagia burdens were observed with Cooperia numbers being significantly higher ( $p < 0.05$ ) in 1982.

#### Paddock 3 - Calves on alternate sheep and cattle grazing

After being grazed by sheep the previous year, calves were first introduced into this area in 1983 and by the end of the season mean Ostertagia burdens of 28,450 (range 13,100 - 52,400) had been acquired plus a mean of 18,583 Cooperia (range 3,800 - 40,200). Following a further year's grazing by sheep in 1984 the



calves in Paddock 3 in 1985 acquired mean worm burdens of 46,400 Ostertagia (range 6,800 - 96,200) and 47,700 Cooperia (range 400 - 111,000). A significantly lower number of Ostertagia ( $p < 0.05$ ) were recovered from the calves in 1983 with no significant differences being noted for the Cooperia populations in either year.

### CONCLUSIONS

In the first year serum pepsinogen levels in the two groups of grazing calves were similar in pattern and magnitude. As both of these paddocks had been regularly grazed by cattle in previous years this was not unexpected. In the second grazing season pepsinogen levels of the calves which followed the sheep were normal throughout the grazing period compared with a progressive increase in pepsinogen concentration in the calves in the permanent cattle paddock. It thus appeared that despite the fact that this area had been grazed by cattle prior to the start of the study the use of sheep in the first year had markedly reduced the numbers of bovine Ostertagia L<sub>3</sub> on this paddock. In the third year of the study the pepsinogen levels of the calves on the alternated cattle/sheep paddock, although slightly higher than normal, were lower than those of the calves on the permanent cattle paddock. This indicated that a sufficient number of Ostertagia L<sub>3</sub> had survived the intervening sheep grazing period to establish a primary infection leading to the pasture contamination which eventually produced moderate worm burdens in the alternate bovine host. Finally in 1985 the calves on the

alternated area showed a pattern and level of pepsinogen values which was almost identical to those observed in the permanent calves indicating a similar level of Ostertagia infection.

Faecal egg count data from the set-stocked control calves in all four years of the study confirmed the presence of a parasite population from four weeks after turnout onwards. Marked increases in egg output in the first grazing season from the calves allocated to the alternate cattle/sheep paddock necessitated two therapeutic anthelmintic treatments which resulted in a sharp drop in egg output. In the second year of the experiment low faecal egg counts of the calves which followed the sheep on Paddock 3 suggested that the alternating strategy had been successful. That this was not sustained over the next two years of observation was clearly demonstrated by the fact that in 1985, similar egg counts were recorded from the calves on the alternated paddock compared with those of the animals on the permanent cattle grazing.

The magnitude of pasture larval numbers recovered from the four areas in 1982 reflected their grazing host. Thus in the control calf paddock and the alternate area grazed by calves in the first year, Ostertagia and Cooperia L<sub>3</sub> levels were consistent with those of a cattle grazing area while from the alternate area first used by sheep and from the set-stocked sheep paddock very few bovine L<sub>3</sub> were recovered. Thereafter in the remaining three years of the study typical seasonal recoveries of L<sub>3</sub> on herbage were noted from the permanent calf paddock. On the alternate

plots, with the exception of 1983, very few bovine L<sub>3</sub> were recovered during the sheep grazing periods. It is remarkable, with such a low demonstrable pasture infectivity in these intervening years, that in the following cattle grazing periods such high recoveries of bovine L<sub>3</sub> occurred.

End of season necropsy data showed that in 1982 both calf groups had amassed considerable Ostertagia worm burdens. Both the numbers and stages of these populations were of interest. Surprisingly the calves on the designated alternate paddock had twice the number of Ostertagia as their control counterparts with a higher level of Cooperia also being present in these animals. In the second year the worm burdens from the calves which had grazed the area previously used by sheep indicated a level of success for the control strategy with a 70% reduction in Ostertagia and a 30% reduction in Cooperia burdens being recorded. By the end of the third year Ostertagia burdens of almost 50% those of the control calves and a substantially higher number of Cooperia were recovered from the calves on the alternate area. In the last year of the study no significant difference in group mean Ostertagia burdens between the control and alternate calf groups was noted while the Cooperia burdens of the latter far outnumbered those of the calves on permanent cattle grazing. This gradual build up of Cooperia may, as in the case of T. axei, have been due to an enhanced infectivity caused by passage in the alternate sheep host.

It is clear from these results that, despite some early indication of success, at the end of the study the alternation of cattle and sheep over a four year period did not prevent the acquisition of substantial burdens of gastrointestinal nematodes by the calves grazed on the alternated pasture. The results of the other parameters measured, i.e. faecal egg and pasture larval counts plus plasma pepsinogen concentrations supported this conclusion.

### CHAPTER THREE

Nematodirus battus: A PATHOGEN OF CALVES?

## INTRODUCTION

Clinical nematodiriasis due to Nematodirus battus in 6 - 8 week old lambs during April - June is a well recognised entity. The clinical signs of a profuse watery diarrhoea with loss of condition are due to disruption of the proximal small intestinal mucosa principally by the developing larval stages (Thomas, 1959a). This genus is peculiar among trichostrongylids in that development to L<sub>3</sub> occurs within the egg and a long period of chill followed by warmth is necessary for hatching. These critical environmental requirements together with a rapid development of immunity following infection result in single annual seasonal increases in pasture larvae which if ingested by susceptible lambs may result in clinical disease (Thomas, 1959b). Thus the condition is transmitted from lamb crop to lamb crop yearly, ewes having a negligible role in outbreaks of disease.

In addition to prophylactic anthelmintic treatment during the period of highest risk, control can be achieved by systems of grazing management. One such integrated system, shown to be effective by Southcott and Barger (1975) involves grazing sheep and cattle alternately each year and relies for its success on the host specificity of many parasite species. However, Helle (1981) showed that calves could contaminate pasture with N. battus eggs while Coop, Jackson, Jackson, Fitzsimons and Lowman (1988) reported Nematodirus infection in lambs after one year's grazing with calves; in this case the alternate bovine host had passaged N. battus infection leading to contamination of pasture. Recently, clinical nematodiriasis in calves due to

N. battus infection has been described (Bairden and Armour, 1987; Armour et al, 1988) with diarrhoea and loss of condition in two five month old Friesian X calves one month after turnout on to experimental grazing. At necropsy jejunal enteritis was observed in association with burdens of 10,000 and 35,000 N. battus. In addition to clinical disease occurring on these experimental areas a further two calves exhibiting gross signs of gastrointestinal parasitism which were referred to Glasgow University Veterinary School in 1988 were found at necropsy to have moderate to severe burdens of N. battus (5,000 -22,000 worms).

The occurrence of these clinical cases of nematodiriasis apparently connected with a history of previous integrated grazing management suggested that it would be useful to define the parasitological and pathological effects of experimental N. battus infection in calves and the relationship between N. battus and other gastrointestinal nematode infections.

#### Experimental Design

The experimental work comprised three phases:

(1) A Field Study. The calves used in this study formed the control group of a larger anthelmintic efficacy trial (Experiment 3 in report by Bairden and Armour, 1987) and were divided into two replicate groups each of five animals. The experimental design is shown in Table 8. The calves were grazed from May to October on areas known to be contaminated with N. battus infective larvae. Fortnightly faecal and herbage

**Table 8. Experimental Design - Field Study**

	GRAZED BY 5 CALVES MAY - OCTOBER	GRAZED BY 5 CALVES MAY - OCTOBER	
--	---	---	--

**2 Tracer calves grazed in each paddock for 2 weeks  
in May and October**



samples were monitored throughout the grazing period for evidence of nematodiriasis and the animals were necropsied 6-8 days post housing. To augment pasture sampling data two tracer calves were introduced into each paddock at the beginning and end of the study. These animals were allowed to graze for two weeks then housed and necropsied two weeks later.

(2) A Dose Titration Study to determine the dose of N. battus L<sub>3</sub> required to give a clinical response. The experimental design is shown in Table 9. Fifteen calves (five groups of three) were infected with a single dose of 5,000, 10,000, 15,000 or 20,000 N. battus infective larvae on Day 1. A group of uninfected control calves was included to provide a baseline for bodyweight and pepsinogen data. When patency was first detected in the infected calves the animals were necropsied and their worm burdens established. Weekly bodyweights, plasma pepsinogen levels and faecal egg counts were recorded and a full histopathological examination carried out at necropsy.

(3) A Combined Infection Study on the effects of N. battus in combination with two of the other common nematode parasites of cattle, namely Ostertagia ostertagi and Cooperia oncophora. The experimental design is shown in Table 10. Twelve calves (three groups of four) were infected with monospecific inocula of O. ostertagi, C. oncophora or N. battus L<sub>3</sub> while a further three groups of four calves were challenged with a combination of O. ostertagi and N. battus, C. oncophora and N. battus or O. ostertagi, C. oncophora and N. battus. The animals were

Table 9

## Experimental Design: Dose Titration Study

Group	No. of Animals	Day 1 <u>N.b.</u> L <sub>3</sub>	Day 33
1	3	5,000	Necropsy
2	3	10,000	Necropsy
3	3	15,000	Necropsy
4	3	20,000	Necropsy
5	3	0	-

Table 10

## Experimental Design: Combined Infection Study

Group	No. of Animals	Day 1	Day 33
1	4	20,000 <u>O.o.</u> L <sub>3</sub>	Necropsy
2	4	20,000 <u>C.o.</u> L <sub>3</sub>	Necropsy
3	4	20,000 <u>N.b.</u> L <sub>3</sub>	Necropsy
4	4	20,000 <u>O.o.</u> + <u>N.b.</u> L <sub>3</sub>	Necropsy
5	4	20,000 <u>C.o.</u> + <u>N.b.</u> L <sub>3</sub>	Necropsy
6	4	20,000 <u>O.o.</u> + <u>C.o.</u> + <u>N.b.</u> L <sub>3</sub>	Necropsy
7	4	0	-

N.b. = N. battus  
O.o. = O. ostertagi  
C.o. = C. oncophora

necropsied shortly after patency (Day 33) to determine their parasitological and pathological status. A further group of four calves was included as uninfected controls.

## RESULTS

### 1. Field Study

#### Clinical

Herbage growth was poor throughout the first half of the grazing season and, to ease grazing pressure, two animals (Nos. W45, R53), one from each paddock, were removed and necropsied on Day 88 (3/8). A further calf (No. R52) was removed and necropsied two weeks later on Day 103 (18/8) due to loss of condition. As both groups showed a further deterioration in condition it was decided to treat the remaining calves therapeutically with thiabendazole (Thibenzole, Merck, Sharp and Dohme) which was given on Day 115 (30/8). In spite of this treatment a second calf, which failed to respond (No. W47) was removed and necropsied on Day 156 (10/10).

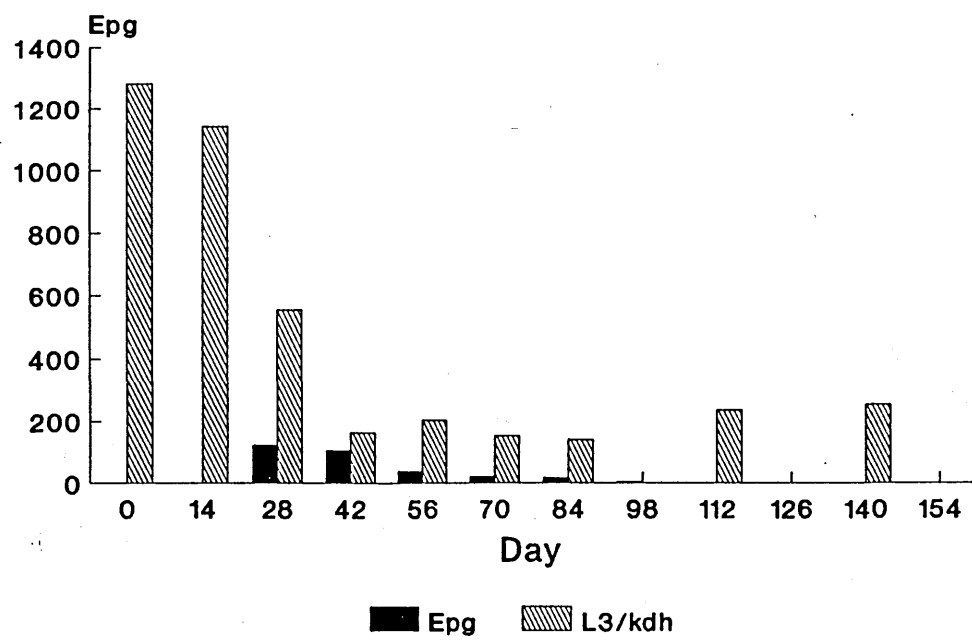
#### Faecal Examination

Mean faecal egg counts are shown in Figure 13 and detailed in Appendix M. By Day 28 (4/6) several of the calves were passing N. battus eggs (mean 120 epg) and continued to do so at a low level until Day 98 (13/8).

#### Pasture Larval Recoveries

Larval recoveries are also shown in Figure 13 and detailed in Appendix N. N. battus infective L<sub>3</sub> were detected early in the grazing season with the maximum recovery of 1,280 L<sub>3</sub>/kdh being

Figure 13. Mean *N. battus* Faecal  
Egg Counts Plus L3/kg Dried Herbage



recorded on Day 0 (7/5). With only three exceptions in the second half of the grazing period, N. battus L<sub>3</sub> were present at each sampling with recoveries ranging from 140 to 1,140 L<sub>3</sub>/kdh.

#### **Worm Burdens**

Tracer worm burdens are given in Table 11 with those of the principal animals detailed in Table 12. Spring tracer burdens ranged from 0 to 88,600 with a recovery range of 1,600 to 8,300 being found in the autumn tracer calves. No N. battus parasites were recovered from any of the principal calves at necropsy at the end of the experimental period while the two calves necropsied earlier on Day 88 had total N. battus burdens of 400 and 1,400.

## **2. Dose Titration Study**

### **Clinical**

Group mean bodyweights, shown in Figure 14, are detailed in Appendix O. Group mean weight gains of between 20 kg and 30 kg were observed over the experimental period. No significant weight differences were observed between groups.

### **Plasma Pepsinogen Levels**

Group mean plasma pepsinogen levels, shown in Figure 15, are detailed in Appendix P. No significant differences were obtained in pepsinogen values between groups with all animals showing normal levels during the experimental period.

### **Faecal Egg Counts**

Faecal egg count data is presented in Appendix Q. With the exception of animal number R34 in Group 2 which had an egg count of less than 50 epg on Day 28 no positive faecal egg counts were

Table 11

Tracer Calf N. battus Worm Burdens

Spring Tracers				
Plot	No.	Adults	L <sub>4</sub>	Total
R1	Y126	0	0	0
R1	Y52	85,400	3,200	88,600
R2	Y98	29,800	0	29,800
R2	Y656	14,800	0	14,800
Autumn Tracers				
Plot	No.	Adults	L <sub>4</sub>	Total
R1	Y193	8,300	0	8,300
R1	Y194	1,700	0	1,700
R2	Y195	1,600	0	1,600
R2	Y196	7,600	0	7,600

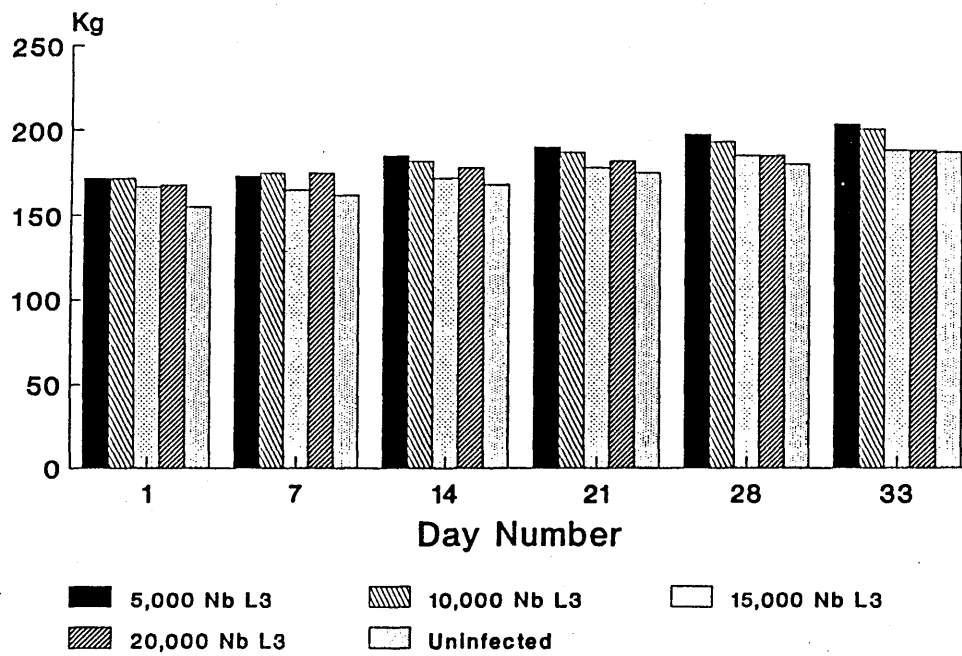
Table 12

Principal Calf N. battus Worm Burdens

Plot	No.	Adults	L <sub>4</sub>	Total
R1	W43	0	0	0
	W44	0	0	0
	* W45	1,400	0	1,400
	W46	0	0	0
	W47	0	0	0
	W48	0	0	0
	Mean	233	0	233
	SD	572	0	572
Plot	No.	Adults	L <sub>4</sub>	Total
R2	R49	0	0	0
	R50	0	0	0
	R51	0	0	0
	R52	0	0	0
	* R53	400	0	400
	R54	0	0	0
	Mean	67	0	67
	SD	163	0	163

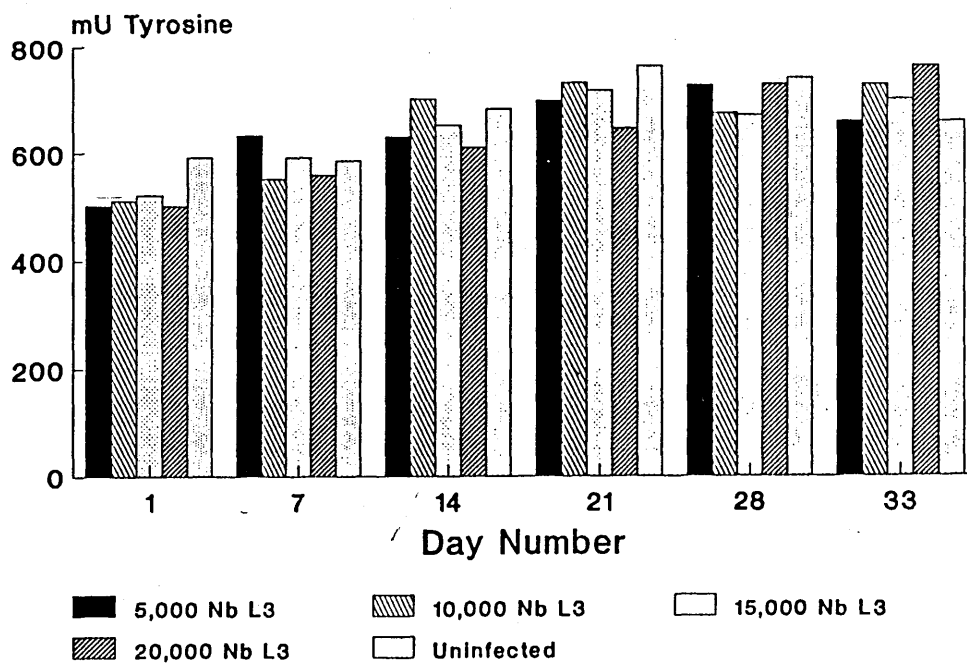
\* Necropsied Day 88 (3/8)

Figure 14 Group Mean Bodyweights



Nb = N.battus

Figure 15. Group Mean Pepsinogen Levels



Nb = N.battus

observed.

#### Post-mortem Worm Burdens

The worm burdens in the three sections of the small intestine for each animal are shown in Table 13. Most worms were recovered from the proximal region. Low numbers of worms were recovered from only one animal in Group 1 compared with two animals in Group 2 while all three calves of Groups 3 and 4 were positive. The highest worm recoveries were from the Group 3 calves (mean 333). No statistical differences were observed between groups.

#### Gross and Histopathological Lesions

Gross lesions were observed along the small intestine. These consisted of 10-15 cm long and 2-3 cm wide areas which were raised 2-3 mm above the surface. They were lightly mottled and contained circular nodules about 3-4 mm in diameter which were embedded in the lesion. The nodules had umbilicated centres some of which were dark and some green in colour. When examined microscopically these nodules were observed to contain both larval and adult stages of N. battus. In addition there were isolated nodules with no reaction around them scattered throughout the small intestine.

Histopathologically an increasing degree of damage was observed with increasing doses of larvae. This was found mainly in the jejunum but also involved the duodenum and ileum at higher doses. The reaction consisted of infiltration of the lamina propria by lymphoid cells, eosinophils and macrophages. Reactive Peyer patches were also seen throughout the small intestine.



Table 13

## Dose Titration Study

Individual and Group Mean N. battus Worm Burdens at Necropsy

Group	Animal No.	Small Intestine			Total
		Duodenum	Jejunum	Ileum	
1	040	0	0	0	0
	041	100	0	0	100
	042	0	0	0	0
Mean		33	0	0	33
SD		58	0	0	58
2	R33	0	0	0	0
	R34	100	0	0	100
	R35	100	0	0	100
Mean		67	0	0	67
SD		58	0	0	58
3	G25	100	0	100	200
	G26	400	0	0	400
	G27	300	100	0	400
Mean		267	33	33	333
SD		153	58	58	115
4	B50	100	100	0	200
	B51	0	100	0	100
	B52	100	0	0	100
Mean		67	67	0	133
SD		58	58	0	58

**N. battus Dose Titration Study**  
**Statistical analysis of N. battus worm burdens**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 16-Jul-91

Experiment title..... Nb Titration Worm Burdens  
 Number of treatments... 4

	Description:	No. of obsvs.
Treatment 1	5000 L3	3
Treatment 2	10000 L3	3
Treatment 3	15000 L3	3
Treatment 4	20000 L3	3

Replicate	Treatments 1	2	3	4
1	0.00	0.00	200.00	200.00
2	100.00	100.00	400.00	100.00
3	0.00	100.00	400.00	100.00

**ONE WAY ANALYSIS OF VARIANCE :**  
 =====

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	5.99	3.00	2.00	2.92
Experimental error	5.48	8.00	0.68	
Total	11.47	11.00		

Critical value for a 5% significance test is  $F_{.95(3,8)} = 4.07$

Since the calculated F-value is less than the critical F-value, then the data suggests that treatment effects are not different.

**NEWMAN KEULS MULTIPLE RANGE TEST :**  
 =====

K	Means	Ranges	L.S.R.	Treatment	St. Error
-	0.67			1	
2	1.34	3.26	1.56	2	0.478
3	2.10	4.04	1.93	4	
4	2.50	4.53	2.16	3	

Treatment Comparison	Significantly Different	GEOMETRIC Treat.MEANS
3 V 1	No	1 3.66
3 V 2	No	2 20.69
3 V 4	No	3 317.54
4 V 1	No	4 126.04
4 V 2	No	
2 V 1	No	

The infiltration was well differentiated and formed aggregates around the degenerating parasites. Villous damage was also seen with the villi becoming flattened and thickened along with disruption of the plica circulares by the lymphoid infiltration. All of these changes were especially marked in the nodular regions previously described.

### 3. Combined Infection Study

#### Clinical

Group mean bodyweights, shown in Figure 16, are detailed in Appendix R. Group mean weight gains of between 15 kg and 30 kg were observed over the experimental period. No statistically significant weight differences were observed between groups.

#### Plasma Pepsinogen Levels

Group mean plasma pepsinogen levels, illustrated in Figure 17, are listed in Appendix S. All animals in Groups 2, 3, 5 and 7 showed normal levels during the experimental period. In contrast gradually increasing plasma pepsinogen levels were observed in the calves of Groups 1, 4 and 6 with mean maximum levels of 1,733, 1,831 and 1,750 mU Tyrosine being recorded. Significantly higher pepsinogen concentrations ( $p < 0.05$ ) were recorded from all calves given O. ostertagi either alone or in combination with C. oncophora or N. battus L<sub>3</sub> compared with those given only the small intestinal species.

#### Faecal Egg Counts

Faecal egg count data is presented in Table 14. No eggs of N. battus were detected in the faeces of any of the infected calves throughout the study. Positive results were obtained from

**Figure 1: Weight of lambs at different ages.**

The chart displays the weight of lambs in kilograms (Kg) at various ages (Day Number) for different infection treatments. The Y-axis represents weight in Kg, ranging from 0 to 200. The X-axis represents the Day Number, with data points at 1, 7, 14, 21, 28, and 33 days. The legend identifies seven treatment groups: Oo L3 (solid black), Co L3 (diagonal lines), Nb L3 (white), Oo+Nb L3 (cross-hatch), Co+Nb L3 (dotted), Oo+Co+Nb L3 (horizontal lines), and Uninfected (vertical lines).

Day Number	Oo L3	Co L3	Nb L3	Oo+Nb L3	Co+Nb L3	Oo+Co+Nb L3	Uninfected
1	145	150	150	145	150	150	150
7	150	155	155	150	155	155	155
14	155	160	155	150	155	155	160
21	160	170	165	155	150	160	165
28	165	175	165	160	165	165	170
33	170	180	170	165	165	170	175

**Legend:**

- Oo L3
- Co L3
- Nb L3
- Oo+Nb L3
- Co+Nb L3
- Oo+Co+Nb L3
- Uninfected

**Abbreviations:** Oo = *O.ostertagi*, Co = *C.ontophora*, Nb = *N.battus*

**mU Tyrosine**

**Day Number**

Legend:

- Oo (solid black)
- Co (diagonal lines)
- Nb (white)
- Oo+Nb (cross-hatched)
- Co+Nb (horizontal lines)
- Oo+Co+Nb (vertical lines)
- Uninfected (dotted)

Approximate data values (mU Tyrosine):

Day Number	Oo	Co	Nb	Oo+Nb	Co+Nb	Oo+Co+Nb	Uninfected
1	750	700	650	700	700	700	650
7	800	700	700	850	700	850	650
14	1050	750	650	1050	700	1050	700
21	1450	750	650	1300	700	1300	700
28	1600	750	700	1600	700	1450	700
33	1700	800	750	1800	750	1750	700

Oo = *O.ostertagi*    Co = *C.ontophora*    Nb = *N.battus*

Table 14

N. battus Combined Infection Study  
Individual and Group Mean Faecal Egg Counts

Group	Calf No.	1	7	14	21	28	33
1	057	0	0	0	100	400	450
	058	0	0	0	150	300	550
	059	0	0	0	400	750	600
	060	0	0	0	350	500	650
	Mean	0	0	0	250	488	563
2	R41	0	0	0	500	750	800
	R42	0	0	0	250	500	650
	R43	0	0	0	300	550	600
	R44	0	0	0	350	650	900
	Mean	0	0	0	350	613	738
3	G32	0	0	0	0	0	0
	G33	0	0	0	0	0	0
	G34	0	0	0	0	0	0
	G35	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
4	B66	0	0	0	650	800	800
	B67	0	0	0	500	650	450
	B68	0	0	0	350	700	550
	B69	0	0	0	250	350	600
	Mean	0	0	0	438	625	600
5	Y80	0	0	0	700	750	600
	Y81	0	0	0	850	650	800
	Y82	0	0	0	950	450	750
	Y83	0	0	0	600	700	550
	Mean	0	0	0	775	638	675

Table 14 (Cont'd)

Group	Calf No.	1	7	14	21	28	33
6	P94	0	0	0	850	1,000	1,200
	P95	0	0	0	900	1,150	1,050
	P96	0	0	0	1,050	1,250	1,100
	P97	0	0	0	650	900	1,200
	Mean	0	0	0	863	1,075	1,138
7	Bk6	0	0	0	0	0	0
	Bk7	0	0	0	0	0	0
	Bk8	0	0	0	0	0	0
	Bk9	0	0	0	0	0	0
	Mean	0	0	0	0	0	0

the animals of Groups 1, 2, 4, 5 and 6 with group mean trichostrongyle egg counts (excluding N. battus) ranging from 250 to 863 epg on Day 21 and 563 to 1,138 epg at necropsy on Day 33.

#### Post-mortem Worm Burdens

The worm burdens in the three sections of the small intestine for each animal are shown in Table 15. Again most of the small intestinal genera present were recovered from the proximal region. N. battus were observed in all but two (No. G35 Group 3; No. P96 Group 6) of the calves challenged with this species. However recoveries were again low with the highest mean count of 325 being obtained from the Group 4 calves. No significant differences were observed between the N. battus worm burdens of the calves from Groups 3, 4, 5 and 6.

O. ostertagi and C. oncophora worm burdens of the calves given either monospecific or mixed infections were as would be expected from the larval dose administered.

#### Gross and Histopathological Lesions

Although the gross lesions in the small intestinal mucosa were much less pronounced in this study the histopathological findings proved to be very similar to those observed from the single infections with N. battus described in the Dose Titration Study.

#### CONCLUSIONS

From the data obtained in the field study it is obvious that N. battus L<sub>3</sub> were present in substantial numbers at the beginning of the grazing season and that these were successfully passaged by the bovine host. It is interesting that even the autumn

Table 15  
Combined Infection Study  
Individual and Group Mean Worm Burdens

Group	Calf No.	O.o.	Duodenum	C.o. Jejunum	Ileum	Duodenum	N.b. Jejunum	Ileum
1	057	5,200	-	-	-	-	-	-
	058	6,100	-	-	-	-	-	-
	059	5,900	-	-	-	-	-	-
	060	7,300	-	-	-	-	-	-
	Mean	6,235	-	-	-	-	-	-
	SD	873						
2	R41	-	4,800	1,200	0	-	-	-
	R42	-	6,100	400	0	-	-	-
	R43	-	5,700	100	0	-	-	-
	R44	-	6,900	600	0	-	-	-
	Mean	-	5,875	575	0	-	-	-
	SD		873	465	0			
3	G32	-	-	-	-	500	0	0
	G33	-	-	-	-	100	100	0
	G34	-	-	-	-	200	0	0
	G35	-	-	-	-	0	0	0
	Mean	-	-	-	-	200	25	0
	SD					216	50	0
4	B66	6,400	-	-	-	300	0	0
	B67	7,200	-	-	-	100	100	0
	B68	5,200	-	-	-	400	0	0
	B69	6,300	-	-	-	300	100	0
	Mean	6,275	-	-	-	275	50	0
	SD	822				126	58	0
5	Y80	-	3,700	1,100	100	200	0	0
	Y81	-	3,900	900	0	100	100	100
	Y82	-	4,600	1,400	100	300	100	0
	Y83	-	3,600	1,200	0	100	100	0
	Mean	-	3,950	1,150	50	175	75	25
	SD		451	208	58	96	50	50



Table 15 (Cont'd)

Group	Calf	O.o.	Duodenum	C.o. Jejunum	Ileum	Duodenum	N.b. Jejunum	Ileum
	No.							
6	P94	6,900	3,500	1,000	100	100	0	0
	P95	4,800	2,900	600	0	0	200	0
	P96	5,100	3,700	800	0	0	0	0
	P97	4,200	3,800	1,100	0	0	200	0
	Mean	5,250	3,475	875	25	25	100	0
	SD	1,162	403	222	50	50	115	0

**N. battus Combined Infection Study**  
**Statistical analysis of N. battus worm burdens**

ANIMAL DESIGNS... One Way anova then Range Test      Date... 16-Jul-91

Experiment title..... Nb + Oo + Co combination

Number of treatments... 4

	Description:	No. of obsvs.
	-----	-----
Treatment 1	Nb only	4
Treatment 2	Nb + Oo	4
Treatment 3	Nb + Co	4
Treatment 4	Nb + Oo + Co	4

Replicate	Treatments			
	1	2	3	4
1	500.00	300.00	200.00	100.00
2	200.00	200.00	300.00	200.00
3	200.00	400.00	400.00	0.00
4	0.00	400.00	200.00	200.00

ONE WAY ANALYSIS OF VARIANCE :

=====

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
-----	-----	-----	-----	-----
Treatment	2.14	3.00	0.71	1.02
Experimental error	8.38	12.00	0.70	
-----	-----	-----	-----	-----
Total	10.52	15.00		

Critical value for a 5% significance test is  $F_{.95}(3,12) = 3.49$

Since the calculated F-value is less than the critical F-value, then the data suggests that treatment effects are not different.

NEWMAN KEULS MULTIPLE RANGE TEST :

=====

K	Means	Ranges	L.S.R.	Treatment	St. Error
-----	-----	-----	-----	-----	-----
	1.65			4	
2	1.83	3.08	1.29	1	0.418
3	2.42	3.77	1.58	3	
4	2.50	4.20	1.75	2	

Treatment Comparison	Significantly Different	GEOMETRIC Treat. MEANS
-----	-----	-----
2 V 4	No	1 66.07
2 V 1	No	2 313.06
2 V 3	No	3 263.26
3 V 4	No	4 43.94
3 V 1	No	
1 V 4	No	

NEWMAN KEULS MULTIPLE RANGE TEST :

=====

K	Means	Ranges	L.S.R.	Treatment	St. Error
-----	-----	-----	-----	-----	-----
1	719.00			3	
2	724.00	2.89	323.25	7	111.850
3	752.33	3.48	389.24	5	
4	775.17	3.84	429.50	2	
5	1189.67	4.11	459.70	6	
6	1233.83	4.30	480.95	4	
7	1235.50	4.46	498.85	1	

Treatment Significantly  
Comparison Different

-----	-----
1 V 3	Yes
1 V 7	Yes
1 V 5	Yes
1 V 2	Yes
1 V 6	No
1 V 4	No
4 V 3	Yes
4 V 7	Yes
4 V 5	Yes
4 V 2	Yes
4 V 6	No
6 V 3	Yes
6 V 7	Yes
6 V 5	Yes
6 V 2	Yes
2 V 3	No
2 V 7	No
2 V 5	No
5 V 3	No
5 V 7	No
7 V 3	No

tracer cattle had acquired moderate N. battus burdens which must either have come from larvae which had survived over the summer or from those derived from an autumn hatch.

However, despite the apparent ease with which natural infections were established, the experimental infection with the field isolate of N. battus proved to be very difficult, at least in terms of demonstrable patency. Zero or very low faecal egg output was observed in the majority of the animals. In the Dose Titration Study, apart from the recovery of worms at necropsy and some histopathological evidence of their presence in the intestinal musoa there was nothing in the other parameters measured, i.e. bodyweight, plasma pepsinogen levels or faecal egg counts, to suggest the presence of N. battus infection. Not surprisingly, in view of the very low level of N. battus establishment, there were no clinical signs of nematodiriasis e.g. diarrhoea and weight loss.

In the Combined Infection Study, while the viability of the Ostertagia and Cooperia components was established by elevated plasma pepsinogen levels, faecal egg output and worm burdens at necropsy, the N. battus L<sub>3</sub> again gave rise to very low worm burdens.

This attempt to mimic the field situation, in which clinical nematodiriasis in calves due to N. battus was observed, failed and the reasons for this lack of success are discussed later.

## **CHAPTER FOUR**

### **GENERAL DISCUSSION**

Parasitic gastroenteritis is well established as one of the major causes of disease in cattle both in Europe and elsewhere (Armour and Ogbourne, 1982). From fundamental studies into the ecology of infective helminth stages, and their pattern of availability to grazing animals (Anderson et al, 1965b; Michel, 1966; Armour, 1974), a variety of control measures to combat bovine PGE have evolved. Many of these have concentrated on the use of therapeutic or prophylactic anthelmintic medication with, latterly, several ingenious methods of their application being made available including topical formulations and long acting intra-rumenal devices. The range and activity spectrum of the anthelmintic drugs currently available has been detailed by Armour and Bogan (1982) while many studies have proven their efficacy against ruminant gastrointestinal parasites (Armour et al, 1973; Duwel, 1974; Jacobs, Fox, Walker, Jones and Bliss, 1981; Armour, Bairden, Oakley and Rowlands, 1985; Armour et al, 1987).

Alternative strategies to chemotherapy have also been devised and, in the U.K., these tend to rely mainly on integrated grazing systems using different host species. However, in many areas farm husbandry systems are not flexible enough to allow their proper implementation thus making them less popular than anthelmintic treatment regimes. A disadvantage of repeated chemotherapy is the possibility of selecting for anthelmintic resistance and it may be that because of this methods of control based on grazing systems will achieve more prominence in future.

With most control measures there are potential problems. For example strategic dosing programmes have been implicated in a reduced or delayed acquisition of immunity to Ostertagia in young cattle (Armour, Bairden and Ryan, 1988) and in alternate grazing systems parasite adaptation between host species has evolved (Helle, 1981; Eysker and Jansen, 1982; Armour et al, 1988; Coop et al, 1991). The findings from two of the field studies described in this thesis, i.e. the strategic anthelmintic treatment study and the cattle and sheep alternate grazing experiment provide additional information on the advantages and disadvantages of currently recommended control methods.

In the anthelmintic prophylactic study carried out during 1988 and 1989 two points are worth noting. First, there were constraints on its management because it was carried out on a commercial farm where productivity took precedence over parasite epidemiology or anthelmintic efficacy data and secondly, several of the principal cattle used in the first year of this study were summer/autumn born calves from the previous season and as such were possibly less susceptible than younger calves to parasitic gastroenteritis. However, the results from the two prophylactically treated groups showed that the use of either ivermectin treatment at 3, 8 and 13 weeks post turnout or the administration of a morantel bolus prior to turnout prevented outbreaks of ostertagiasis in young cattle in their first grazing season. The efficacy of the ivermectin 3, 8 and 13 week programme, which is based on a 14 day persistent effect of the

drug after administration, has been demonstrated previously in the U.K. and U.S.A. (Armour et al, 1980; Armour et al, 1987; Jacobs, Fox and Ryan, 1987; Williams, Knox, Barras and Hawkins, 1990) while in Denmark three treatments with ivermectin at four week intervals during the early part of the grazing season were effective in preventing the acquisition of large burdens of O. ostertagi and C. oncophora by young calves (Steffan and Nansen, 1990). In Europe a morantel tartrate bolus has also proven effective in the control of bovine parasitic gastroenteritis during a first grazing season (Jones, 1981; Armour et al, 1981; Jacobs et al, 1981; Tharaldsen and Helle, 1982; Talty, 1985).

Despite the inclusion of some fairly mature calves in the ivermectin/MSRT study reported in this thesis, the control group cycled the available overwintered infective larvae and as an outbreak of Type I ostertagiasis appeared inevitable therapeutic anthelmintic treatment was administered to this group in mid-season. Thus, in terms of productivity, although the weight gains of the ivermectin and MSRT treated animals when compared to the control calves were not marked, the reduced food intake which has been associated with heavy nematode infections in cattle (Randall and Gibbs, 1981) makes it probable that without the midseason therapeutic anthelmintic treatment of the first year controls there would have been a much greater weight difference between them and the ivermectin and MSRT treated groups at housing. However, appetite loss in ruminants during outbreaks of



PGE is thought to be a transient feature returning to normal after a few weeks (Symons, Steel and Jones, 1981; Coop, Sykes and Angus, 1982) so the mid-season treatment might have been expected to allow the control calves to show a greater weight improvement than that observed. In fact, these animals failed to fully compensate in growth and were several kilograms lighter than the treated groups at the end of the first grazing season. This was also the case in a previous report by Entrocasso, Parkins, Armour, Bairden and McWilliam (1986a). In the situation where dairy replacement heifers are reared to calve at two years of age, when weighing approximately 500 kg, any retardation in liveweight gain during their first year might be significant and could lead to the postponement of breeding and thus economic loss.

Climatic conditions throughout the first grazing season were typical of those normally occurring in southwest Scotland as was the low level of pasture infectivity prior to the commencement of grazing. Although it has been frequently shown that a low level of pasture infectivity in spring is sufficient to establish a disease producing population of  $L_3$  on pasture late in the season (Michel, 1969; Armour, 1980) the previous grazing history of this area together with the disappearance of pasture larvae by the time of turnout and the low worm burdens of the spring tracer calves, suggested that a subsequent outbreak of disease was unlikely. However positive faecal egg counts were detected in the control calves by the end of June at levels in excess of those found in either of the treated groups of cattle and the

resulting contamination led to marked increases in infective larvae on pasture and as mentioned previously a potential disease outbreak in late August in the untreated animals. Such a pattern of early contamination followed by the onset of clinical PGE in untreated calves has been demonstrated on several occasions previously (Taylor, Mallon and Kenny, 1985; Armour et al, 1985). Positive faecal egg counts were recorded from the treated cattle but these were low throughout the summer and pasture larval recoveries from the paddocks grazed by these animals showed little increase until the autumn. This is the pattern which has been observed in several recent grazing studies using anthelmintics prophylactically (Jacobs et al, 1987; Armour et al, 1987).

From the worm burdens of the autumn tracer calves grazed on the ivermectin and MSRT treatment paddocks, which ranged from 11,200 to 18,500, it was clear that there was a moderate level of pasture larval infection on these two areas at the end of the grazing period. The Ostertagia burdens of the principal cattle of the two treatment groups at this time were similar and higher than the tracers and probably consisted mainly of worms acquired in the latter half of the grazing season. Since the post-treatment decrease in pepsinogen values and faecal egg counts of the control calves in August showed that treatment was successful, it was therefore a reflection of pasture larval levels during the latter part of the grazing season that the control animals necropsied in October had such high worm burdens.

These larvae were assumed to have originated from pasture contamination with eggs in the early part of the season and a high subsequent level of pasture infectivity was therefore not unexpected. However what is interesting is the source of the rise in pasture larval numbers in both the ivermectin and MSRT paddocks so near the end of the grazing season. From the very low faecal egg output of the treated cattle and the assumption that eggs deposited after late July do not greatly contribute to pasture contamination (Michel, 1969) these were unexpected. It is possible that these late increases simply reflected a more efficient larval recovery from the relatively short herbage available at this time but they may also have come from development of eggs passed after July, perhaps representing an autumn extension of the "concertina effect". The overall picture from the results obtained in the first grazing season however was consistent with the successful use of two methods of prophylactic treatment to control bovine PGE.

As it had been previously shown that second year grazing cattle, treated with ivermectin at 3, 8 and 13 weeks in their first grazing season, accumulated high numbers of fourth stage Ostertagia larvae (Armour et al, 1988), the purpose of the second year of the study was to determine whether previously morantel bolused cattle would also accumulate high larval burdens. The control area was chosen as being most likely to have the highest level of overwintered larvae to provide an initial challenge.

One of the major constraints on the second year was the restricted grazing area for the study. As a result the total

group size had to be kept to a maximum of twelve animals i.e. four from each of the first year grazing groups. Because of availability, there was a marked difference in group mean bodyweights at turnout but interestingly the ex-control cattle, which began the second season with a heavier group mean bodyweight, maintained their weight advantage until the end of the second grazing season.

The zero to very low faecal egg counts recorded from all of the second year cattle were as expected and similar to those obtained in other studies from previously bolused cattle turned out to graze for a second season (Borgsteede, Kloosterman, Oostendorp and Tarrij, 1985; Jacobs, Pitt, Foster and Fox, 1987). From this faecal egg count data it appeared that all twelve yearlings had acquired some degree of immunity to Ostertagia.

The pepsinogen levels obtained from the second season grazing cattle are interesting. All twelve animals had been treated with ivermectin at housing and therefore were virtually parasite-free during the housed period. This should have allowed recovery to relatively healthy, functional abomasal mucosae by the time of turnout. However at the beginning of the second grazing season normal values were recorded only from the ex-ivermectin and ex-bolused cattle those of the former controls being in excess of 2,000 mU Tyrosine. Armour, Bairden, Duncan, Jennings and Parkins (1979) observed similarly high pepsinogen concentrations in cattle, treated at housing with levamisole, at the time of turnout for a second year. While these increased

pepsinogen levels noted by Armour et al (1979) were probably due to the maturation of inhibited early fourth stage larvae which would not have been removed by the levamisole housing treatment, the high levels in this study cannot be so attributed as the housing treatment was carried out using ivermectin, which is highly efficient against hypobiotic larvae. If, for some reason a significant number of parasites remained after the housing treatment and the elevated pepsinogen levels were caused by larval emergence and development, much higher faecal egg counts would have been expected than those recorded after turnout. It may be that the abomasal damage incurred in the first season was so severe that complete recovery of the mucosa during housing did not occur and that this was responsible for the observed high pepsinogen levels in the ex-control cattle at turnout.

The subsequent rises in pepsinogen levels in all three groups observed four weeks after turnout in the second grazing season were consistent with a degree of acquired immunity to O. ostertagi as it has been demonstrated that previously infected and treated animals although sufficiently immune to prevent the establishment of significant patent infections, show an initial elevation in pepsinogen concentration after larval challenge (Anderson, 1972; 1973; McKellar et al, 1986). In this study all three groups of calves had received prophylactic or therapeutic anthelmintic treatment during their first grazing period and at housing. In the Dutch study by Borgsteede et al (1985), previously bolused cattle also showed a pattern of rising pepsinogen levels in the early part of the second grazing season.

However, as the second grazing season progressed, there was a decrease in pepsinogen levels in the ex-control cattle while those of the two ex-treated groups continued to rise until the end of the season. The findings in the control cattle in this study were similar to those recorded by Armour *et al* (1979) who noted a decrease in pepsinogen levels in therapeutically treated cattle after turnout for a second season. A fall in pepsinogen levels in second year cattle previously grazed as untreated controls in their first season, has been reported by Entrocasso *et al* (1986a; 1986b). In this case and that reported by Berghen, Dorny, Hilderson, Vercruysse and Hollanders (1990) no immediate post-turnout increase in pepsinogen levels was observed but in both of these studies plasma pepsinogens were high at the beginning of the second season (4,000 - 6,000 mU Tyrosine). This may have been due to the onset of Type II ostertagiasis four to six weeks prior to the start of grazing as no housing treatment had been given to the animals in either study at the end of their first grazing season. However, the calves in Entrocasso's study had received a mid-season therapeutic treatment with levamisole while those in the Belgian study were given two salvage anthelmintic treatments in their first grazing season.

With all twelve cattle grazing on the same paddock and therefore exposed to the same level of larval challenge from pasture, fluctuations in pepsinogen levels might have been expected to show a similar trend in all of the animals. The difference in response between the animals from the two ex-

treatment groups and the ex-control cattle, may simply reflect a low level of larval challenge which in the ex-controls was insufficient to cause a sustained rise in pepsinogen concentrations in animals where the abomasal mucosa was returning to normality. In the ex-ivermectin and ex-MSRT cattle, which could be expected to have relatively intact abomasal mucosae, the greater response in pepsinogen levels might have been a truer reflection of larval challenge causing a hypersensitivity reaction similar to that observed in previously immunised rats when re-infected with N. braziliensis (Barth, Jarrett and Urquhart, 1966).

At the end of the second year the relatively low O. ostertagi burdens of the ex-control animals, compared with those of the prophylactically treated calves suggested that the persistence or continuous release of anthelmintic in the early part of their first grazing season had subdued or altered the immune response to gastrointestinal parasites in the calves given ivermectin or the morantel bolus. However Entrocasso (1984) maintained that the morantel bolus did not interfere with the immunity to O. ostertagi acquired by yearling cattle but in Entrocasso's work, as in other studies involving a second grazing season (Prosl, Supperer, Jones, Lockwood and Bliss, 1983; Conder, Jones and Bliss, 1983), a second bolus had been given to the yearlings at turnout. Despite the first bolus treatment Entrocasso found more than 17,500 O. ostertagi, with a 70% level of inhibition, in the cattle necropsied at the end of the first grazing season although it was not considered that this level of

infection was significant in terms of potential development of Type II ostertagiasis.

Interactions between anthelmintic prophylaxis and the development of immunity to cattle helminths have been discussed by Armour (1989) who suggested that the level of immunity which developed was inversely proportional to the efficacy of the anthelmintics used. This view was based on a number of studies which monitored the parasitological status, during a second grazing season, of calves treated strategically during their first year at grass. For example the use of a morantel bolus did not appear to impair the acquisition of immunity to subsequent experimental Ostertagia challenge (Entrocasso et al, 1986a, 1986b). A different picture emerges, however, from studies involving the use of more efficient anthelmintics in strategic dosing programmes. Thus Jacobs et al (1987) found a reduced immunity to challenge with both gastrointestinal helminths and lungworms in animals previously given an oxfendazole pulse release bolus (OPRB, Repidose, Coopers Animal Health) which releases therapeutic doses of oxfendazole at three week intervals during the grazing season. Higher faecal egg counts were also noted from second year grazing cattle following first season OPRB treatment in field studies conducted by Herbert and Probert (1987) and in Eire by Downey (1988).

Using ivermectin at 3, 8 and 13 weeks post-turnout Taylor et al (1985) and Armour et al (1987) obtained excellent control of Ostertagia and Cooperia in first season grazing calves. In



the following year, as judged by faecal egg counts and pepsinogen levels, there appeared to be a high degree of immunity in the ex-treated animals (Taylor, Mallon, Green, McLoughlin and Bryson, 1988; Armour et al, 1988) but on necropsy it was found that very high levels of inhibited Ostertagia fourth stage larvae had accumulated in these cattle (Armour et al, 1988).

With both ivermectin, which has a persistent effect for 2 to 3 weeks, and any continuous release device, it is difficult to determine at which stage parasites are killed. The duration of larval survival or degree of development after treatment could have an important bearing on the acquisition of immunity if, as in H. contortus in sheep (Adams, 1982), maturation through exsheathing L<sub>3</sub> and L<sub>4</sub> stages within the host is necessary to confer protection against reinfection. If this is so then repeated treatments with ivermectin, which is highly larvicidal, would not be expected to enhance acquired immunity. In contrast in the case of morantel the level of drug released from a bolus has been shown to be relatively inefficient (40 - 60% efficacy) against experimental O. ostertagi challenge during the first three to six weeks after administration (Ibarra-Silva, 1988; Holmes, Bairden, Ibarra-Silva, Salman and McWilliam, 1989). This might be expected to allow bolused animals exposure to a sufficient parasite challenge from pasture to develop a significant immunity but from the worm burdens at necropsy in the previously bolused animals in this two year study this did not appear to be the case.

Irrespective of previous treatment the Ostertagia worm

burdens of all the yearling cattle in this study were substantial and consisted mainly of early fourth stage larvae. Armour et al (1988) also recorded high levels of inhibited fourth stage Ostertagia larvae in second year grazing cattle treated prophylactically the previous year with ivermectin. This was attributed in part to the fact that these animals were grazed together with a group of young susceptible calves and were therefore subjected to an abnormally high larval challenge from pasture. In the study reported here, however, the yearlings were grazed alone with only the overwintered larval population from the previous season as an initial source of infection. Eighteen month to two year old cattle are considered unable to cycle infection sufficiently to produce a significant level of pasture contamination at the end of the grazing season although there are instances however when the immune status of such animals may be impaired. For example although cattle show a good immunity to Ostertagia at the end of their first grazing season it has been suggested that this can wane during a period of winter housing (Ross and Dow, 1965; Armour, 1967). Also, immunity is not necessarily a permanent feature in older cattle and may breakdown particularly in heifers during calving and early lactation (Michel, Lancaster and Hong, 1979; Petrie, Armour and Stevenson, 1984).

With the very low level of egg output from all twelve cattle in this study and the consequent low level of pasture infectivity for most of the grazing season, the source of the infective

larvae giving rise to the large numbers of inhibited Ostertagia seen at necropsy in this study remains uncertain. It is possible that late in the season when herbage was rather sparse the animals were forced to graze near to faecal pats and thus ingested pasture which had the highest concentrations of infective larvae. Whatever the source of infection the significance of large numbers of inhibited larvae in the yearling cattle is still unknown although there are reports of such larvae being responsible for outbreaks of Type II ostertagiasis in dairy heifers around the time of calving (Hotson, 1967; Wedderburn, 1970; Raynaud and Bouchet, 1976a; 1976b).

The findings of this study highlight a number of important points. First it was established that, as in calves treated prophylactically with ivermectin in their first grazing season, animals given a morantel tartrate bolus in their first year and put to graze for a second season without further anthelmintic prophylaxis acquired large burdens of inhibited Ostertagia fourth stage larvae. It was also shown that these animals, while showing no clinical signs of gastrointestinal helminthiasis, contaminated the pasture sufficiently to cause an autumn rise in numbers of infective larvae thus establishing an overwintering larval population. Young susceptible calves going to grass for the first time on such an area which might be considered "clean" would be exposed to a potential infection in the following season. The results of this study indicate a possible requirement for further anthelmintic prophylaxis in second year grazing cattle.

Early attempts to control helminthiasis by grazing different host species together were criticised in that the lack of host specificity of a number of parasite genera, for example T. axei, could cause problems in each host species (Hall, 1917; Tetley, 1934). This view was contested by Snell (1935) who maintained that grazing two different host species together could be to their mutual benefit.

The use of cattle to reduce the number of H. contortus larvae on a former sheep pasture was first investigated by Ross (1931) who found that after eight weeks of cattle grazing the pasture was "safe" for subsequent grazing by sheep. A subsequent attempt by Roberts (1942) to determine the feasibility of cleaning sheep pasture by grazing it with calves for a short period typifies the approach common to most experimental integrated grazing studies at that time. In Robert's study where pasture was contaminated by grazing it with three artificially infected lambs for six weeks, the faecal egg counts of calves which subsequently grazed the area were then monitored for a further six weeks. The results of this brief study suggested that alternate host grazing could be beneficial in the control of a number of parasite genera, for example Haemonchus.

In two more recent Australian studies (Southcott and Barger, 1975; Barger and Southcott, 1975) former cattle paddocks were stocked with sheep for 6, 12 or 24 weeks. By using tracer calves it was found that levels of O. ostertagi and C. oncophora remained the same after six weeks, a reduction in O. ostertagi

numbers had occurred after 12 weeks and after 24 weeks there were significantly lower recoveries of both genera. Morley and Donald (1980) have also suggested that parasite cross transmission seemed to be sufficiently restricted to enable grazing management systems to have substantial benefits to offer for parasite control in cool temperate regions where the dominant cattle parasites were Ostertagia and Cooperia. In their review of helminth control systems three levels of cross transmission of ruminant nematodes were recognised. These were (1) very low without reproduction, (2) moderate but with a short patency and (3) with minor differences which were likely to disappear over a few generations making them equally infective to both host species. Interestingly both Ostertagia and Nematodirus were included in the first category despite the fact that in studies carried out in Canberra O. ostertagi burdens in excess of 10,000 parasites were found in tracer lambs grazing a former calf paddock. Although 70% of the O. ostertagi present were classed as inhibited fourth stage larvae, the fact that faecal cultures from sheep grazing on the area failed to reveal the presence of any O. ostertagi L<sub>3</sub> suggested to the authors that any cross-infection was transient.

In a recent UK survey in an agricultural area of southwest Scotland it was found that a high percentage of farmers already utilised alternate grazing as an aid in the control of ruminant parasitism (Gettinby, Armour, Bairden and Plenderleith, 1987). For example, of 25 beef/sheep units, 21 practised annual

alternate grazing of sheep and cattle and of these, 11 also moved calves to aftermath after weaning. Also in beef/dairy/sheep units in this area seven out of nine practised alternate grazing of cattle and sheep with three also moving weaned calves to aftermath grazing.

In a number of experimental and grazing studies over several decades, very few O. ostertagi have ever been shown to become established in sheep (Porter, 1953; Dikmans and Kates, 1955; Arundel and Hamilton, 1975) and those which did mature did not produce patent infections (Herlich and Stewart, 1954; Pandey, 1971). This lent support to the concept of alternate grazing of cattle and sheep, as a method of control of gastrointestinal parasitism. However, the comment by Southcott and Barger (1973) that "while alternate grazing has been recommended and theoretically justified by many authorities it is only recently that the practice has become experimentally evaluated" is still pertinent in terms of studies into the long term success of these methods of parasite control. The pasture management study reported here was designed to prevent bovine PGE without the use of anthelmintic treatment and was undertaken in the full knowledge of the fact that helminth control, based on some grazing management systems, has been criticised. For example, Michel (1969) stated "It is evident that any practicable system of rotational grazing can have no relevance to the control of trichostrongyloid worms" although in a later review on the control of bovine PGE (Michel, 1972), it was suggested that to economically utilise pasture grazed and contaminated early in the

season by susceptible calves subsequently transferred to aftermath, the area should then be grazed by a different host species or older cattle. However the view that helminth control must conflict with agricultural objectives may only be true where management systems have been devised without considering the nature of the parasitism involved (Spedding, 1969).

In the four year study described in this thesis the annual seasonal pattern of infective larvae on pasture and the composition of worm burdens at necropsy in cattle grazing either a permanent calf paddock or paddocks annually rotated with sheep were monitored. It was hoped that this would provide information which would be useful in assessing the potential advantages or disadvantages which might accompany such management practices.

In the first year serum pepsinogen levels in the two groups of grazing calves were similar in pattern and magnitude and were within the same range as those found in other studies in this area (Armour, et al, 1985; 1987). As both of these paddocks had been regularly grazed by cattle in previous years this was not unexpected. In the second grazing season, serum pepsinogen levels of the calves which followed the sheep were normal throughout the grazing period whereas there was again a progressive increase in pepsinogen values in the calves in the permanent cattle paddock. It thus appeared that the use of sheep in the first year had effectively reduced the numbers of bovine Ostertagia L<sub>3</sub> available to infect calves grazing this area in the following year.

In the third year of the study the pepsinogen levels of the calves on the alternated cattle/sheep paddock, although slightly higher than normal, were lower than those of the calves on the permanent cattle paddock. This suggested that a sufficient number of Ostertagia L<sub>3</sub> had survived two winters and the intervening sheep grazing period to establish moderate worm burdens in the alternate bovine host. Finally, and most interestingly, in the fourth year of the study the calves on the alternated sheep/cattle paddock, where calves in the second year of the study had reduced pepsinogen levels, showed a pattern and levels of serum pepsinogen which were almost identical to those observed in the animals grazing the permanent cattle paddock indicating a similar exposure to Ostertagia infection. This return to pepsinogen levels normally associated with clinical ostertagiasis was completely unexpected and the source of infection, whether from soil or elsewhere is discussed later.

Faecal egg count data from the set-stocked control calves in all four years of the study followed the expected pattern from four weeks after turnout onwards. However in the first year calves allocated to the alternate cattle/sheep paddock, marked increases in egg output with clinical signs of ostertagiasis occurred which necessitated two therapeutic anthelmintic treatments six weeks and 14 weeks after turnout. As the calves grazing both of these paddocks were expected to be exposed to a similar overwintered larval challenge, the reasons for this outbreak of disease in one group so soon after turnout remains



obscure. In the second year of the study the low faecal egg counts in the calves which followed the sheep suggested that the alternating strategy had been successful. That this was not sustained over the next two years of observation was clearly demonstrated by the fact that in the final year similar egg counts were recorded from both calves on the sheep/cattle paddock and those on the permanent cattle grazing. In contrast, in The Netherlands the same levels of egg output from alternated and set-stocked calves were observed in each grazing season over a four year period (Borgsteede and Oostendorp, 1982).

The recoveries of infective larvae of cattle parasites from the four areas in 1982 reflected their grazing host species. Thus in the control calf paddock and the alternate area grazed by calves in the first year, Ostertagia and Cooperia L<sub>3</sub> levels were consistent with those of a cattle grazing area while from the alternate paddock first used by sheep and from the set-stocked sheep area very few bovine parasite L<sub>3</sub> were recovered. The negligible bovine parasite larval recoveries from the latter paddock were not unexpected since for a number of years prior to this study it had always been used to graze sheep. In contrast the alternate paddock allocated to sheep in the first year had been grazed for several years previously by calves, with sheep only being allowed a short spell of winter grazing. Initially a similar pattern of overwintered bovine larval recoveries might therefore have been expected but the close cropping grazing habits of the sheep probably resulted in "cleaning" the pasture

of surface dwelling larvae.

In the remaining three years of the study seasonal recoveries of bovine parasite  $L_3$  from the permanent calf paddock were typical. On the alternate plots, however, such a pattern was seen only when they were grazed by calves. In the intervening sheep grazing periods very few bovine  $L_3$  were recovered although following the heavy contamination by calves in the first year of the study, relatively high numbers of bovine  $L_3$  were recovered from the alternated cattle/sheep paddock early in the second year, despite the fact that it was grazed by sheep during this period.

The Cooperia larval recoveries recorded during this study were interesting. Adult parasites of this species had been regularly recovered from sheep which had previously grazed the set-stocked sheep plot and isolates of these larvae had been used in successful experimental infections of lambs in other studies (unpublished data). In a four year alternation study by Borgsteede and Oostendorp (1982) it was also found that less host specific species were easily transferred to the other host with C. oncophora being frequently found in lambs and H. contortus in calves. The Cooperia herbage  $L_3$  recoveries during the sheep grazing periods in this alternate grazing study however did not suggest a significant cycling of infection. One possible explanation may be that in addition to being passaged by the "wrong" host a rapid immunity develops against C. oncophora in grazing sheep and that such infections are quickly eliminated. Despite this apparent lack of cycling, it is clear that as with

Ostertagia, a sufficient number of Cooperia survived, or were produced, during the sheep grazing periods to overwinter and infect calves in the following spring. With hindsight it would have been advantageous to culture faeces from some of the sheep from the alternated areas to determine the extent of their role in the cycling of this parasite species.

The overall picture of larval recoveries from the two alternated areas in this study does not indicate that an intervening year's grazing by sheep would be sufficient to clean the pasture of bovine nematode larvae. Indeed the upward trend of bovine L<sub>3</sub> recoveries from the alternated paddocks in the third and fourth years suggests an increasing establishment and cycling of the relatively small numbers of larvae remaining after the sheep grazing period.

End of season necropsy data showed that in the first year animals from both calf paddocks had acquired considerable O. ostertagi worm burdens. Both the numbers and stages of these populations were of interest. The calves on the designated alternate paddock had twice the number of Ostertagia of their control counterparts together with higher Cooperia burdens. A much higher level of O. ostertagi larval inhibition was also seen in these calves i.e. 54% compared with 27% in the controls. While the difference in numbers could be explained by the greater larval challenge during the grazing period, which was confirmed by the herbage analysis data, it was more difficult to account for the difference in population structure; interpretation of

these results was also complicated by the fact that although all of the control calves were necropsied in September, two of the animals from the alternate paddock had to be slaughtered in August. The most likely explanation however is that the therapeutic anthelmintic treatment on two occasions had somehow interfered with the normal acquisition of acquired immunity in the treated calves.

In the second year of the study the worm burdens from the calves which had grazed the area previously used by sheep indicated a level of success for the control strategy with a 70% reduction in Ostertagia and a 30% reduction in Cooperia burdens being recorded. However by the end of the third year, although overall Ostertagia burdens were lower in the calves from the cattle/sheep alternated paddock, the success rate against Ostertagia had fallen to 50% and substantially higher numbers of Cooperia were recovered from the calves on this area compared with the controls. The most interesting finding in the fourth year of the study was that there was no significant difference in group mean Ostertagia burdens between the controls and the sheep/cattle alternate group; in addition the Cooperia burdens of the latter far outnumbered those of the calves on permanent cattle grazing. This gradual build up of Cooperia may, as in the case of T. axei, have been due to an enhanced establishment caused by passage in the alternate sheep host although, as mentioned earlier, the possibility of this species cycling in the sheep host was not considered in this study.

It is perhaps of significance that the design of the study

reported here differed from most other assessments of helminth control by grazing management in that data was recorded over four consecutive years rather than a two to three year period. One exception was a sheep and cattle alternate grazing study over a five year period in Switzerland (Inderbitzen, Eckert and Hoffman, 1981) but in this, the animals were changed over in mid-season. In the Swiss study, as in the work reported here, an increase in abomasal parasite numbers, mainly Ostertagia, was observed in calves after the second year (from a mean of approximately 40,000 in the first year to almost 180,000 in the second) and although marginally smaller mean numbers were recorded from the calves in the third and final year individual animals still had abomasal burdens of over 140,000 worms. The work of Helle (1981) is perhaps more comparable with the study described in this thesis. The results of his five year assessment of both mixed and alternate grazing systems indicated that alternation of cattle and sheep was the most effective method of markedly reducing the numbers of parasite larvae available to either host species. Interestingly the one exception was N. battus although only the sheep required anthelmintic treatment to combat the effects of infection with this species.

It is a generally held view that larvae, which have overwintered from the previous season, are cycled by susceptible calves giving rise to disease producing parasite populations in the late summer or autumn. Such larvae, if not ingested by calves, are thought to die off during the warmer dry conditions

of summer. The results of this study suggest that sufficient numbers of  $L_3$  must have survived for at least 18 months to initiate the infection in the calves on the alternately grazed paddocks. Prolonged third stage larval survival of up to 18 months has been demonstrated (Al-saqr et al, 1982; Bairden et al, 1985) and possibly occurs in faecal pats or soil, both providing protection from the environment (Krecek and Murrell, 1988). However in the area of this field study, bovine faecal pats have usually disintegrated by spring (Duncan, Armour, Bairden, Urquhart and Jorgensen, 1979) which would limit their usefulness in the protection and survival of infective larvae. It may also be that the pre-infective stages (eggs or first/second stage larvae) can survive in faeces. It has been shown for example that hatching of eggs can be temperature/moisture dependent taking between 3-4 days in summer, when the faeces are relatively warm and dry, to up to 68 days in waterlogged faeces in winter (Lehane, 1981). This could result in pre-infective stages being present in faeces during the winter. A major limitation to such survival would again be faecal pat degradation, an unhappy prospect for these early parasite forms which are almost totally dependent on faecal pat protection.

Unexplained outbreaks of PGE which have occurred on presumed safe pasture have been attributed to infective  $L_3$  in such reservoirs as herbage root-mat or soil (Nelson, 1977; Oakley, 1977; Duncan et al, 1979). That such larvae are not "soil bound" has been shown by Fincher and Stewart (1979) who demonstrated the

upward migration of Cooperia spp L<sub>3</sub> from soil to herbage while Gruner, Mauleon and Sauve (1982) reported similar findings for L<sub>3</sub> of sheep trichostrongylids. Although survival of L<sub>3</sub> on herbage for 18 months seems unlikely because of exposure to the environment, it has been suggested that in countries such as Canada with a long cold winter, L<sub>3</sub> survival on pasture is prolonged and probably represents an adaptation to climatic conditions (Armour, 1982).

It is clear from the results of the four year grazing study described in this thesis that the annual alternate grazing of cattle and sheep did not prevent the occurrence of bovine ostertagiasis. Mitchell and Fitzsimons (1983) have also demonstrated that pastures in southern Scotland are not helminthologically safe after resting for 18 months but maintain that the various systems for control of PGE which depend on integrated pasture management are not to be disparaged but should be operated on a planned basis. Although not practicable on many farm enterprises it is likely that if a longer "resting" period can be allowed, as in the three year sheep/cattle/crop system devised by Rutter (1975), a greater degree of control could be achieved. Also it is interesting to speculate what the result might have been if instead of continuing with the planned cattle/sheep alternation, an older less susceptible group of cattle had been substituted in the third year.

In practice alternate grazing systems, in common with all other control methods, depend on individual choice and

applicability with economic considerations being of great importance. To be practical, management systems for the control of parasitism must be flexible. For example, it would be unrealistic to expect forward planning to take into account a complex programme of control which might rely on future unpredictable weather conditions. In this context it has been suggested in a detailed review of the economic impact of farm management systems (Morley and Donald, 1980) that control procedures, based on epidemiological principles, have seldom been tested realistically to assess feasibility and benefits in actual, or even simulated, farming systems.

In the final field study reported in this thesis it was noted that substantial numbers of Nematodirus battus could be acquired by set-stocked grazing calves. This prompted the experiments which were designed to determine the dose of N. battus larvae required to produce clinical nematodiriasis in calves and to examine any interaction between infections with this species and the two other commonly occurring cattle parasites in the U.K., namely O. ostertagi and C. oncophora.

A relationship between the seasonal incidence of helminth disease and weather conditions has been shown for a number of nematode genera (Gordon, 1948; Levine, 1963; Kates, 1965). N. battus however, shows such dependence on climatic conditions that it is possible to predict in which years outbreaks of disease due to this parasite are likely to occur (Ollerenshaw and Smith, 1969). Unlike ovine PGE, nematodiriasis occurs in young lambs in spring and is sudden in onset with acute signs including



severe diarrhoea and dehydration.

Cross transmission of N. battus from sheep to cattle according to the previously mentioned categorisation by Morley and Donald (1980), should be very low with little reproduction potential and thus be negligible and of little significance. However, it has been shown in Norway by Helle (1981) that calves which graze pastures previously grazed by sheep, can excrete N. battus eggs. Furthermore the results of studies in the U.K. (Bairden and Armour, 1987; Armour et al, 1988) show that such patent infections in calves can give rise to a marked increase in the numbers of infective N. battus larvae on the pasture in the following spring and that these larvae are still infective to calves. Similar results have been obtained by Coop et al (1991).

The results of a field study by Bairden and Armour (1987), some of which are included in this thesis, raise several important questions. First the use of a management system considered effective in the control of bovine PGE in which young cattle and sheep graze a pasture in alternate years may not prevent the establishment of N. battus in grazing calves in subsequent years. Secondly it highlights the question of possible adverse effects of N. battus infections on the productivity of young cattle.

In this field study there was an increase in the numbers of N. battus infective larvae present on the pasture during the autumn. Autumn hatching of N. battus eggs has been shown to occur on experimentally contaminated plots (Gibson and Everett,

1981) and can result in autumn nematodiriasis under field conditions (McKellar, Bairden, Duncan and Armour, 1983; Rodger, 1983). The strong post-infection immunity, which characterises lamb nematodiriasis, appeared to develop in the calves in this study in that all had excreted N. battus eggs in the spring but no N. battus worms were recovered from them at necropsy in the autumn. Although these animals had been given a mid-season salvage anthelmintic treatment, the considerable numbers of N. battus acquired by the autumn grazed tracer calves indicated a high level of immunity of the principal calves to this parasite.

In studies by Mitchell, Mathieson and Fitzsimons (1985) in which a three year rotation involving cattle, sheep and a crop was involved, moderate infections of N. battus were recorded in the lambs which was suggested to be due to a combination of the cycling of the infection by the young beef cattle and the longevity of the free-living larval stages of the parasite. If it were possible to substitute suckler cows and their calves for weaned young cattle in such three year rotational systems the presence of the immune adult cows would be likely to reduce the level of infection of N. battus in the calves and thus reduce the risk to sheep in the following year. It is perhaps of interest to record that in field studies currently underway (1991 unpublished data) and those of the 1989/90 grazing seasons (MacLean, Bairden, Holmes, Mulligan and McWilliam, 1991) clinical disease, in part attributed to N. battus infection, has necessitated very early therapeutic treatment of young calves i.e. between eight and ten days post-turnout.

In the first of the experimental studies, designed to determine the infective dose of N. battus necessary to produce clinical signs in calves, the almost total lack of parasite establishment was disappointing. There are several possible reasons for the very low numbers of worms which were recovered at necropsy. It may have been that the infective larvae completed their development to adults and were then rapidly eliminated as has been shown to occur between 18 and 34 days post-infection in lambs (Mapes, Coop and Angus, 1973). If this were the case higher egg counts than those observed (<50 eggs/gram) should have been recorded during this period. Alternatively the life cycle may not have been completed in the calves with either poor larval establishment taking place or only partial development occurring. Finally it is possible that the level of challenge was simply not high enough for significant parasite establishment to occur. In this respect, however, lambs given small numbers of N. battus e.g. 2,000 L<sub>3</sub> as compared with higher levels (up to 60,000 L<sub>3</sub>) have been shown to carry the infection for much longer without degenerative changes in the parasites (Martin and Lee, 1976).

In the field situation mixed infections are the rule and may influence successful establishment of N. battus in calves and the second experimental study was designed to test this hypothesis. Again, as in the first dose titration experiment very low levels of N. battus parasites established in the experimentally infected animals. Considering the high field infectivity of the larvae from which this isolate was derived this result was surprising.

However it should also be remembered that in the field situation there is a daily intake of infective larvae and perhaps a trickle challenge would have produced a higher level of worm establishment.

The level of challenge in these studies was determined partly by larval availability. A number of problems were encountered in the recovery of N. battus eggs from bovine faeces and their subsequent culture to the infective stage. The main difficulty found was that with the low egg output from the donor animals a very large amount of faeces had to be processed. In addition the consistency of bovine faeces, compared to that of sheep, made it more difficult to process in all respects. This was illustrated by the fact that the recovery of eggs from lamb faeces was much higher (>80%) than that from a similar quantity of bovine faeces.

The main egg recovery method used was that detailed in the Ministry of Agriculture Fisheries and Food handbook (1986) by which a relatively clean suspension of N. battus eggs is recovered from faeces by a combination of flotation and sieving. Cleanliness of the suspension was critical as, following incubation, the eggs had to be physically "cracked" to liberate the larvae. With regard to the time and temperature of incubation in N. battus L<sub>3</sub> production, many combinations with varying degrees of success have been claimed. In one of the simplest methods used (Dr. R. Connan - personal communication) eggs, dirty or clean, were left in water under a laboratory bench

for up to three months and then cracked by refrigeration at 4°C for one to two weeks. By far the most successful technique was that of Coop and Jackson (personal communication) where small volumes of the egg suspension were pressed between glass plates and the liberated larvae washed into a container. An alternative means of "cracking" the eggs was to add glass beads to the suspension and shake vigorously. A variable success rate was obtained using this technique.

Another exasperating feature of our N. battus larval culture was that individual eggs appeared to require different periods of incubation. This led to a system of "milking" the culture by cracking and removing the motile larvae by Baermannisation and then reincubating the remaining non-hatched eggs before repeating the procedure. The larvae obtained from each successive "milking" were visually less motile and from general appearance probably less viable than the first crop.

When the gross and histopathological findings are considered it would appear that there was a considerable immunological reaction which seemed to trap larval stages and prevent them from completing their development. Increased numbers of eosinophils were observed in nodules associated with the presence of parasite debris. There was also extensive mononuclear infiltration with a scarcity of polymorphonuclear leucocytes which might indicate that the animals were effectively overcoming the infection. It is interesting to note that one animal, given 15,000 N. battus L<sub>3</sub> in the dose titration study, exhibited transient diarrhoea on days 13-14 and had higher numbers of worms at post-mortem. This

may have been due to a less effective immunological response in this individual animal.

While age immunity does occur in lambs (Gibson, 1959; Mapes and Coop, 1973) it is unlikely to have affected the results of this study since as shown by Armour et al, 1988, bovine clinical nematodiriasis occurred in calves which were approximately the same age as the animals used in this study. Also calves of a similar age which were currently grazing the area from which the larval strain was isolated were shown to be cycling N. battus.

Patency has been observed in the field by several workers (Helle, 1981; Bairden and Armour, 1987; Coop et al, 1988) with alternate grazing systems possibly exerting selection pressure for a strain of N. battus that is adapted for calves. Calves grazing pastures on which N. battus larval levels were similar to those in corresponding sheep paddocks had lower faecal egg outputs than the sheep (Coop et al, 1988). This was despite the fact that calves, by ingesting more herbage, would be exposed to higher levels of challenge. Although it would be unwise to assume that all the N. battus L<sub>3</sub> were infective to calves it is possible that the bovine host is better equipped to deal with such an infection than its ovine counterpart. It may also prove to be the case that given time this "adapting strain" might increase in virulence resulting in a more severe clinical syndrome of nematodiriasis in cattle.

There may be other contributory factors which are important in the manifestation of clinical nematodiriasis in calves. Apart

from age or acquired immunity the plane of nutrition may be a factor influencing the percentage establishment and development of N. battus stages (Rowlands and Probert, 1972). Concurrent infection with other parasites such as Eimeria spp may also compromise the immune response of Nematodirus allowing increased establishment and higher faecal egg outputs as has been shown in lambs (Catchpole and Harris, 1989). In this context it is interesting to note that Haemonchus appears able to decrease the percentage take of N. battus in lambs by altering the chemical environment in the abomasum/small intestine, preventing initiation of the mucosal stage of development (Mapes and Coop, 1970).

The results of the studies described in this thesis while showing that there are a number of ways of effectively controlling bovine parasitic gastroenteritis (PGE), draw attention to several potentially serious problems associated with such control measures. For example prophylactic anthelmintic treatment of first year grazing calves appeared to impair the acquisition of immunity during a second grazing season. Also, while control of bovine PGE by grazing management over a four year period seemed to be effective in the second and third years there was a subsequent failure to prevent a significant level of pasture contamination and the acquisition of substantial worm burdens. The unexpected cycling of N. battus and the resultant clinical nematodiriasis in set stocked calves several years after the end of the grazing management study illustrates a potentially serious drawback to parasite control by grazing management in

areas where N. battus occurs.

Finally, in a short communication, "The Eradication of Parasitic Disease", Spedding (1969) concludes that it may be doubted whether many general conclusions could apply to all parasitic diseases of all farm animals and that methods of disease eradication have all to be assessed in relation to whole husbandry systems. This remains a valid assessment of present day helminth control.



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## **APPENDICES**

# Appendix A

## Anthelmintic Prophylaxis Study

### Individual and Group Mean Bodyweights - 1988

#### Group 1 - Ivermectin at 3, 8 and 13 Weeks

No.	5/5	28/5	30/6	4/8	1/9	29/9	20/10
Y31	390	380	410	430	420	-	-
Y32	295	310	340	380	380	-	-
Y33	280	280	325	365	375	380	380
Y34	260	270	305	325	340	320	315
Y35	210	205	285	320	330	235	340
Y36	185	230	240	275	265	265	270
Y37	185	205	245	280	285	300	295
Y38	175	190	235	260	260	270	275
Y39	170	195	230	270	285	300	-
Y40	155	180	220	255	265	255	-
Y41	145	160	205	235	235	-	-
Y42	120	135	175	210	230	-	-
Y43	245	250	285	315	330	325	-
Y44	170	180	210	240	255	245	-
Y45	190	205	255	280	290	280	-
Y46	160	170	210	220	225	235	-
Y47	325	320	365	385	380	375	-
Y48	225	235	260	265	260	270	-
Y49	285	300	340	370	370	365	360
Y50	230	240	275	295	305	300	295
Y51	210	220	270	305	320	310	305
Y52	205	215	245	260	260	-	-
Y53	195	205	240	270	295	-	-
Y54	250	265	300	325	335	330	335
Y55	175	190	230	275	290	295	300
Y56	230	240	280	305	315	320	320
Y57	155	170	205	230	245	-	-
Y58	150	160	200	210	220	-	-
Mean	213	225	264	291	299	299	316
SD	61	56	55	56	53	43	33

# Appendix A (Cont'd)

## Group 2 - Untreated Control

No.	5/5	28/5	30/6	4/8	1/9	29/9	20/10
B61	355	360	395	415	420	-	-
B62	315	310	350	365	360	-	-
B63	280	295	335	355	340	355	340
B64	265	270	315	345	355	355	355
B65	210	220	255	280	300	305	300
B66	200	215	265	285	280	270	265
B67	180	200	240	265	270	265	265
B68	175	185	215	240	240	245	250
B69	265	170	195	220	210	220	-
B70	160	180	210	255	245	260	-
B71	135	145	190	195	180	-	-
B72	130	140	180	205	215	-	-
B73	210	210	235	245	250	255	-
B74	205	215	260	275	280	265	-
B75	200	210	255	275	270	270	-
B76	160	170	215	250	245	250	-
B77	280	285	320	335	335	335	-
B78	245	245	285	300	300	310	-
B79	285	280	340	365	380	385	380
B80	230	240	280	310	320	335	320
B81	210	215	260	285	280	290	290
B82	205	210	240	255	260	-	-
B83	200	220	260	295	290	-	-
B84	250	255	295	315	300	295	285
B85	205	220	260	280	285	285	270
B86	225	240	295	330	335	320	315
B87	155	175	220	240	220	-	-
B88	115	125	155	185	185	-	-
Mean	213	222	261	284	284	294	303
SD	56	53	56	55	50	43	40



Appendix A (Cont'd)

Group 3 - MSRT Bolus at Turnout

No.	5/5	28/5	30/6	4/8	1/9	29/9	20/10
G1	405	405	440	455	445	-	-
G2	295	305	345	375	380	-	-
G3	270	280	325	350	380	370	365
G4	265	270	315	330	240	345	340
G5	210	220	255	280	300	305	290
G6	175	190	235	260	280	275	270
G7	180	195	230	260	280	285	270
G8	180	195	245	275	285	280	275
G9	165	185	220	250	260	265	-
G10	160	175	200	230	255	260	-
G11	135	155	185	205	240	-	-
G12	130	140	175	195	215	-	-
G13	240	245	295	305	315	310	-
G14	205	215	260	275	290	285	-
G15	170	170	205	235	245	245	-
G16	160	170	210	245	265	265	-
G17	265	270	305	310	310	320	-
G18	260	270	300	315	320	330	-
G19	255	270	305	340	335	345	335
G20	235	250	310	340	360	370	365
G21	215	220	265	305	330	320	295
G22	205	220	265	300	320	-	-
G23	200	220	260	285	300	-	-
G24	235	250	300	335	350	350	345
G25	200	220	275	310	325	325	320
G26	250	275	320	345	365	350	355
G27	155	160	195	220	240	-	-
G28	115	120	150	175	190	-	-
Mean	212	224	264	289	301	310	319
SD	60	59	61	60	56	38	37

**Anthelmintic Prophylaxis Study**  
**Statistical analysis of liveweight data**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 17-Jul-91

Experiment title..... Appendix A  
 Number of treatments... 3

	Description:	No. of obsvs.
	-----	-----
Treatment 1	Ivermectin	7
Treatment 2	Control	7
Treatment 3	MSRT	7

Replicate	Treatments 1	2	3
1	213.00	213.00	212.00
2	225.00	222.00	224.00
3	264.00	261.00	264.00
4	291.00	284.00	289.00
5	299.00	284.00	301.00
6	299.00	294.00	310.00
7	316.00	303.00	319.00

ONE WAY ANALYSIS OF VARIANCE :  
 =====

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	267.81	2.00	133.90	0.09
Experimental error	27797.43	18.00	1544.30	
Total	28065.24	20.00		

Critical value for a 5% significance test is  $F_{.95}(2,18) = 3.55$

Since the calculated F-value is less than the critical F-value, then the data suggests that treatment effects are not different.

NEWMAN KEULS MULTIPLE RANGE TEST :  
 =====

K	Means	Ranges	L.S.R.	Treatment	St. Error
-	-----	-----	-----	-----	-----
2	265.86			2	
2	272.43	2.97	44.11	1	14.853
3	274.14	3.61	53.62	3	

Treatment Significantly  
 Comparison      Different

3 V 2	No
3 V 1	No
1 V 2	No

# Appendix B

## Anthelmintic Prophylaxis Study

### Individual and Group Mean Pepsinogen Levels - 1988

#### Group 1 - Ivermectin at 3, 8 and 13 Weeks

No.	5/5	28/5	30/6	4/8	1/9	29/9	20/10
Y31	640	1,253	873	901	1,026	-	-
Y32	532	955	678	701	838	-	-
Y33	780	1,007	911	1,046	1,076	1,034	954
Y34	611	956	743	781	825	796	670
Y35	815	828	1,012	1,046	1,114	953	880
Y36	690	1,443	676	766	698	655	584
Y37	858	788	875	673	861	782	719
Y38	598	1,035	848	995	1,178	1,071	867
Y39	610	686	487	594	837	732	-
Y40	961	738	875	914	861	1,009	-
Y41	529	711	607	727	673	-	-
Y42	506	970	1,197	1,832	1,645	-	-
Y43	597	1,021	795	899	1,036	1,017	-
Y44	598	982	959	1,114	1,228	1,130	-
Y45	716	867	813	954	1,216	1,133	-
Y46	543	659	624	741	900	717	-
Y47	663	1,097	1,028	875	886	908	-
Y48	939	1,380	1,235	1,247	1,190	1,079	-
Y49	594	956	736	713	900	910	880
Y50	569	956	777	753	1,152	956	830
Y51	635	993	982	861	1,013	1,358	929
Y52	544	738	662	769	874	-	-
Y53	687	1,058	973	1,086	1,216	-	-
Y54	451	1,021	716	741	1,114	970	736
Y55	437	594	621	713	862	708	733
Y56	776	1,228	1,009	1,100	1,252	1,134	730
Y57	477	801	697	832	824	-	-
Y58	677	1,343	1,034	927	989	-	-
Mean	644	967	837	904	1,010	953	793
SD	136	219	182	242	208	180	113

# Appendix B (Cont'd)

## Group 2 - Untreated Control

No.	5/5	28/5	30/6	4/8	1/9	29/9	20/10
B61	843	1,203	1,399	1,418	4,611	-	-
B62	577	1,434	1,188	1,838	3,833	-	-
B63	693	764	989	1,470	3,202	2,598	1,654
B64	825	985	1,641	2,409	5,686	4,056	4,174
B65	617	906	1,275	1,863	3,535	2,912	2,497
B66	694	738	763	1,523	2,869	4,470	3,261
B67	519	841	773	1,279	3,772	2,848	2,407
B68	892	1,126	1,223	2,005	4,067	4,355	3,384
B69	511	597	875	1,041	2,634	2,136	-
B70	734	791	943	1,569	3,981	2,149	-
B71	796	1,190	1,327	1,383	3,485	-	-
B72	722	726	1,078	1,342	4,932	-	-
B73	475	1,100	1,281	1,873	5,067	4,645	-
B74	817	1,113	1,346	1,645	4,576	4,483	-
B75	841	751	864	1,205	2,707	2,548	-
B76	788	778	1,030	1,458	5,640	5,011	-
B77	938	1,152	1,249	1,760	5,032	4,093	-
B78	795	856	861	1,340	5,166	2,920	-
B79	772	970	955	1,559	6,369	2,341	2,918
B80	744	867	938	1,090	3,560	1,931	1,768
B81	724	984	923	1,598	6,060	4,123	3,020
B82	850	971	734	1,116	3,922	-	-
B83	475	738	1,008	1,968	6,876	-	-
B84	452	984	944	1,052	2,892	1,713	1,361
B85	624	1,062	1,084	1,241	4,316	2,507	2,249
B86	1,088	1,216	1,500	2,363	4,957	3,049	3,416
B87	689	919	816	1,335	4,826	-	-
B88	709	1,086	1,666	2,998	8,521	-	-
Mean	722	959	1,095	1,598	4,539	3,244	2,676
SD	151	193	261	453	1,364	1,043	836

# Appendix B (Cont'd)

## Group 3 - MSRT Bolus at Turnout

No.	5/5	28/5	30/6	4/8	1/9	29/9	20/10
G1	674	938	944	885	956	-	-
G2	1,078	757	727	804	750	-	-
G3	829	953	959	923	1,270	1,553	2,331
G4	660	771	805	860	739	841	671
G5	564	707	830	734	930	1,222	888
G6	787	1,108	909	881	1,013	1,147	837
G7	553	615	655	604	696	830	658
G8	709	723	768	797	871	1,136	824
G9	725	879	1,208	1,007	1,129	1,591	-
G10	1,650	1,056	939	901	1,426	1,628	-
G11	697	719	961	969	1,614	-	-
G12	710	993	1,128	1,070	1,355	-	-
G13	594	836	889	834	841	1,235	-
G14	555	718	966	705	757	778	-
G15	643	656	709	808	1,053	1,363	-
G16	563	759	839	932	1,034	1,501	-
G17	647	915	880	755	682	830	-
G18	760	861	1,001	858	1,066	1,375	-
G19	616	786	806	793	792	740	646
G20	1,103	1,017	1,071	905	953	1,197	1,041
G21	676	1,060	980	984	1,065	1,223	812
G22	688	706	932	781	1,158	-	-
G23	714	850	885	881	1,065	-	-
G24	728	850	903	894	1,085	1,133	888
G25	505	758	850	770	857	956	773
G26	898	953	1,219	1,045	1,528	2,048	1,182
G27	780	629	514	657	913	-	-
G28	700	669	902	962	1,621	-	-
Mean	743	830	899	857	1,044	1,216	963
SD	226	140	153	112	268	339	458

**Anthelmintic Prophylaxis Study**  
**Statistical analysis of pepsinogen data**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 17-Jul-91

Experiment title..... Appendix B  
 Number of treatments... 3

	Description:	No. of obsvs.
	-----	-----
Treatment 1	Ivermectin	7
Treatment 2	Control	7
Treatment 3	MSRT	7

Replicate	Treatments 1	2	3
1	644.00	722.00	743.00
2	967.00	959.00	830.00
3	837.00	1095.00	899.00
4	904.00	1598.00	857.00
5	1010.00	4539.00	1044.00
6	953.00	3244.00	1216.00
7	793.00	2676.00	963.00

ONE WAY ANALYSIS OF VARIANCE :  
 =====

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	6899891.52	2.00	3449945.76	5.05
Experimental error	12291489.71	18.00	682860.54	
Total	19191381.24	20.00		

Critical value for a 5% significance test is  $F_{.95}(2,18) = 3.55$

Since the calculated F-value is greater than or equal to the critical F-value, then the data suggests that treatment effects are different.

NEWMAN KEULS MULTIPLE RANGE TEST :  
 =====

K	Means	Ranges	L.S.R.	Treatment	St. Error
-	-----	-----	-----	-----	-----
	872.57			1	
2	936.00	2.97	927.63	3	312.332
3	2119.00	3.61	1127.52	2	

Treatment Significantly  
 Comparison Different

2 V 1	Yes
2 V 3	Yes
3 V 1	No

# Appendix C

## Anthelmintic Prophylaxis Study

### Individual and Group Mean Faecal Egg Counts - 1988

#### Group 1 - Ivermectin at 3, 8 and 13 Weeks

No.	5/5	28/5	30/6	4/8	1/9	29/9	20/10
Y31	0	0	100	50	50	-	-
Y32	0	0	0	0	50	-	-
Y33	0	50	0	0	50	50	0
Y34	0	0	50	200	50	0	250
Y35	0	0	0	50	0	100	100
Y36	0	0	0	0	0	50	0
Y37	0	0	0	0	0	150	50
Y38	0	0	0	0	0	NS	150
Y39	0	0	50	0	0	100	-
Y40	0	0	0	0	50	150	-
Y41	0	0	0	50	50	-	-
Y42	0	0	0	0	50	-	-
Y43	0	0	0	0	0	150	-
Y44	0	0	0	0	0	0	-
Y45	0	0	0	0	0	0	-
Y46	0	0	0	0	0	100	-
Y47	0	0	0	0	50	100	-
Y48	0	0	0	0	50	50	-
Y49	0	0	0	0	0	100	150
Y50	0	0	100	100	50	0	100
Y51	0	0	0	100	0	0	50
Y52	0	0	0	100	0	-	-
Y53	0	0	0	0	0	-	-
Y54	0	0	50	100	100	700	NS
Y55	0	0	0	0	200	150	100
Y56	0	0	0	0	0	50	0
Y57	0	0	0	50	50	-	-
Y58	0	0	0	50	50	-	-
Mean	0	2	13	30	32	105	86
SD	0	9	29	50	43	154	78

# Appendix C (Cont'd)

## Group 2 - Untreated Control

No.	5/5	28/5	30/6	4/8	1/9	29/9	20/10
B61	0	0	50	0	50	-	-
B62	0	0	250	900	550	-	-
B63	0	0	300	100	50	50	0
B64	0	0	0	50	50	50	0
B65	0	0	50	50	200	50	50
B66	0	0	300	0	250	0	100
B67	0	0	50	200	100	0	50
B68	0	0	100	0	50	150	100
B69	0	0	0	200	50	50	-
B70	0	0	250	200	100	50	-
B71	0	0	150	0	150	-	-
B72	0	0	150	100	250	-	-
B73	0	0	0	0	600	50	-
B74	0	0	100	100	900	0	-
B75	0	0	150	100	150	0	-
B76	0	0	0	150	300	0	-
B77	0	0	150	50	250	50	-
B78	0	0	200	100	250	50	-
B79	0	0	350	0	100	0	0
B80	0	0	300	200	200	0	50
B81	0	0	250	0	250	0	150
B82	0	0	0	400	500	-	-
B83	0	0	150	50	350	-	-
B84	0	0	400	400	200	0	150
B85	0	0	100	350	150	50	200
B86	0	0	150	0	100	0	50
B87	0	0	150	450	150	-	-
B88	0	0	0	0	50	-	-
Mean	0	0	146	148	227	30	75
SD	0	0	118	200	199	38	66



Appendix C (Cont'd)

Group 3 - MSRT Bolus at Turnout

No.	5/5	28/5	30/6	4/8	1/9	29/9	20/10
G1	0	0	0	0	0	-	-
G2	0	0	0	0	0	-	-
G3	0	0	0	0	0	50	0
G4	0	0	0	NS	0	300	0
G5	0	0	0	0	0	0	0
G6	0	0	0	0	0	100	50
G7	0	0	0	0	0	50	0
G8	0	0	0	0	50	100	250
G9	0	0	50	0	0	100	-
G10	0	0	0	50	0	50	-
G11	0	0	0	0	100	-	-
G12	0	0	0	0	50	-	-
G13	0	0	50	0	0	0	-
G14	0	0	0	0	0	100	-
G15	0	0	0	0	0	0	-
G16	0	0	0	50	0	0	-
G17	0	0	0	0	0	100	-
G18	0	0	0	0	0	100	-
G19	0	0	0	0	50	0	100
G20	0	0	0	50	0	0	150
G21	0	0	0	0	0	150	0
G22	0	0	0	50	0	-	-
G23	0	0	0	0	0	-	-
G24	0	0	0	0	0	400	0
G25	0	0	0	50	0	0	100
G26	0	0	0	0	0	0	50
G27	0	0	0	0	0	-	-
G28	0	0	0	0	0	-	-
Mean	0	0	4	9	9	80	58
SD	0	0	13	20	24	106	79

**Anthelmintic Prophylaxis Study**  
**Statistical analysis of faecal egg count data**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 17-Jul-91

Experiment title..... Appendix C  
 Number of treatments... 3

		Description:	No. of obsvs.
		-----	-----
Treatment 1	Ivermectin		7
Treatment 2	Control		7
Treatment 3	MSRT		7

Replicate	Treatments		
	1	2	3
1	0.00	0.00	0.00
2	2.00	0.00	0.00
3	13.00	146.00	4.00
4	30.00	148.00	9.00
5	32.00	227.00	9.00
6	105.00	30.00	80.00
7	86.00	75.00	58.00

ONE WAY ANALYSIS OF VARIANCE :

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	0.99	2.00	0.49	0.68
Experimental error	13.08	18.00	0.73	
Total	14.07	20.00		

Critical value for a 5% significance test is  $F_{.95}(2,18) = 3.55$

Since the calculated F-value is less than the critical F-value, then the data suggests that treatment effects are not different.

NEWMAN KEULS MULTIPLE RANGE TEST :

K	Means	Ranges	L.S.R.	Treatment	St. Error
-	0.91			3	
2	1.23	2.97	0.96	1	0.322
3	1.44	3.61	1.16	2	

Treatment Comparison	Significantly Different
2 V 3	No
2 V 1	No
1 V 3	No

GEOMETRIC Treat.MEANS	
1	15.92
2	26.46
3	7.15

# Appendix D

## Anthelmintic Prophylaxis Study

### Ostertagia and Cooperia Larval Recoveries per

#### Kilogram Dried Herbage - 1988

##### Paddock 1 - Grazed by Ivermectin Treated Calves

	5/5	28/5	30/6	4/8	1/9	29/9	20/10
O.o.	0	47	462	256	123	588	1,795
C.o.	0	0	154	0	0	0	1,026

##### Paddock 2 - Grazed by Control Calves

	5/5	28/5	30/6	4/8	1/9	29/9	20/10
O.o.	143	50	200	1,233	6,400	6,486	7,037
C.o.	0	0	0	129	400	1,892	926

##### Paddock 3 - Grazed by MSRT Bolused Calves

	5/5	28/5	30/6	4/8	1/9	29/9	20/10
O.o.	0	142	0	0	0	606	1,220
C.o.	0	0	0	0	0	303	244

**Anthelmintic Prophylaxis Study**  
**Statistical analysis of O.ostertagi pasture**  
**larval recovery data**

ANIMAL DESIGNS... One Way anova then Range Test      Date... 29-Jul-91

Experiment title..... Appendix D OoL3

Number of treatments... 3

	Description:	No. of obsvs.
Treatment 1	Ivermectin	7
Treatment 2	Control	7
Treatment 3	MSRT	7

Replicate	Treatments 1	2	3
1	0.00	143.00	0.00
2	47.00	50.00	142.00
3	462.00	200.00	0.00
4	256.00	1233.00	0.00
5	123.00	6400.00	0.00
6	588.00	6486.00	606.00
7	1795.00	7037.00	1220.00

**ONE WAY ANALYSIS OF VARIANCE :**

=====

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	11.55	2.00	5.77	4.26
Experimental error	24.39	18.00	1.35	
Total	35.93	20.00		

Critical value for a 5% significance test is  $F_{.95}(2,18) = 3.55$

Since the calculated F-value is greater than or equal to the critical F-value, then the data suggests that treatment effects are different.

**NEWMAN KEULS MULTIPLE RANGE TEST :**

=====

K	Means	Ranges	L.S.R.	Treatment	St. Error
-	1.15			3	
2	2.12	2.97	1.31	1	0.440
3	2.96	3.61	1.59	2	

Treatment Significantly  
Comparison Different

2 V 3	Yes
2 V 1	No
1 V 3	No

GEOMETRIC  
Treat.MEANS

1	132.33
2	912.87
3	13.01

**Anthelmintic Prophylaxis Study**  
**Statistical analysis of C. oncophora pasture**  
**larval recovery data**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 29-Jul-91

Experiment title..... Appendix D CoL3

Number of treatments... 3

	Description:	No. of obsvs.
Treatment 1	Ivermectin	7
Treatment 2	Control	7
Treatment 3	MSRT	7

Replicate	Treatments 1	2	3
1	0.00	0.00	0.00
2	0.00	0.00	0.00
3	154.00	0.00	0.00
4	0.00	129.00	0.00
5	0.00	400.00	0.00
6	0.00	1892.00	303.00
7	1026.00	926.00	244.00

ONE WAY ANALYSIS OF VARIANCE :

=====

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	3.35	2.00	1.68	0.94
Experimental error	32.11	18.00	1.78	
Total	35.46	20.00		

Critical value for a 5% significance test is  $F_{.95}(2,18) = 3.55$

Since the calculated F-value is less than the critical F-value, then the data suggests that treatment effects are not different.

NEWMAN KEULS MULTIPLE RANGE TEST :

=====

K	Means	Ranges	L.S.R.	Treatment	St. Error
-	0.70			3	
2	0.74	2.97	1.50	1	0.505
3	1.57	3.61	1.82	2	

Treatment Comparison	Significantly Different
----------------------	-------------------------

		GEOMETRIC Treat. MEANS
2 V 3	No	1    4.54
2 V 1	No	2    35.80
1 V 3	No	3    3.97

# Appendix E

## Anthelmintic Prophylaxis Study

### Individual and Group Mean Bodyweights - 1989

No.	4/5	1/6	6/7	3/8	1/9	4/10
Y44	295	355	400	425	440	450
Y46	270	305	380	420	440	430
Y48	310	330	360	380	365	360
Y58	265	300	370	405	405	400
Mean	285	323	378	408	413	410
SD	21	25	17	20	36	39
No.	4/5	1/6	6/7	3/8	1/9	4/10
B72	285	325	365	390	410	440
B75	345	385	440	470	365	480
B76	255	325	370	405	410	415
B78	315	350	375	400	400	410
Mean	300	346	388	416	421	436
SD	39	28	35	36	30	32
No.	4/5	1/6	6/7	3/8	1/9	4/10
G5	275	330	375	395	400	405
G15	270	300	360	400	420	415
G16	330	365	410	440	440	455
G27	270	295	355	390	395	400
Mean	286	323	375	406	414	419
SD	29	32	25	23	21	25

**Anthelmintic Prophylaxis Study**  
**Statistical analysis of liveweight data**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 17-Jul-91

Experiment title..... Appendix E

Number of treatments... 3

	Description:	No. of obsvs.
	-----	-----
Treatment 1      Ex-Ivermectin		6
Treatment 2      Ex-Control		6
Treatment 3      Ex-MSRT		6

Replicate	Treatments 1	2	3
1	285.00	300.00	286.00
2	323.00	346.00	323.00
3	378.00	388.00	375.00
4	408.00	416.00	406.00
5	413.00	421.00	414.00
6	410.00	436.00	419.00

ONE WAY ANALYSIS OF VARIANCE :

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	844.00	2.00	422.00	0.15
Experimental error	42922.50	15.00	2861.50	
Total	43766.50	17.00		

Critical value for a 5% significance test is  $F_{.95}(2,15) = 3.68$

Since the calculated F-value is less than the critical F-value, then the data suggests that treatment effects are not different.

NEWMAN KEULS MULTIPLE RANGE TEST :

K	Means	Ranges	L.S.R.	Treatment	St. Error
-	-----	-----	-----	-----	-----
	369.50			1	
2	370.50	3.01	65.73	3	21.838
3	384.50	3.67	80.15	2	

Treatment Significantly  
Comparison      Different

2 V 1	No
2 V 3	No
3 V 1	No

# Appendix F

## Anthelmintic Prophylaxis Study

### Individual and Group Mean Pepsinogen Levels - 1989

No.	4/5	1/6	6/7	7/8	1/9	4/10
Y44	719	1,188	1,318	1,641	2,268	2,528
Y46	724	825	1,059	1,753	1,546	1,843
Y48	1,022	1,211	1,237	1,408	1,523	1,775
Y58	588	1,253	1,228	1,086	1,528	1,692
Mean	763	1,119	1,211	1,472	1,716	1,960
SD	184	190	109	205	368	384
No.	4/5	1/6	6/7	7/8	1/9	4/10
B72	1,495	1,351	1,273	1,889	1,789	1,720
B75	1,088	1,824	1,564	1,347	1,563	1,555
B76	2,210	2,532	2,150	2,048	2,124	1,898
B78	3,759	6,447	5,575	3,682	4,033	3,611
Mean	2,138	3,039	2,641	2,242	2,377	2,196
SD	1,176	2,324	1,990	1,006	1,120	954
No.	4/5	1/6	6/7	7/8	1/9	4/10
G5	1,099	1,663	1,350	1,483	1,752	2,021
G15	1,066	1,626	1,448	1,508	2,454	2,364
G16	731	1,221	1,408	1,717	2,177	2,432
G27	681	1,600	1,582	1,507	2,386	2,337
Mean	894	1,528	1,447	1,554	2,192	2,289
SD	219	206	99	109	316	183



**Anthelmintic Prophylaxis Study**  
**Statistical analysis of pepsinogen data**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 17-Jul-91

Experiment title..... Appendix F

Number of treatments... 3

	Description:	No. of obsvs.
Treatment 1	Ex-Ivermectin	6
Treatment 2	Ex-Control	6
Treatment 3	Ex-MSRT	6

Replicate	Treatments 1	2	3
1	763.00	2138.00	894.00
2	1119.00	3039.00	1528.00
3	1211.00	2641.00	1447.00
4	1472.00	2242.00	1554.00
5	1716.00	2377.00	2192.00
6	1960.00	2196.00	2289.00

ONE WAY ANALYSIS OF VARIANCE :

=====

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	3665926.33	2.00	1832963.17	9.59
Experimental error	2866911.67	15.00	191127.44	
Total	6532838.00	17.00		

Critical value for a 5% significance test is  $F_{.95}(2,15) = 3.68$

Since the calculated F-value is greater than or equal to the critical F-value, then the data suggests that treatment effects are different.

NEWMAN KEULS MULTIPLE RANGE TEST :

=====

K	Means	Ranges	L.S.R.	Treatment	St. Error
-	1373.50			1	
2	1650.67	3.01	537.22	3	178.478
3	2438.83	3.67	655.02	2	

Treatment Significantly  
Comparison Different

2 V 1	Yes
2 V 3	Yes
3 V 1	No

# Appendix G

## Anthelmintic Prophylaxis Study

### Individual and Group Mean Faecal Egg Counts - 1989

No.	4/5	1/6	6/7	3/8	1/9	4/10
Y44	0	0	0	0	150	0
Y46	50	0	0	0	150	0
Y48	0	0	0	0	NS	0
Y58	0	0	0	0	50	0
Mean	13	0	0	0	117	0
SD	25	0	0	0	58	0
No.	4/5	1/6	6/7	3/8	1/9	4/10
B72	0	0	100	0	50	0
B75	50	0	50	0	0	0
B76	50	200	50	0	50	0
B78	50	0	0	0	0	0
Mean	38	50	50	0	25	0
SD	25	100	41	0	29	0
No.	4/5	1/6	6/7	3/8	1/9	4/10
G5	0	0	50	0	0	100
G15	0	100	0	0	50	0
G16	50	0	50	0	50	0
G27	50	0	0	0	150	0
Mean	25	25	25	0	63	0
SD	29	50	29	0	63	50

**Anthelmintic Prophylaxis Study**  
**Statistical analysis of faecal egg count data**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 17-Jul-91

Experiment title..... Appendix G  
 Number of treatments... 3

	Description:	No. of obsvs.
Treatment 1	Ex-Ivermectin	6
Treatment 2	Ex-Control	6
Treatment 3	Ex-MSRT	6

Replicate	Treatments 1	2	3
1	13.00	38.00	25.00
2	0.00	50.00	25.00
3	0.00	50.00	25.00
4	0.00	0.00	0.00
5	117.00	25.00	63.00
6	0.00	0.00	0.00

ONE WAY ANALYSIS OF VARIANCE :

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	1.02	2.00	0.51	0.73
Experimental error	10.54	15.00	0.70	
Total	11.56	17.00		

Critical value for a 5% significance test is  $F_{.95}(2,15) = 3.68$

Since the calculated F-value is less than the critical F-value, then the data suggests that treatment effects are not different.

NEWMAN KEULS MULTIPLE RANGE TEST :

K	Means	Ranges	L.S.R.	Treatment	St. Error
-	0.54			1	
2	1.01	3.01	1.03	3	0.342
3	1.07	3.67	1.26	2	

Treatment Comparison	Significantly Different	GEOMETRIC Treat.MEANS
2 V 1	No	1 2.44
2 V 3	No	2 10.75
3 V 1	No	3 9.20

Appendix H

Anthelmintic Prophylaxis Study

Ostertagia and Cooperia Larval Recoveries per Kilogram

Dried Herbage - 1989

Paddock 2 - Former Control Area Grazed by All Cattle

	4/5	1/6	6/7	3/8	1/9	4/10
O.o.	1,053	385	526	588	4,230	3,333
C.o.	0	0	0	294	1,438	0

# Appendix I

## Alternate Grazing Study

### Individual and Group Mean Pepsinogen Levels

#### Paddock 1 - Permanent Cattle Grazing

Year - 1982												
Date												
No.	10/5	24/5	7/6	21/6	5/7	19/7	2/8	16/8	30/8	13/9	Nec	
21	754	843	1,564	2,046	2,113	2,461	3,172	3,264	4,111	4,095	5,632	-
37	843	1,011	1,453	1,955	2,548	2,573	2,954	3,011	3,178	3,999	4,154	-
48	762	976	1,784	1,843	2,067	2,340	2,862	3,143	3,842	4,65	4,724	-
53	659	859	1,275	1,752	2,347	2,641	2,970	3,652	4,153	4,821	5,430	-
56	872	1,011	1,407	1,948	2,488	2,782	3,112	3,420	3,872	4,111	4,963	-
60	904	1,102	1,153	1,346	1,972	2,074	2,134	2,947	3,011	3,240	3,652	-
Mean	799	967	1,439	1,815	2,256	2,479	2,867	3,240	3,695	4,153	4,759	-
SD	91	99	221	251	238	249	376	265	484	558	755	-
Year - 1983												
Date												
No.	9/5	23/5	6/6	20/6	4/7	18/7	1/8	15/8	29/8	12/9	26/9	10/10 Nec
15	516	753	1,143	1,153	1,340	1,406	1,683	1,640	2,011	2,064	3,120	2,967 3,111
18	803	841	1,065	1,206	1,157	1,531	1,640	1,482	1,948	2,133	2,541	2,640 2,998
32	716	608	1,317	1,069	1,217	1,333	1,087	1,395	1,752	2,111	2,356	2,951 -
38	802	648	1,030	1,042	1,147	1,853	3,274	4,133	5,304	4,875	5,603	4,172 5,073
39	1,002	992	1,539	1,481	1,759	2,188	4,040	4,610	4,922	4,997	5,120	5,431 5,116
50	854	860	1,524	1,547	2,019	2,254	2,642	2,540	3,041	3,140	2,997	3,462 4,130
Mean	782	784	1,270	1,250	1,440	1,761	2,394	2,633	3,163	3,220	3,623	3,604 4,086
SD	101	143	226	214	364	399	1,125	1,415	1,580	1,389	1,384	1,043 1,021
Year - 1984												
Date												
No.	1/5	15/5	29/5	12/6	26/6	10/7	24/7	7/8	21/8	4/9	18/9	2/10 Nec
51	1,134	1,246	2,107	2,805	3,667	3,582	3,582	3,820	2,653	3,124	3,640	2,978 3,400
52	2,343	1,355	4,106	2,835	3,071	3,987	3,253	2,795	1,958	1,885	1,564	2,198 3,126
54	840	1,041	4,534	3,703	2,684	2,989	2,508	2,129	2,213	-	-	- -
57	902	1,279	2,244	1,734	1,436	1,575	1,698	1,827	1,319	2,028	1,970	2,436 2,895
88	2,073	1,205	3,641	3,727	3,420	2,729	1,800	1,700	1,972	2,822	7,163	5,922 5,843
90	802	664	4,244	2,504	2,260	2,172	5,260	6,244	4,145	3,771	5,440	4,910 5,126
Mean	1,349	1,132	3,479	2,885	2,669	2,853	3,017	3,086	2,377	2,726	3,955	3,689 4,078
SD	681	252	1,051	756	726	903	1,333	1,733	968	783	2,359	1,641 1,321
Year 1985												
Date												
No.	7/5	21/5	4/6	18/6	2/7	16/7	30/7	13/8	27/8	10/9	Nec	
29	764	813	1,205	1,670	1,744	2,055	2,240	3,041	3,655	3,852	3,955	- -
30	843	1,019	1,530	1,455	1,840	2,741	2,911	3,511	3,721	3,432	4,611	- -
31	605	847	1,411	1,732	1,920	2,630	2,738	3,264	3,483	3,611	4,120	- -
32	711	955	1,159	1,644	2,053	2,411	2,954	3,411	3,656	3,240	3,782	- -
33	624	843	1,063	1,348	1,743	2,011	2,742	3,024	3,422	4,071	4,132	- -
36	608	886	1,243	1,466	1,865	2,455	2,763	3,643	3,884	3,977	4,540	- -
Mean	693	894	1,269	1,553	1,861	2,384	2,725	3,316	3,637	3,697	4,190	- -
SD	98	78	172	150	117	297	255	252	167	325	325	- -

Appendix I (Cont'd)

Paddock 2 - Alternate Cattle and Sheep Grazing

Year - 1982													
No.	10/5	24/5	7/6	21/6	5/7	Date 19/7	2/8	16/8	30/8	13/9	Nec		
1	628	1,441	1,955	2,492	2,740	3,715	5,469	5,976	6,819	7,312	4,726	-	-
14	698	930	2,264	1,625	2,031	3,400	5,519	4,401	3,837	5,604	7,482	-	-
34	627	1,074	2,427	2,384	1,868	2,116	3,530	3,215	4,992	8,273	9,411	-	-
38	497	1,414	2,790	2,370	1,828	2,444	2,695	-	-	-	-	-	-
44	641	1,104	3,034	3,319	2,086	1,749	2,391	2,108	2,909	3,573	4,136	-	-
54	498	1,103	2,063	2,885	2,631	3,466	5,533	6,430	5,433	4,811	5,430	-	-
Mean	598	1,178	2,422	2,513	2,197	2,815	4,190	4,426	4,798	5,915	6,237	-	-
SD	82	204	420	568	392	817	1,491	1,821	1,501	1,892	2,198		

Year - 1984													
No.	1/5	15/5	29/5	12/6	26/6	Date 10/7	24/7	7/8	21/8	4/9	18/9	2/10	Nec
3	883	1,524	2,332	2,466	2,260	1,666	1,402	1,185	1,062	1,291	2,081	2,086	3,900
17	723	2,575	2,546	1,733	1,474	1,433	1,120	1,324	1,547	1,448	1,786	1,699	2,015
45	1,079	2,513	3,879	3,457	2,859	2,495	2,585	2,418	2,520	2,785	2,647	2,860	2,977
53	1,468	2,368	3,011	2,370	2,110	1,783	1,980	1,751	1,803	1,719	2,204	2,186	2,603
55	1,018	2,942	3,552	2,565	2,124	1,757	1,518	1,776	1,368	1,458	2,020	2,024	2,604
56	773	1,940	2,810	2,676	2,509	2,328	2,199	1,890	1,841	2,242	2,831	3,723	5,368
Mean	991	2,310	3,022	2,545	2,223	1,910	1,801	1,724	1,690	1,824	2,262	2,430	3,245
SD	271	504	594	556	463	411	544	439	499	578	398	739	1,212

Paddock 3 - Alternate Sheep and Cattle Grazing

Year - 1983													
No.	9/5	23/5	6/6	20/6	4/7	Date 18/7	1/8	15/8	29/8	12/9	26/9	10/10	Nec
44	986	1,889	1,103	981	1,074	851	740	747	940	1,041	1,130	1,540	1,481
62	903	1,239	1,049	956	1,045	839	810	1,166	1,161	1,212	1,304	1,286	1,488
78	842	1,054	968	877	964	726	657	605	731	906	1,201	1,134	1,320
79	611	1,413	1,360	1,046	1,144	809	752	803	1,030	1,131	1,067	1,240	1,977
80	648	920	901	968	1,061	1,028	781	662	837	900	1,040	1,204	1,199
81	772	883	968	930	1,017	716	742	634	811	765	943	1,154	1,321
Mean	794	1,233	1,058	960	1,051	828	747	770	918	993	1,114	1,260	1,464
SD	146	378	164	56	60	113	52	208	158	166	127	148	274

Year - 1985						Date								
No.	7/5	21/5	4/6	18/6	2/7	16/7	30/7	13/8	27/8	10/9	Nec			
34	654	1,031	1,560	1,770	2,053	2,640	3,070	4,950	4,731	5,420	5,167	-	-	
35	877	914	1,432	1,640	1,789	2,111	2,911	3,211	3,688	4,037	-	-	-	
37	724	1,123	1,532	1,831	2,111	2,301	2,702	3,641	3,978	4,211	4,688	-	-	
38	638	977	1,351	1,742	1,972	2,416	2,815	3,211	3,554	4,032	5,120	-	-	
39	651	854	1,862	1,901	2,350	2,644	2,720	3,337	3,682	4,112	-	-	-	
40	720	903	1,544	1,744	2,011	2,310	3,060	3,525	3,887	4,560	5,136	-	-	
Mean	711	967	1,547	1,771	2,048	2,404	2,880	3,646	3,920	4,395	5,028	-	-	
SD	89	98	174	89	184	209	162	662	426	539	227			

Alternate Grazing Study  
Statistical analysis of pepsinogen data 1982

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App. I Permanents 1982  
Replicates in group 1 : 11

Group 2 description : App I. Alternates 1982  
Replicates in group 2 : 11

Group 1 data :

1	799.00
2	967.00
3	1439.00
4	1815.00
5	2256.00
6	2479.00
7	2867.00
8	3240.00
9	3695.00
10	4153.00
11	4759.00

Group 2 data :

1	598.00
2	1178.00
3	2422.00
4	2513.00
5	2197.00
6	2815.00
7	4190.00
8	4426.00
9	4798.00
10	5915.00
11	6237.00

Results:  
=====

Group 1 : App. I Permanents 1982

Mean            = 2588.09  
Variance       = 1675885.69  
St. Dev.       = 1294.56  
St. Error      = 390.32  
Sample size = 11

Group 2 : App I. Alternates 1982

Mean            = 3389.91  
Variance       = 3432502.89  
St. Dev.       = 1852.70  
St. Error      = 558.61  
Sample size = 11

Test statistic =    - 1.18

Critical value = +/- 2.23

No evidence that groups are different at 5% significance level.

Alternate Grazing Study  
Statistical analysis of pepsinogen data 1983

ANIMAL DESIGNS... Two-sample t-test Date... 03-Jul-90

Group 1 description : App I. Permanents 1983  
Replicates in group 1 : 13

Group 2 description : App I. Alternates 1983  
Replicates in group 2 : 13

Group 1 data :

1	782.00
2	784.00
3	1270.00
4	1250.00
5	1440.00
6	1761.00
7	2394.00
8	2633.00
9	3163.00
10	3220.00
11	3623.00
12	3604.00
13	4086.00

Group 2 data :

1	794.00
2	1233.00
3	1058.00
4	960.00
5	1051.00
6	828.00
7	747.00
8	770.00
9	918.00
10	993.00
11	1114.00
12	1260.00
13	1464.00

Results:  
=====

Group 1 : App I. Permanents 1983

Mean	= 2308.46
Variance	= 1345550.44
St. Dev.	= 1159.98
St. Error	= 321.72
Sample size	= 13

Group 2 : App I. Alternates 1983

Mean	= 1014.62
Variance	= 45889.26
St. Dev.	= 214.22
St. Error	= 59.41
Sample size	= 13

Test statistic = 3.95

Critical value = +/- 2.18

Evidence that groups are different at 5% significance level.



Alternate Grazing Study  
Statistical analysis of pepsinogen data 1984

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App I. Permanents 1984  
Replicates in group 1 : 13

Group 2 description : App I. Alternates 1984  
Replicates in group 2 : 13

Group 1 data :

1	1349.00
2	1132.00
3	3479.00
4	2885.00
5	2669.00
6	2853.00
7	3017.00
8	3086.00
9	2377.00
10	2726.00
11	3955.00
12	3689.00
13	4078.00

Group 2 data :

1	991.00
2	2310.00
3	3022.00
4	2545.00
5	2223.00
6	1910.00
7	1801.00
8	1724.00
9	1690.00
10	1824.00
11	2262.00
12	2430.00
13	3245.00

Results:

=====

Group 1 : App I. Permanents 1984

Mean	= 2868.85
Variance	= 782096.97
St. Dev.	= 884.36
St. Error	= 245.28
Sample size	= 13

Group 2 : App I. Alternates 1984

Mean	= 2152.08
Variance	= 354940.41
St. Dev.	= 595.77
St. Error	= 165.24
Sample size	= 13

Test statistic = 2.42

Critical value = +/- 2.18

Evidence that groups are different at 5% significance level.

Alternate Grazing Study  
Statistical analysis of pepsinogen data 1985

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App I. Permanents 1985  
Replicates in group 1 : 11

Group 2 description : App I. Alternates 1985  
Replicates in group 2 : 11

Group 1 data :

1	693.00
2	894.00
3	1269.00
4	1553.00
5	1861.00
6	2384.00
7	2725.00
8	3316.00
9	3637.00
10	3697.00
11	4190.00

Group 2 data :

1	711.00
2	967.00
3	1547.00
4	1771.00
5	2048.00
6	2404.00
7	2880.00
8	3646.00
9	3920.00
10	4395.00
11	5028.00

Results:

=====

Group 1 : App I. Permanents 1985

Mean            = 2383.55  
Variance       = 1482741.27  
St. Dev.       = 1217.68  
St. Error      = 367.14  
Sample size = 11

Group 2 : App I. Alternates 1985

Mean            = 2665.18  
Variance       = 2035956.96  
St. Dev.       = 1426.87  
St. Error      = 430.22  
Sample size = 11

Test statistic =    - 0.50

Critical value = +/- 2.23

No evidence that groups are different at 5% significance level.

# Appendix J

## Alternate Grazing Study

### Individual and Group Mean Faecal Egg Counts

#### Paddock 1 - Permanent Cattle Grazing

Year - 1982													Date	
No.	10/5	24/5	7/6	21/6	5/7	19/7	2/8	16/8	30/8	13/9	Nec			
21	0	0	50	50	50	900	700	1,100	600	950	1,500	-	-	
37	0	0	0	50	0	1,000	800	800	700	850	1,350	-	-	
48	0	0	0	50	0	1,200	800	500	400	1,200	3,000	-	-	
53	0	0	50	50	0	1,000	900	2,000	600	950	850	-	-	
56	0	0	50	100	100	650	700	700	950	750	500	-	-	
60	0	0	0	0	50	900	600	650	800	450	700	-	-	
Mean	0	0	25	50	33	942	750	958	675	842	1,258	-	-	
SD	0	0	27	32	41	180	105	548	189	246	906	-	-	
Year - 1983													Date	
No.	9/5	23/5	6/6	20/6	4/7	18/7	1/8	15/8	29/8	12/9	26/9	10/10	Nec	
15	0	0	0	50	50	100	100	50	100	150	200	350	200	
18	0	0	50	0	50	100	150	200	150	150	300	450	350	
32	0	0	0	0	0	0	0	150	100	100	50	17,600	-	
38	0	0	0	0	50	150	150	150	200	200	350	450	1,300	
39	0	0	50	50	100	200	200	150	250	250	600	650	550	
50	0	0	0	0	0	50	150	50	0	50	0	100	350	
Mean	0	0	17	17	42	100	125	125	133	150	250	3,267	733	
SD	0	0	26	26	38	71	69	61	88	71	219	7,024	501	
Year - 1984													Date	
No.	1/5	15/5	29/5	12/6	26/6	10/7	24/7	7/8	21/8	4/9	18/9	2/10	Nec	
51	0	150	1,900	800	900	700	400	650	400	150	850	350	450	
52	0	250	2,600	1,200	450	0	300	400	250	350	800	350	550	
54	0	250	2,500	800	2,350	1,500	700	1,950	850	-	-	-	-	
57	0	50	2,500	900	1,000	1,350	450	500	1,050	50	950	400	300	
88	0	100	450	200	250	0	50	150	100	200	150	150	100	
90	0	200	1,450	850	400	750	500	250	350	50	1,750	500	350	
Mean	0	167	1,900	792	892	717	400	650	500	160	900	350	350	
SD	0	82	838	326	773	639	217	661	369	124	570	127	170	
Year - 1985													Date	
No.	7/5	21/5	4/6	18/6	2/7	16/7	30/7	13/8	27/8	10/9	Nec			
29	0	0	150	350	350	250	300	250	50	50	100	-	-	
30	0	0	150	1,150	400	1,000	1,300	850	300	150	0	-	-	
31	0	0	100	1,300	200	300	150	200	250	400	0	-	-	
32	0	0	250	500	300	1,450	100	200	250	100	950	-	-	
33	0	0	50	900	350	650	500	350	200	150	350	-	-	
36	0	0	100	750	300	850	450	400	250	200	350	-	-	
Mean	0	0	133	825	317	750	467	375	217	175	350	-	-	
SD	0	0	68	367	68	453	438	246	88	121	369	-	-	

Appendix J (Cont'd)

Paddock 2 - Alternate Cattle and Sheep Grazing

Year - 1982												Date	
No.	10/5	24/5	7/6	21/6	5/7	19/7	2/8	16/8	30/8	13/9	Nec		
1	0	0	150	3,050	0	50	1,250	1,350	0	50	1,000	-	-
14	0	0	100	2,150	0	100	400	1,300	0	800	950	-	-
34	0	0	50	6,500	0	0	100	200	0	200	450	-	-
38	0	0	50	5,000	0	50	550	-	-	-	-	-	-
44	0	0	100	300	0	0	250	50	0	0	0	-	-
54	0	0	50	1,450	0	50	1,650	4,000	600	800	1,000	-	-
Mean	0	0	83	3,075	0	42	700	1,380	0	263	680	-	-
SD	0	0	41	2,308	0	38	613	1,584	0	368	445		
Year - 1984													
No.	1/5	15/5	19/5	12/6	26/6	10/7	24/7	7/8	21/8	4/9	18/9	2/10	Nec
3	0	0	50	200	500	300	250	250	0	50	300	150	450
17	0	0	150	850	1,250	450	200	450	450	150	350	150	600
45	0	0	500	500	550	350	100	300	300	600	300	200	0
53	0	0	200	700	950	2,600	1,100	0	0	150	250	100	50
55	0	0	700	700	950	500	200	450	50	150	250	100	250
56	0	0	850	550	2,000	1,700	1,100	650	450	400	750	100	1,400
Mean	0	0	408	583	1,033	983	492	350	208	250	367	150	458
SD	0	0	325	225	550	950	474	221	218	207	191	41	515

Paddock 3 - Alternate Sheep and Cattle Grazing

Year - 1983						Date								
No.	9/5	23/5	6/6	20/6	4/7	18/7	1/8	15/8	29/8	12/9	26/9	10/10	Nec	
44	0	0	0	0	0	0	0	0	0	50	50	50	50	
62	0	0	0	0	0	0	100	50	0	50	150	250	0	
78	0	0	0	0	0	0	0	0	50	0	200	100	200	
79	0	0	50	100	0	0	0	0	100	50	250	100	200	
80	0	0	0	150	0	150	0	50	200	50	300	300	150	
81	0	0	0	0	0	0	50	0	0	0	200	100	150	
Mean	0	0	8	42	0	25	25	17	58	33	192	150	125	
SD	0	0	20	66	0	61	42	26	80	26	86	100	82	
Year - 1985						Date								
No.	7/5	21/5	4/6	18/6	2/7	16/7	30/7	13/8	27/8	10/9	Nec			
34	0	0	50	0	50	100	250	350	300	800	350	-	-	
35	0	0	100	100	50	150	350	1,100	700	600	-	-	-	
37	0	0	50	150	0	0	50	0	50	0	50	-	-	
38	0	0	300	150	400	0	-	500	400	2,100	1,550	-	-	
39	0	0	50	150	100	100	-	550	300	200	-	-	-	
40	0	0	50	100	100	50	-	100	150	100	150	-	-	
Mean	0	0	100	108	117	67	217	433	317	633	525	-	-	
SD	0	0	100	58	144	61	153	392	225	968	695	-	-	

**Alternate Grazing Study**  
**Statistical analysis of faecal egg count data 1982**

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App J. Permanents 1982  
Replicates in group 1 : 11

Group 2 description : App J. Alternates 1982  
Replicates in group 2 : 11

**Group 1 data :**

```
-----
1      0.00
2      0.00
3     25.00
4     50.00
5     33.00
6    942.00
7    750.00
8    958.00
9    675.00
10   842.00
11  1258.00
```

**Group 2 data :**

```
-----
1      0.00
2      0.00
3     83.00
4    3075.00
5      0.00
6     42.00
7    700.00
8    1380.00
9      0.00
10   263.00
11   680.00
```

**Results:**  
=====

**Group 1 : App J. Permanents 1982**

```
Mean      = 2.03
Variance  = 1.41
St. Dev.  = 1.19
St. Error = 0.36
Sample size = 11
```

**Group 2 : App J. Alternates 1982**

```
Mean      = 1.66
Variance  = 2.00
St. Dev.  = 1.41
St. Error = 0.43
Sample size = 11
```

Test statistic = 0.66

Critical value = +/- 2.23

No evidence that groups are different at 5% significance level.

**Geometric means:**

```
-----
Group 1.... 106.42
Group 2.... 44.96
```

**Alternate Grazing Study**  
**Statistical analysis of faecal egg count data 1983**

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App J. Permanents 1983  
Replicates in group 1 : 13

Group 2 description : App J. Alternates 1983  
Replicates in group 2 : 13

Group 1 data :

```
-----
1      0.00
2      0.00
3     17.00
4     17.00
5     42.00
6    100.00
7    125.00
8    125.00
9    133.00
10   150.00
11   250.00
12  3267.00
13   733.00
```

Group 2 data :

```
-----
1      0.00
2      0.00
3      8.00
4     42.00
5      0.00
6     25.00
7     25.00
8     17.00
9     58.00
10    33.00
11   192.00
12   150.00
13   125.00
```

Results:  
=====

Group 1 : App J. Permanents 1983

```
Mean      = 1.80
Variance  = 1.00
St. Dev.  = 1.00
St. Error = 0.28
Sample size = 13
```

Group 2 : App J. Alternates 1983

```
Mean      = 1.27
Variance  = 0.66
St. Dev.  = 0.82
St. Error = 0.23
Sample size = 13
```

Test statistic = 1.48

Critical value = +/- 2.18

No evidence that groups are different at 5% significance level.

Geometric means:

```
-----
Group 1.... 62.49
Group 2.... 17.72
```

**Alternate Grazing Study**  
**Statistical analysis of faecal egg count data 1984**

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App J. Permanents 1984  
Replicates in group 1 : 13

Group 2 description : App J. Alternates 1984  
Replicates in group 2 : 13

Group 1 data :

1	0.00
2	167.00
3	1900.00
4	792.00
5	892.00
6	717.00
7	400.00
8	650.00
9	500.00
10	160.00
11	900.00
12	350.00
13	350.00

Group 2 data :

1	0.00
2	0.00
3	408.00
4	583.00
5	1033.00
6	983.00
7	492.00
8	350.00
9	208.00
10	250.00
11	367.00
12	150.00
13	458.00

Results:

=====

Group 1 : App J. Permanents 1984

Mean            = 2.51  
Variance        = 0.66  
St. Dev.        = 0.81  
St. Error       = 0.22  
Sample size = 13

Group 2 : App J. Alternates 1984

Mean            = 2.21  
Variance        = 1.02  
St. Dev.        = 1.01  
St. Error       = 0.28  
Sample size = 13

Test statistic =        0.82

Critical value = +/- 2.18

No evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1.... 319.70  
Group 2.... 161.76

Alternate Grazing Study  
Statistical analysis of faecal egg count data 1985

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App J. Permanents 1985  
Replicates in group 1 : 11

Group 2 description : App J. Alternates 1985  
Replicates in group 2 : 11

Group 1 data :

-----

1	0.00
2	0.00
3	133.00
4	825.00
5	317.00
6	750.00
7	467.00
8	375.00
9	217.00
10	175.00
11	350.00

Group 2 data :

-----

1	0.00
2	0.00
3	100.00
4	108.00
5	117.00
6	67.00
7	217.00
8	433.00
9	317.00
10	633.00
11	525.00

Results:

=====

Group 1 : App J. Permanents 1985

Mean	=	2.07
Variance	=	1.11
St. Dev.	=	1.05
St. Error	=	0.32
Sample size	=	11

Group 2 : App J. Alternates 1985

Mean	=	1.90
Variance	=	0.99
St. Dev.	=	0.99
St. Error	=	0.30
Sample size	=	11

Test statistic =        0.39

Critical value = +/- 2.23

No evidence that groups are different at 5% significance level.



# Appendix K

## Alternate Grazing Study

### O. ostertagi and C. oncophora Larval Recoveries per Kilogram Dried Herbage

#### Paddock 1 - Permanent Cattle Grazing

Year - 1982													Date	
	10/5	24/5	7/6	21/6	5/7	19/7	2/8	16/8	30/8	13/9	27/9			
<u>O.o.</u>	0	0	355	126	338	176	212	3,544	13,664	6,980	3,333	-	-	
<u>C.o.</u>	513	0	77	160	76	0	636	1,392	3,282	1,611	1,250	-	-	
Year - 1983													Date	
	9/5	23/5	6/6	20/6	4/7	18/7	1/8	15/8	29/8	12/9	26/9	10/10	24/10	
<u>O.o.</u>	0	0	0	0	275	615	1,133	2,135	9,126	12,381	17,931	10,454	18,592	
<u>C.o.</u>	0	0	0	0	0	0	0	0	1,941	1,905	10,345	31,812	20,304	
Year - 1984													Date	
	1/5	15/5	29/5	12/6	26/6	10/7	24/7	7/8	21/8	4/9	18/9	2/10	16/10	
<u>O.o.</u>	563	526	0	185	292	169	1,125	2,424	7,692	23,233	16,000	102,222	92,307	
<u>C.o.</u>	0	175	0	0	58	0	125	303	769	4,511	4,000	35,555	17,692	
Year - 1985													Date	
	7/5	21/5	4/6	18/6	2/7	16/7	30/7	13/8	27/8	10/9	24/9			
<u>O.o.</u>	500	204	0	976	795	612	1,620	1,364	256	3,728	8,896	-	-	
<u>C.o.</u>	125	0	0	488	1,136	816	1,350	1,364	512	932	2,542	-	-	

Appendix K (Cont'd)

Paddock 2 - Alternate Cattle and Sheep Grazing

Grazed by Cattle

Year - 1982													Date	
	10/5	24/5	7/6	21/6	5/7	19/7	2/8	16/8	30/8	13/9	27/9			
<u>O.o.</u>	823	1,900	2,479	96	2,873	15,544	7,458	4,865	25,877	24,797	48,342	-	-	
<u>C.o.</u>	566	205	263	288	1,470	7,227	8,693	3,599	11,995	5,209	11,274	-	-	

Grazed by Sheep

Year - 1983														Date	
	9/5	23/5	6/6	20/6	4/7	18/7	1/8	15/8	29/8	12/9	26/9	10/10	24/10		
<u>O.o.</u>	0	154	499	0	0	0	307	3,487	0	0	0	0	0		
<u>C.o.</u>	0	0	0	0	0	0	0	0	0	290	0	0	0		

Grazed by Cattle

Year - 1984														Date	
	1/5	15/5	29/5	12/6	26/6	10/7	24/7	7/8	21/8	4/9	18/9	2/10	16/10		
<u>O.o.</u>	135	143	111	53	581	65	0	2,407	799	44,040	38,954	57,770	44,105		
<u>C.o.</u>	0	132	0	0	0	130	0	0	620	31,313	19,587	18,209	35,219		

Grazed by Sheep

Year - 1985													Date	
	7/5	21/5	4/6	18/6	2/7	16/7	30/7	13/8	27/8	10/9	14/9			
<u>O.o.</u>	1,224	150	278	0	351	370	369	0	0	0	0	-	-	
<u>C.o.</u>	599	94	0	0	0	0	0	0	0	0	0	0	0	

Appendix K (Cont'd)

Paddock 3 - Alternate Sheep and Cattle Grazing

Grazed by Sheep

Year - 1982												
	Date											
	10/5	24/5	7/6	21/6	5/7	19/7	2/8	16/8	30/8	13/9	27/9	
<u>O.o.</u>	0	0	0	0	0	0	0	0	0	0	0	-
<u>C.o.</u>	0	0	0	0	0	0	0	0	0	0	0	-

Grazed by Cattle

Year - 1983													
	Date												
	9/5	23/5	6/6	20/6	4/7	18/7	1/8	15/8	29/8	12/9	26/9	10/10	24/10
<u>O.o.</u>	0	42	0	0	67	0	50	263	72	1,076	2,203	829	271
<u>C.o.</u>	0	0	0	0	0	0	0	44	49	1,794	2,763	522	151

Grazed by Sheep

Year - 1984													
	Date												
	1/5	15/5	29/5	12/6	26/6	10/7	24/7	7/8	21/8	4/9	18/9	2/10	16/10
<u>O.o.</u>	139	364	0	233	0	0	0	0	0	0	270	0	0
<u>C.o.</u>	0	0	0	191	0	0	0	0	0	0	0	0	0

Grazed by Cattle

Year - 1985												
	Date											
	7/5	21/5	4/6	18/6	2/7	16/7	30/7	13/8	27/8	10/9	24/9	
<u>O.o.</u>	0	48	70	0	119	705	306	306	254	4,872	0	-
<u>C.o.</u>	0	0	0	0	382	705	730	439	84	4,488	0	-

Appendix K (Cont'd)

Paddock 4 - Permanent Sheep Grazing

Year - 1982													
Date													
	10/5	24/5	7/6	21/6	5/7	19/7	2/8	16/8	30/8	13/9	27/9		
<u>O.o.</u>	0	0	0	0	0	0	217	0	0	0	0	-	-
<u>C.o.</u>	0	0	0	0	0	0	217	0	0	0	0	-	-
Year - 1983													
Date													
	9/5	23/5	6/6	20/6	4/7	18/7	1/8	15/8	29/8	12/9	26/9	10/10	24/10
<u>O.o.</u>	0	0	0	0	0	0	0	166	0	0	0	0	0
<u>C.o.</u>	0	0	0	0	0	0	0	0	0	0	0	0	0
Year - 1984													
Date													
	1/5	15/5	29/5	12/6	26/6	10/7	24/7	7/8	21/8	4/9	18/9	2/10	16/10
<u>O.o.</u>	0	0	0	0	0	0	0	0	0	0	0	0	0
<u>C.o.</u>	0	0	0	0	0	0	0	0	0	0	0	0	0
Year - 1985													
Date													
	7/5	21/5	4/6	18/6	2/7	16/7	30/7	13/8	27/8	10/9	24/9		
<u>O.o.</u>	0	0	0	0	0	0	0	0	0	0	0	-	-
<u>C.o.</u>	0	0	0	0	0	0	0	0	0	0	0	-	-

**Alternate Grazing Study**  
**Statistical analysis of Oostertagi pasture larval**  
**recoveries from P1 (calves) and P2 (calves - 1982)**

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanent Oo 1982  
Replicates in group 1 : 11

Group 2 description : App K. Alternates Oo 1982  
Replicates in group 2 : 11

Group 1 data :

1	0.00
2	0.00
3	355.00
4	126.00
5	338.00
6	176.00
7	212.00
8	3544.00
9	13664.00
10	6980.00
11	3333.00

Group 2 data :

1	823.00
2	1900.00
3	2479.00
4	96.00
5	2873.00
6	15544.00
7	7458.00
8	4865.00
9	25877.00
10	24797.00
11	48342.00

Results:  
=====

Group 1 : App K. Permanent Oo 1982

Mean            = 2.44  
Variance       = 1.93  
St. Dev.       = 1.39  
St. Error      = 0.42  
Sample size = 11

Group 2 : App K. Alternates Oo 198

Mean            = 3.66  
Variance       = 0.61  
St. Dev.       = 0.78  
St. Error      = 0.24  
Sample size = 11

Test statistic =    - 2.55

Critical value = +/- 2.23

Evidence that groups are different at 5% significance level.

Geometric means:

Group 1.... 272.92  
Group 2.... 4586.89

Alternate Grazing Study  
 Statistical analysis of O.ostertagi pasture larval  
 recoveries from P1 (calves) and P3 calves) - 1983

ANIMAL DESIGNS... Two-sample t-test Date... 03-Jul-90

Group 1 description : App K. Permanent Oo 1983  
 Replicates in group 1 : 13

Group 2 description : App K. Alternate Oo 1983  
 Replicates in group 2 : 13

Group 1 data :

1	0.00
2	0.00
3	0.00
4	0.00
5	275.00
6	165.00
7	1133.00
8	2135.00
9	9126.00
10	12381.00
11	17931.00
12	10454.00
13	18592.00

Group 2 data :

1	0.00
2	42.00
3	0.00
4	0.00
5	67.00
6	0.00
7	50.00
8	263.00
9	72.00
10	1076.00
11	2203.00
12	829.00
13	271.00

Results:

=====

Group 1 : App K. Permanent Oo 1983

Mean = 2.43  
 Variance = 3.27  
 St. Dev. = 1.81  
 St. Error = 0.50  
 Sample size = 13

Group 2 : App K. Alternate Oo 1982

Mean = 1.63  
 Variance = 1.55  
 St. Dev. = 1.24  
 St. Error = 0.34  
 Sample size = 13

Test statistic = 1.32

Critical value = +/- 2.18

No evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1.... 270.58  
 Group 2.... 41.64

Alternate Grazing Study  
 Statistical analysis of O.ostertagi pasture larval  
 recoveries form P1 (calves) and P2 (calves) - 1984

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanents Oo1984  
 Replicates in group 1 : 13

Group 2 description : App K. Alternates Oo 1984  
 Replicates in group 2 : 13

Group 1 data :

1	563.00
2	526.00
3	0.00
4	185.00
5	292.00
6	169.00
7	1125.00
8	2424.00
9	7692.00
10	23233.00
11	16000.00
12	102222.00
13	92307.00

Group 2 data :

1	135.00
2	143.00
3	111.00
4	53.00
5	581.00
6	65.00
7	0.00
8	2407.00
9	799.00
10	44040.00
11	38954.00
12	57770.00
13	44105.00

Results:

=====

Group 1 : App K. Permanents Oo1984

Mean            = 3.18  
 Variance       = 1.86  
 St. Dev.       = 1.36  
 St. Error      = 0.38  
 Sample size = 13

Group 2 : App K. Alternates Oo 198

Mean            = 2.89  
 Variance       = 2.12  
 St. Dev.       = 1.46  
 St. Error      = 0.40  
 Sample size = 13

Test statistic =     0.52

Critical value = +/- 2.18

No evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1..... 1503.83  
 Group 2..... 777.09

**Alternate Grazing Study**  
**Statistical analysis of O.ostertagi pasture larval**  
**recoveries from P1 (calves) and P3 (calves) - 1985**

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanent Oo 1985  
Replicates in group 1 : 11

Group 2 description : App K. Alternate Oo 1985  
Replicates in group 2 : 11

**Group 1 data :**

1	500.00
2	204.00
3	0.00
4	976.00
5	795.00
6	612.00
7	1620.00
8	1364.00
9	256.00
10	3728.00
11	8896.00

**Group 2 data :**

1	0.00
2	48.00
3	70.00
4	0.00
5	119.00
6	705.00
7	306.00
8	306.00
9	254.00
10	4872.00
11	0.00

**Results:**

=====

**Group 1 : App K. Permanent Oo 1985**

Mean            = 2.72  
Variance        = 1.04  
St. Dev.        = 1.02  
St. Error       = 0.31  
Sample size = 11

**Group 2 : App K. Alternate Oo 1985**

Mean            = 1.78  
Variance        = 1.58  
St. Dev.        = 1.26  
St. Error       = 0.38  
Sample size = 11

Test statistic =        1.94

Critical value = +/- 2.23

No evidence that groups are different at 5% significance level.

**Geometric means:**

-----

Group 1.... 528.83  
Group 2.... 58.73



**Alternate Grazing Study**  
**Statistical analysis of O.ostertagi pasture larval**  
**recoveries from P1 (calves) and P3 (sheep) - 1982**

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanent Oo 1982  
Replicates in group 1 : 11

Group 2 description : App K. Alternate Oo 1982  
Replicates in group 2 : 11

Group 1 data :

1	0.00
2	0.00
3	355.00
4	126.00
5	338.00
6	176.00
7	212.00
8	3544.00
9	13664.00
10	6980.00
11	3333.00

Group 2 data :

1	0.00
2	0.00
3	0.00
4	0.00
5	0.00
6	0.00
7	0.00
8	0.00
9	0.00
10	0.00
11	0.00

Results:

=====

Group 1 : App K. Permanent Oo 1982

Mean            = 2.44  
Variance        = 1.93  
St. Dev.        = 1.39  
St. Error       = 0.42  
Sample size = 11

Group 2 : App K. Alternate Oo 1982

Mean            = 0.00  
Variance        = 0.00  
St. Dev.        = 0.00  
St. Error       = 0.00  
Sample size = 11

Test statistic =        5.81

Critical value = +/- 2.23

Evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1.... 272.92  
Group 2.... 0.00

Alternate Grazing Study  
 Statistical analysis of O.ostertagi pasture larval  
 recoveries from P1 (calves) and P2 (sheep) - 1983

ANIMAL DESIGNS... Two-sample t-test Date... 03-Jul-90

Group 1 description : App K. Permanents Oo 1983  
 Replicates in group 1 : 13

Group 2 description : App K. Alternates Oo1983  
 Replicates in group 2 : 13

Group 1 data :

1	0.00
2	0.00
3	0.00
4	0.00
5	275.00
6	615.00
7	1133.00
8	2135.00
9	9126.00
10	12381.00
11	17931.00
12	10454.00
13	18592.00

Group 2 data :

1	0.00
2	154.00
3	499.00
4	0.00
5	0.00
6	0.00
7	307.00
8	3487.00
9	0.00
10	0.00
11	0.00
12	0.00
13	0.00

Results:

=====

Group 1 : App K. Permanents Oo 198

Mean = 2.48  
 Variance = 3.27  
 St. Dev. = 1.81  
 St. Error = 0.50  
 Sample size = 13

Group 2 : App K. Alternates Oo1983

Mean = 0.84  
 Variance = 1.80  
 St. Dev. = 1.34  
 St. Error = 0.37  
 Sample size = 13

Test statistic = 2.62

Critical value = +/- 2.18

Evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1.... 299.40  
 Group 2.... 5.92

Alternate Grazing Study  
Statistical analysis of O.ostertagi pasture larval  
recoveries from P1 (calves) and P3 (sheep) - 1984

ANIMAL DESIGNS... Two-sample t-test Date... 03-Jul-90

Group 1 description : App K. Permanent Oo 1984  
Replicates in group 1 : 13

Group 2 description : App K. Alternate Oo 1984  
Replicates in group 2 : 13

Group 1 data :

1	563.00
2	526.00
3	0.00
4	185.00
5	292.00
6	169.00
7	1125.00
8	2424.00
9	7692.00
10	23233.00
11	16000.00
12	102222.00
13	92307.00

Group 2 data :

1	139.00
2	364.00
3	0.00
4	233.00
5	0.00
6	0.00
7	0.00
8	0.00
9	0.00
10	0.00
11	270.00
12	0.00
13	0.00

Results:  
=====

Group 1 : App K. Permanent Oo 1984

Mean = 3.18  
Variance = 1.86  
St. Dev. = 1.36  
St. Error = 0.38  
Sample size = 13

Group 2 : App K. Alternate Oo 1984

Mean = 0.73  
Variance = 1.31  
St. Dev. = 1.15  
St. Error = 0.32  
Sample size = 13

Test statistic = 4.95

Critical value = +/- 2.18

Evidence that groups are different at 5% significance level.

Geometric means:

Group 1.... 1503.83  
Group 2.... 4.39

Alternate Grazing Study  
 Statistical analysis of O.ostertagi pasture larval  
 recoveries from P1 (calves) and P2 (sheep) - 1985

ANIMAL DESIGNS... Two-sample t-test Date... 03-Jul-90

Group 1 description : App K. Permanent Oo 1985  
 Replicates in group 1 : 11

Group 2 description : App K. Alternate Oo 1985  
 Replicates in group 2 : 11

Group 1 data :

1	500.00
2	204.00
3	0.00
4	976.00
5	795.00
6	612.00
7	1620.00
8	1364.00
9	256.00
10	3728.00
11	8896.00

Group 2 data :

1	1224.00
2	150.00
3	278.00
4	0.00
5	351.00
6	370.00
7	369.00
8	0.00
9	0.00
10	0.00
11	0.00

Results:  
 =====

Group 1 : App K., Permanent Oo 1985

Mean = 2.72  
 Variance = 1.04  
 St. Dev. = 1.02  
 St. Error = 0.31  
 Sample size = 11

Group 2 : App K. Alternate Oo 1985

Mean = 1.40  
 Variance = 1.84  
 St. Dev. = 1.36  
 St. Error = 0.41  
 Sample size = 11

Test statistic = 2.59

Critical value = +/- 2.23

Evidence that groups are different at 5% significance level.

Geometric means:

-----  
 Group 1.... 528.83  
 Group 2.... 24.10

**Alternate Grazing Study**  
**Statistical analysis of C. oncophora pasture larval**  
**recoveries from P1 (calves) and P2 (calves) - 1982**

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanent Co 1982  
Replicates in group 1 : 11

Group 2 description : App K. Alternate Co 1982  
Replicates in group 2 : 11

Group 1 data :

1	513.00
2	0.00
3	77.00
4	160.00
5	76.00
6	0.00
7	636.00
8	1392.00
9	3282.00
10	1611.00
11	1250.00

Group 2 data :

1	566.00
2	205.00
3	263.00
4	288.00
5	1470.00
6	7227.00
7	8693.00
8	3599.00
9	11995.00
10	5209.00
11	11274.00

Results:

=====

Group 1 : App K. Permanent Co 1982

Mean            = 2.22  
Variance        = 1.49  
St. Dev.        = 1.22  
St. Error       = 0.37  
Sample size = 11

Group 2 : App K. Alternate Co 1982

Mean            = 3.30  
Variance        = 0.49  
St. Dev.        = 0.70  
St. Error       = 0.21  
Sample size = 11

Test statistic =    - 2.54

Critical value = +/- 2.23

Evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1.... 166.54  
Group 2.... 2002.67

**Alternate Grazing Study**  
**Statistical analysis of C. oncophora pasture larval**  
**recoveries from P1 (calves) and P3 (calves) - 1983**

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanent Co 1983  
Replicates in group 1 : 13

Group 2 description : App K. Alternate Co 1983  
Replicates in group 2 : 13

Group 1 data :

1	0.00
2	0.00
3	0.00
4	0.00
5	0.00
6	0.00
7	0.00
8	0.00
9	1941.00
10	1905.00
11	10345.00
12	31812.00
13	20304.00

Group 2 data :

1	0.00
2	0.00
3	0.00
4	0.00
5	0.00
6	0.00
7	0.00
8	44.00
9	49.00
10	1794.00
11	2763.00
12	522.00
13	151.00

Results:  
=====

Group 1 : App K. Permanent Co 1983

Mean            = 1.49  
Variance        = 3.97  
St. Dev.        = 1.99  
St. Error       = 0.55  
Sample size = 13

Group 2 : App K. Alternate Co 1983

Mean            = 1.15  
Variance        = 1.92  
St. Dev.        = 1.38  
St. Error       = 0.38  
Sample size = 13

Test statistic =        0.51

Critical value = +/- 2.18

No evidence that groups are different at 5% significance level.

Geometric means:

Group 1.... 30.03  
Group 2.... 13.12

Alternate Grazing Study  
Statistical analysis of C. oncophora pasture larval  
recoveries from P1 (calves) and P2 (calves) - 1984

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanent Co 1984  
Replicates in group 1 : 13

Group 2 description : App K. Alternate Co 1984  
Replicates in group 2 : 13

Group 1 data :

1	0.00
2	175.00
3	0.00
4	0.00
5	58.00
6	0.00
7	125.00
8	303.00
9	769.00
10	4511.00
11	4000.00
12	35555.00
13	17692.00

Group 2 data :

1	0.00
2	132.00
3	0.00
4	0.00
5	0.00
6	130.00
7	0.00
8	0.00
9	620.00
10	31313.00
11	19587.00
12	18209.00
13	35219.00

Results:

Group 1 : App K. Permanent Co 1984

Mean            = 2.12  
Variance       = 2.82  
St. Dev.       = 1.68  
St. Error      = 0.47  
Sample size = 13

Group 2 : App K. Alternate Co 1984

Mean            = 1.89  
Variance       = 3.97  
St. Dev.       = 1.99  
St. Error      = 0.55  
Sample size = 13

Test statistic =        0.31

Critical value = +/- 2.18

No evidence that groups are different at 5% significance level.

Geometric means:

Group 1.... 130.39  
Group 2.... 77.44

Alternate Grazing Study  
Statistical analysis of C. oncophora pasture larval  
recoveries from P1 (calves) and P3 (calves) - 1985

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanent Co 1985  
Replicates in group 1 : 11

Group 2 description : App K. Alternate Co 1985  
Replicates in group 2 : 11

Group 1 data :

1	125.00
2	0.00
3	0.00
4	488.00
5	1136.00
6	816.00
7	1350.00
8	1364.00
9	512.00
10	932.00
11	2542.00

Group 2 data :

1	0.00
2	0.00
3	0.00
4	0.00
5	382.00
6	705.00
7	730.00
8	439.00
9	84.00
10	4488.00
11	0.00

Results:

=====

Group 1 : App K. Permanent Co 1985

Mean            = 2.37  
Variance       = 1.49  
St. Dev.       = 1.22  
St. Error       = 0.37  
Sample size = 11

Group 2 : App K. Alternate Co 1985

Mean            = 1.50  
Variance       = 2.22  
St. Dev.       = 1.49  
St. Error       = 0.45  
Sample size = 11

Test statistic =        1.50

Critical value = +/- 2.23

No evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1.... 235.33  
Group 2.... 30.76



Alternate Grazing Study  
Statistical analysis of C. oncophora pasture larval  
recoveries from P1 (calves) and P3 (sheep) - 1982

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanent Co 1982  
Replicates in group 1 : 11

Group 2 description : App K. Alternate Co 1982  
Replicates in group 2 : 11

Group 1 data :

1	513.00
2	0.00
3	77.00
4	160.00
5	76.00
6	0.00
7	636.00
8	1392.00
9	3282.00
10	1611.00
11	1250.00

Group 2 data :

1	0.00
2	0.00
3	0.00
4	0.00
5	0.00
6	0.00
7	0.00
8	0.00
9	0.00
10	0.00
11	0.00

Results:

=====

Group 1 : App K. Permanent Co 1982

Mean            = 2.22  
Variance       = 1.49  
St. Dev.       = 1.22  
St. Error      = 0.37  
Sample size = 11

Group 2 : App K. Alternate Co 1982

Mean            = 0.00  
Variance       = 0.00  
St. Dev.       = 0.00  
St. Error      = 0.00  
Sample size = 11

Test statistic =       6.04

Critical value = +/- 2.23

Evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1..... 166.54  
Group 2..... 0.00

Alternate Grazing Study  
Statistical analysis of C.oncophora pasture larval  
recoveries from P1 (calves) and P2 (sheep) - 1983

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanent Co 1983  
Replicates in group 1 : 13

Group 2 description : App K. Alternate Co 1983  
Replicates in group 2 : 13

Group 1 data :

1	0.00
2	0.00
3	0.00
4	0.00
5	0.00
6	0.00
7	0.00
8	0.00
9	1941.00
10	1905.00
11	10345.00
12	31812.00
13	20304.00

Group 2 data :

1	0.00
2	0.00
3	0.00
4	0.00
5	0.00
6	0.00
7	0.00
8	0.00
9	0.00
10	290.00
11	0.00
12	0.00
13	0.00

Results:  
=====

Group 1 : App K. Permanent Co 1983

Mean            = 1.49  
Variance       = 3.97  
St. Dev.       = 1.99  
St. Error      = 0.55  
Sample size = 13

Group 2 : App K. Alternate Co 1983

Mean            = 0.19  
Variance       = 0.47  
St. Dev.       = 0.68  
St. Error      = 0.19  
Sample size = 13

Test statistic =        2.23

Critical value = +/- 2.18

Evidence that groups are different at 5% significance level.

Geometric means:

-----/  
Group 1.... 30.03  
Group 2.... 0.55

Alternate Grazing Study  
Statistical analysis of C. oncophora pasture larval  
recoveries from P1 (calves) and P3 (sheep) - 1984

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanent Co 1984  
Replicates in group 1 : 13

Group 2 description : App K. Alternate Co 1984  
Replicates in group 2 : 13

Group 1 data :

1	0.00
2	175.00
3	0.00
4	0.00
5	58.00
6	0.00
7	125.00
8	303.00
9	769.00
10	4511.00
11	4000.00
12	35555.00
13	17692.00

Group 2 data :

1	0.00
2	0.00
3	0.00
4	191.00
5	0.00
6	0.00
7	0.00
8	0.00
9	0.00
10	0.00
11	0.00
12	0.00
13	0.00

Results:  
=====

Group 1 : App K. Permanent Co 1984

Mean            = 2.12  
Variance       = 2.82  
St. Dev.       = 1.68  
St. Error      = 0.47  
Sample size = 13

Group 2 : App K. Alternate Co 1984

Mean            = 0.18  
Variance       = 0.40  
St. Dev.       = 0.63  
St. Error      = 0.18  
Sample size = 13

Test statistic =        3.90

Critical value = +/- 2.18

Evidence that groups are different at 5% significance level.

Geometric means:

-----  
Group 1.... 130.39  
Group 2.... 0.50

Alternate Grazing Study  
Statistical analysis of C. oncophora pasture larval  
recoveries from P1 (calves) and P2 (sheep) - 1985

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App K. Permanent Co 1985  
Replicates in group 1 : 11

Group 2 description : App K. Alternate Co 1985  
Replicates in group 2 : 11

Group 1 data :

1	125.00
2	0.00
3	0.00
4	488.00
5	1136.00
6	816.00
7	1350.00
8	1364.00
9	512.00
10	932.00
11	2542.00

Group 2 data :

1	599.00
2	94.00
3	0.00
4	0.00
5	0.00
6	0.00
7	0.00
8	0.00
9	0.00
10	0.00
11	0.00

Results:

=====

Group 1 : App K. Permanent Co 1985

Mean            = 2.37  
Variance       = 1.49  
St. Dev.       = 1.22  
St. Error      = 0.37  
Sample size = 11

Group 2 : App K. Alternate Co 1985

Mean            = 0.43  
Variance       = 0.96  
St. Dev.       = 0.98  
St. Error      = 0.30  
Sample size = 11

Test statistic =        4.12

Critical value = +/- 2.23

Evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1.... 235.33  
Group 2.... 1.71

Appendix L  
Alternate Grazing Study  
Individual and Group Mean Worm Burdens

Paddock 1 - Permanent Cattle Grazing

Year - 1982							
Calf No.	Necropsy Date	Adult	<u>Ostertagia</u> L <sub>4</sub>	Total	Adult	<u>Cooperia</u> L <sub>4</sub>	Total
21	28/09	42,600	21,000	63,600	2,700	1,100	3,800
37	28/09	51,000	13,700	64,700	1,100	100	1,200
48	29/09	14,700	8,900	23,600	3,900	1,400	5,300
53	28/09	59,400	13,200	72,600	2,200	500	2,700
56	28/09	61,300	22,700	84,000	400	200	600
60	29/09	52,100	24,300	76,400	1,300	200	1,500
Mean		46,850	17,300	64,150	1,933	583	2,517
SD		17,104	6,200	21,262	1,263	542	1,781
Year - 1983							
Calf No.	Necropsy Date	Adult	<u>Ostertagia</u> L <sub>4</sub>	Total	Adult	<u>Cooperia</u> L <sub>4</sub>	Total
15	15/10	46,400	54,800	101,200	5,400	14,100	19,500
18	15/10	35,200	54,700	89,900	10,100	22,900	33,000
32	03/10	51,400	81,400	132,800	18,700	45,100	63,800
38	18/10	49,800	88,000	137,800	5,300	21,200	26,500
39	18/10	25,600	44,400	70,000	400	2,000	2,400
50	18/10	11,000	30,700	41,700	2,600	9,800	12,400
Mean		36,567	59,000	95,567	7,083	19,183	26,267
SD		15,923	21,877	36,849	6,555	14,824	21,269
Year - 1984							
Calf No.	Necropsy Date	Adult	<u>Ostertagia</u> L <sub>4</sub>	Total	Adult	<u>Cooperia</u> L <sub>4</sub>	Total
51	11/10	3,500	44,100	47,600	2,500	9,000	11,500
52	11/10	4,200	10,000	14,200	10,500	32,300	42,800
54	28/08	13,600	2,200	15,800	800	2,400	3,200
57	15/10	4,700	26,600	31,300	1,000	2,200	3,200
88	15/10	12,300	75,700	88,000	0	200	200
90	15/10	14,800	75,100	89,900	1,400	6,100	7,500
Mean		8,850	38,950	47,800	2,700	8,700	11,400
SD		5,241	31,702	34,099	3,908	11,983	15,881
Year - 1985							
Calf No.	Necropsy Date	Adult	<u>Ostertagia</u> L <sub>4</sub>	Total	Adult	<u>Cooperia</u> L <sub>4</sub>	Total
29	23/09	24,300	59,000	83,300	500	700	1,200
30	09/09	9,600	10,200	19,800	1,200	2,300	3,500
31	19/09	12,700	25,700	38,400	400	2,000	2,400
32	23/09	28,000	47,000	75,000	2,600	10,300	12,900
33	23/09	9,700	38,100	47,800	3,500	3,900	7,400
36	23/09	28,400	32,000	60,400	300	2,300	2,600
Mean		18,783	35,333	54,117	1,417	3,583	5,000
SD		9,074	16,967	23,630	1,335	3,445	4,413

Appendix L (Cont'd)

Paddock 2 - Alternate Cattle and Sheep Grazing

Year - 1982							
Calf No.	Necropsy Date	Adult	<u>Ostertagia</u> L <sub>4</sub>	Total	Adult	<u>Cooperia</u> L <sub>4</sub>	Total
1	23/09	75,600	34,800	110,400	6,500	6,500	13,000
14	23/09	89,200	23,000	112,200	13,000	14,700	27,700
34	23/09	24,300	275,500	299,800	4,500	2,200	6,700
38	03/08	30,000	13,500	43,500	8,400	4,900	13,300
44	23/09	35,600	18,900	54,500	3,600	4,300	7,900
54	09/08	74,200	32,200	106,400	31,700	8,300	40,000
Mean		54,817	66,317	121,133	11,283	6,817	18,100
SD		27,951	102,790	92,521	10,545	4,376	13,075

Year - 1984							
Calf No.	Necropsy Date	Adult	<u>Ostertagia</u> L <sub>4</sub>	Total	Adult	<u>Cooperia</u> L <sub>4</sub>	Total
3	10/10	9,600	29,200	38,800	6,400	13,700	20,100
17	12/10	4,500	11,600	16,100	3,500	9,100	12,600
45	12/10	3,200	11,900	15,100	1,500	3,700	5,200
53	12/10	4,300	10,900	15,200	5,700	9,100	14,800
55	10/10	200	1,300	1,500	6,400	13,800	20,200
56	10/10	19,000	33,400	52,400	53,200	189,700	242,900
Mean		6,800	16,383	23,183	12,783	39,850	52,633
SD		6,704	12,283	18,692	19,893	73,506	93,376

Paddock 3 - Alternate Sheep and Cattle Grazing

Year - 1983							
Calf No.	Necropsy Date	Adult	<u>Ostertagia</u> L <sub>4</sub>	Total	Adult	<u>Cooperia</u> L <sub>4</sub>	Total
44	18/10	3,600	9,500	13,100	4,200	8,400	12,600
62	18/10	10,000	28,800	38,800	6,400	13,500	19,900
78	19/10	5,600	9,600	15,200	3,900	10,900	14,800
79	18/10	4,800	11,800	16,600	1,400	2,400	3,800
80	19/10	19,200	33,200	52,400	2,500	17,700	20,200
81	19/10	12,300	22,300	34,600	11,300	28,900	40,200
Mean		9,250	19,200	28,450	4,950	13,633	18,583
SD		5,898	10,381	15,938	3,541	9,063	12,168

Year - 1985							
Calf No.	Necropsy Date	Adult	<u>Ostertagia</u> L <sub>4</sub>	Total	Adult	<u>Cooperia</u> L <sub>4</sub>	Total
34	19/19	28,900	28,000	56,900	26,400	32,400	58,800
35	09/09	63,100	33,100	96,200	68,200	42,800	111,000
37	23/10	2,500	4,300	6,800	0	400	400
38	19/09	33,400	31,200	64,600	24,400	20,000	44,400
39	09/09	4,200	16,200	20,400	39,000	24,400	63,400
40	16/10	12,100	21,400	33,500	3,500	4,700	8,200
Mean		24,033	22,367	46,400	26,917	20,783	47,700
SD		22,966	10,866	32,656	25,019	16,171	40,460

Alternate Grazing Study  
Statistical analysis of O.ostertagi worm burdens - 1982

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App L. Permanent Oo 1982  
Replicates in group 1 : 6

Group 2 description : App L. Alternate Oo 1982  
Replicates in group 2 : 6

Group 1 data :

1	63600.00
2	64700.00
3	23600.00
4	72600.00
5	84000.00
6	76400.00

Group 2 data :

1	110400.00
2	112200.00
3	299800.00
4	43500.00
5	54500.00
6	106400.00

Results:

=====

Group 1 : App L. Permanent Oo 1982

Mean            = 4.78  
Variance       = 0.04  
St. Dev.       = 0.20  
St. Error      = 0.08  
Sample size = 6

Group 2 : App L. Alternate Oo 1982

Mean            = 5.00  
Variance       = 0.09  
St. Dev.       = 0.29  
St. Error      = 0.12  
Sample size = 6

Test statistic =    - 1.50

Critical value = +/- 2.57

No evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1.... 59694.08  
Group 2.... 98917.01

**Alternate Grazing Study**  
**Statistical analysis of O.ostertagi worm burdens - 1983**

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App L. Permanent Oo 1983  
Replicates in group 1 : 6

Group 2 description : App L. Alternate Oo 1983  
Replicates in group 2 : 6

Group 1 data :

1	101200.00
2	89900.00
3	132800.00
4	137800.00
5	70000.00
6	41700.00

Group 2 data :

1	13100.00
2	38800.00
3	15200.00
4	16600.00
5	52400.00
6	34600.00

Results:

=====

Group 1 : App L. Permanent Oo 1983

Mean            = 4.95  
Variance        = 0.04  
St. Dev.        = 0.19  
St. Error       = 0.08  
Sample size = 6

Group 2 : App L. Alternate Oo 1983

Mean            = 4.39  
Variance        = 0.06  
St. Dev.        = 0.25  
St. Error       = 0.10  
Sample size = 6

Test statistic =        4.26

Critical value = +/- 2.57

Evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1.... 88668.78  
Group 2.... 24797.78



Alternate Grazing Study  
Statistical analysis of Oostertagi worm burdens - 1984

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App L. Permanent Oo 1984  
Replicates in group 1 : 6

Group 2 description : App L. Alternate Oo 1984  
Replicates in group 2 : 6

Group 1 data :

1	47600.00
2	14200.00
3	15800.00
4	31300.00
5	88000.00
6	89900.00

Group 2 data :

1	38800.00
2	16100.00
3	15100.00
4	15200.00
5	1500.00
6	52400.00

Results:

=====

Group 1 : App L. Permanent Oo 1984

Mean	= 4.57
Variance	= 0.12
St. Dev.	= 0.35
St. Error	= 0.14
Sample size	= 6

Group 2 : App L. Alternate Oo 1984

Mean	= 4.18
Variance	= 0.29
St. Dev.	= 0.54
St. Error	= 0.22
Sample size	= 6

Test statistic = 1.50

Critical value = +/- 2.57

No evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1....	37187.19
Group 2....	14974.53

Alternate Grazing Study  
Statistical analysis of O.ostertagi worm burdens - 1985

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App L. Permanent Oo 1985  
Replicates in group 1 : 6

Group 2 description : App L. Alternate Oo 1985  
Replicates in group 2 : 6

Group 1 data :

1	83300.00
2	19800.00
3	38400.00
4	75000.00
5	47800.00
6	60400.00

Group 2 data :

1	56900.00
2	96200.00
3	6800.00
4	64600.00
5	20400.00
6	33500.00

Results:  
=====

Group 1 : App L. Permanent Oo 1985

Mean	= 4.69
Variance	= 0.05
St. Dev.	= 0.23
St. Error	= 0.09
Sample size	= 6

Group 2 : App L. Alternate Oo 1985

Mean	= 4.54
Variance	= 0.17
St. Dev.	= 0.42
St. Error	= 0.17
Sample size	= 6

Test statistic =        0.79

Critical value = +/- 2.57

No evidence that groups are different at 5% significance level.

Geometric means:

Group 1....	48924.81
Group 2....	34352.43

**Alternate Grazing Study**  
**Statistical analysis of C. oncophora worm burdens - 1982**

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App L. Permanent Co 1982  
Replicates in group 1 : 6

Group 2 description : App L. Alternate Co 1982  
Replicates in group 2 : 6

Group 1 data :

1	3800.00
2	1200.00
3	5300.00
4	2700.00
5	600.00
6	1500.00

Group 2 data :

1	13000.00
2	27700.00
3	6700.00
4	13300.00
5	7900.00
6	40000.00

Results:  
=====

Group 1 : App L. Permanent Co 1982

Mean            = 3.30  
Variance       = 0.12  
St. Dev.       = 0.35  
St. Error      = 0.14  
Sample size = 6

Group 2 : App L. Alternate Co 1982

Mean            = 4.17  
Variance       = 0.09  
St. Dev.       = 0.30  
St. Error      = 0.12  
Sample size = 6

Test statistic =    - 4.62

Critical value = +/- 2.57

Evidence that groups are different at 5% significance level.

Geometric means:

-----  
Group 1.... 1971.86  
Group 2.... 14712.23

Alternate Grazing Study  
Statistical analysis of C.oncophora worm burdens - 1983

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App L. Permanent Co 1983  
Replicates in group 1 : 6

Group 2 description : App L. Alternate Co 1983  
Replicates in group 2 : 6

Group 1 data :

1	19500.00
2	33000.00
3	63800.00
4	26500.00
5	2400.00
6	12400.00

Group 2 data :

1	12600.00
2	19900.00
3	14800.00
4	3800.00
5	20200.00
6	40200.00

Results:

=====

Group 1 : App L. Permanent Co 1983

Mean	= 4.25
Variance	= 0.24
St. Dev.	= 0.49
St. Error	= 0.20
Sample size	= 6

Group 2 : App L. Alternate Co 1983

Mean	= 4.18
Variance	= 0.12
St. Dev.	= 0.34
St. Error	= 0.14
Sample size	= 6

Test statistic =        0.31

Critical value = +/- 2.57

No evidence that groups are different at 5% significance level.

Geometric means:

-----

Group 1....	17853.75
Group 2....	15013.58

**Alternate Grazing Study**  
**Statistical analysis of C.oncophora worm burdens - 1984**

ANIMAL DESIGNS... Two-sample t-test Date... 03-Jul-90

Group 1 description : App L. Permanent Co 1984  
Replicates in group 1 : 6

Group 2 description : App L. Alternate Co 1984  
Replicates in group 2 : 6

**Group 1 data :**

1	11500.00
2	42800.00
3	3200.00
4	3200.00
5	200.00
6	7500.00

**Group 2 data :**

1	20100.00
2	12600.00
3	5200.00
4	14800.00
5	20200.00
6	242900.00

**Results:**  
=====

**Group 1 : App L. Permanent Co 1984**

Mean = 3.65  
Variance = 0.61  
St. Dev. = 0.78  
St. Error = 0.32  
Sample size = 6

**Group 2 : App L. Alternate Co 1984**

Mean = 4.33  
Variance = 0.31  
St. Dev. = 0.56  
St. Error = 0.23  
Sample size = 6

Test statistic = - 1.74

Critical value = +/- 2.57

No evidence that groups are different at 5% significance level.

**Geometric means:**

Group 1.... 4433.51  
Group 2.... 21385.21

Alternate Grazing Study  
Statistical analysis of C.oncophora worm burdens - 1985

ANIMAL DESIGNS... Two-sample t-test    Date... 03-Jul-90

Group 1 description : App L. Permanent Co 1985  
Replicates in group 1 : 6

Group 2 description : App L. Alternate Co 1985  
Replicates in group 2 : 6

Group 1 data :

```
-----
1      1200.00
2      3500.00
3      2400.00
4      12900.00
5      7400.00
6      2600.00
```

Group 2 data :

```
-----
1      58800.00
2      111000.00
3      400.00
4      44400.00
5      63400.00
6      8200.00
```

Results:  
=====

Group 1 : App L. Permanent Co 1985

```
Mean      = 3.57
Variance  = 0.14
St. Dev.  = 0.37
St. Error = 0.15
Sample size = 6
```

Group 2 : App L. Alternate Co 1985

```
Mean      = 4.30
Variance  = 0.84
St. Dev.  = 0.91
St. Error = 0.37
Sample size = 6
```

Test statistic =    - 1.81

Critical value = +/- 2.57

No evidence that groups are different at 5% significance level.

Geometric means:

```
-----
Group 1.... 3684.79
Group 2.... 19808.28
```

# Appendix M

## N. battus Field Study

### N. battus Faecal Egg Counts

Animal No.	Day											
	0	14	28	42	56	70	84	98	112	126	140	154
W43	0	0	0	0	0	0	0	0	0	0	0	0
W44	0	0	0	0	0	0	0	0	0	0	0	0
W45	0	0	0	250	0	50	0	-	-	-	-	-
W46	0	0	150	50	0	0	0	0	0	0	0	0
W47	0	0	0	250	150	50	0	0	0	0	0	-
W48	0	0	200	50	0	0	0	0	0	0	0	0
R49	0	0	250	300	50	50	200	50	0	0	0	0
R50	0	0	0	0	150	0	0	0	0	0	0	0
R51	0	0	150	0	0	0	0	0	0	0	0	0
R52	0	0	400	350	0	0	0	0	-	-	-	-
R53	0	0	300	0	0	50	0	-	-	-	-	-
R54	0	0	0	0	100	50	0	0	0	0	0	0
Mean	0	0	121	104	38	21	17	5	0	0	0	0
SD	0	0	142	139	61	26	58	16	0	0	0	0

# Appendix N

## N. battus Field Study

### N. battus L<sub>3</sub> Recoveries/kg Dried Herbage

Animal No.	Day											
	0	14	28	42	56	70	84	98	112	126	140	154
R1	1,330	963	610	126	251	211	136	0	250	0	322	0
R2	1,230	1,317	500	202	155	97	144	0	222	0	188	0
Mean	1,280	1,140	555	164	203	154	140	0	236	0	255	0

# Appendix O

## N. battus Dose Titration Study

### Individual and Group Mean Bodyweights

Group	Animal No.	Day					
		1	7	14	21	28	33
1	040	175	175	185	190	195	200
	041	170	170	180	190	200	205
	042	170	175	190	190	195	205
Mean		172	173	185	190	197	203
SD		3	3	5	0	3	3
2	R33	180	185	190	195	200	205
	R34	165	165	175	185	195	200
	R35	170	175	180	180	185	195
Mean		172	175	182	187	193	200
SD		8	10	8	8	8	5
3	G25	165	160	170	180	185	185
	G26	165	165	175	180	190	195
	G27	170	170	170	175	180	185
Mean		167	165	172	178	185	188
SD		3	5	3	3	5	6
4	B50	170	175	180	180	185	190
	B51	165	175	175	180	185	185
	B52	170	175	180	185	185	190
Mean		168	175	178	182	185	188
SD		3	0	3	3	0	3
5	Y61	150	155	165	170	175	185
	Y62	155	160	165	170	175	180
	Y63	160	170	175	185	190	195
Mean		155	162	168	175	180	187
SD		5	8	6	9	9	8



**N. battus Dose Titration Study**  
**Statistical analysis of liveweight data**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 17-Jul-91

Experiment title..... Appendix O

Number of treatments... 5

Description:

No. of  
obsvs.

Treatment 1	Gp1	6
Treatment 2	Gp2	6
Treatment 3	Gp3	6
Treatment 4	Gp4	6
Treatment 5	Gp5	6

Replicate	Treatments 1	2	3	4	5
1	172.00	172.00	167.00	168.00	155.00
2	173.00	175.00	165.00	175.00	162.00
3	185.00	182.00	172.00	178.00	168.00
4	190.00	187.00	178.00	182.00	175.00
5	197.00	193.00	185.00	185.00	180.00
6	203.00	200.00	188.00	188.00	187.00

**ONE WAY ANALYSIS OF VARIANCE :**

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	976.20	4.00	244.05	2.20
Experimental error	2769.17	25.00	110.77	
Total	3745.37	29.00		

Critical value for a 5% significance test is  $F_{.95(4,25)} = 2.78$

Since the calculated F-value is less than the critical F-value, then the data suggests that treatment effects are not different.

**NEWMAN KEULS MULTIPLE RANGE TEST :**

K	Means	Ranges	L.S.R.	Treatment	St. Error
2	171.17			5	
3	175.83	2.92	12.55	3	4.297
4	179.33	3.53	15.17	4	
5	184.83	3.90	16.76	2	
6	186.67	4.17	17.92	1	

Treatment Significantly  
Comparison Different

1 V 5	No
1 V 3	No
1 V 4	No
1 V 2	No
2 V 5	No
2 V 3	No
2 V 4	No
4 V 5	No
4 V 3	No
3 V 5	No

# Appendix P

## N. battus Dose Titration Study

### Individual and Group Mean Plasma Pepsinogen Levels (mU Tyrosine)

Group	Animal No.	Day					
		1	7	14	21	28	33
1	040	496	535	573	612	667	652
	041	480	717	770	823	769	872
	042	501	644	543	655	741	449
	Mean	501	632	629	697	726	658
	SD	0	92	123	112	53	212
2	R33	521	599	766	731	676	756
	R34	455	586	679	772	706	714
	R35	556	467	655	689	640	711
	Mean	511	551	700	731	674	727
	SD	51	73	58	42	33	25
3	G25	614	616	744	797	679	819
	G26	481	599	613	680	690	623
	G27	467	561	600	670	640	654
	Mean	521	592	652	716	670	699
	SD	81	28	80	71	26	105
4	B50	526	557	600	603	755	785
	B51	540	578	642	678	705	760
	B52	436	543	589	657	723	744
	Mean	501	559	610	646	728	763
	SD	56	18	28	39	25	21
5	Y61	553	544	558	639	586	567
	Y62	645	610	835	1,007	962	766
	Y63	577	603	654	643	670	641
	Mean	592	586	682	763	739	658
	SD	48	36	141	211	197	101

**N. battus Dose Titration Study**  
**Statistical analysis of pepsinogen data**

ANIMAL DESIGNS... One Way anova then Range Test      Date... 17-Jul-91

Experiment title..... Appendix P

Number of treatments... 5

		Description:	No. of obsvs.
		-----	-----
Treatment 1	Gp1		6
Treatment 2	Gp2		6
Treatment 3	Gp3		6
Treatment 4	Gp4		6
Treatment 5	Gp5		6

Replicate	Treatments				
	1	2	3	4	5
1	501.00	511.00	521.00	501.00	592.00
2	632.00	551.00	592.00	559.00	586.00
3	629.00	700.00	652.00	610.00	682.00
4	697.00	731.00	716.00	646.00	763.00
5	726.00	674.00	670.00	728.00	739.00
6	658.00	727.00	699.00	763.00	658.00

ONE WAY ANALYSIS OF VARIANCE :

=====

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	4559.13	4.00	1139.78	0.16
Experimental error	178252.33	25.00	7130.09	
Total	182811.47	29.00		

Critical value for a 5% significance test is  $F_{.95}(4,25) = 2.78$

Since the calculated F-value is less than the critical F-value, then the data suggests that treatment effects are not different.

NEWMAN KEULS MULTIPLE RANGE TEST :

=====

K	Means	Ranges	L.S.R.	Treatment	St. Error
-----	-----	-----	-----	-----	-----
1	634.50			4	
2	640.50	2.92	100.66	1	34.472
3	641.67	3.53	121.69	3	
4	649.00	3.90	134.44	2	
5	670.00	4.17	143.75	5	

Treatment Significantly  
Comparison Different

-----	-----
5 V 4	No
5 V 1	No
5 V 3	No
5 V 2	No
2 V 4	No
2 V 1	No
2 V 3	No
3 V 4	No
3 V 1	No
1 V 4	No

# Appendix Q

## N. battus Dose Titration Study

### Individual and Group Mean Faecal Egg Counts

Group	Animal No.	Day					
		1	7	14	21	28	33
1	040	0	0	0	0	0	0
	041	0	0	0	0	0	0
	042	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
	SD	0	0	0	0	0	0
2	R33	0	0	0	0	0	0
	R34	0	0	0	0	250	0
	R35	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
	SD	0	0	0	0	29	0
3	G25	0	0	0	0	0	0
	G26	0	0	0	0	0	0
	G27	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
	SD	0	0	0	0	0	0
4	B50	0	0	0	0	0	0
	B51	0	0	0	0	0	0
	B52	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
	SD	0	0	0	0	0	0
5	Y61	0	0	0	0	0	0
	Y62	0	0	0	0	0	0
	Y63	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
	SD	0	0	0	0	0	0

# Appendix R

## N. battus Combined Infection Study

### Individual and Group Mean Bodyweights

Group	Animal No.	Day					
		1	7	14	21	28	33
1	057	145	150	160	165	170	175
	058	150	150	155	165	170	170
	059	145	150	150	155	165	170
	060	150	155	150	155	160	165
Mean		145	151	154	160	166	170
SD		4	3	5	6	5	4
2	R41	150	155	160	165	175	180
	R42	150	160	165	175	175	185
	R43	145	155	160	170	175	175
	R44	150	155	160	165	175	185
Mean		149	156	161	169	175	181
SD		3	3	3	5	0	5
3	G32	145	150	155	160	165	170
	G33	150	155	155	160	165	170
	G34	150	160	160	165	170	175
	G35	150	155	160	165	165	170
Mean		149	155	158	163	166	171
SD		3	4	3	3	3	3
4	B66	150	150	155	160	165	170
	B67	140	150	150	155	165	170
	B68	145	155	150	150	160	165
	B69	145	150	150	155	160	170
Mean		145	151	151	155	163	169
SD		4	3	3	4	3	3
5	Y80	150	155	150	150	160	165
	Y81	150	150	155	150	155	160
	Y82	145	150	150	155	160	160
	Y83	150	155	150	150	160	170
Mean		149	153	151	151	159	164
SD		3	3	3	3	3	5

Appendix R (Cont'd)

Group	Animal No.	Day					
		1	7	14	21	28	33
6	P94	145	145	145	150	160	165
	P95	155	155	155	165	170	170
	P96	150	155	160	165	165	170
	P97	145	150	150	160	165	170
Mean		149	151	153	160	165	169
SD		5	5	6	7	4	3
7	Bk6	150	155	160	170	175	180
	Bk7	150	160	165	170	180	180
	Bk8	145	150	155	165	170	180
	Bk9	150	155	160	165	170	175
Mean		149	155	160	168	174	179
SD		3	4	4	3	5	3

**N. battus Combined Infection Study**  
**Statistical analysis of liveweight data**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 17-Jul-91

Experiment title..... Appendix R

Number of treatments... 7

		Description:	No. of obsvs.
		-----	-----
Treatment 1	Gp1		6
Treatment 2	Gp2		6
Treatment 3	Gp3		6
Treatment 4	Gp4		6
Treatment 5	Gp5		6
Treatment 6	Gp6		6
Treatment 7	Gp7		6

Replicate	Treatments				
	1	2	3	4	5
1	145.00	149.00	149.00	145.00	149.00
2	151.00	156.00	155.00	151.00	153.00
3	154.00	161.00	158.00	151.00	151.00
4	160.00	169.00	163.00	155.00	151.00
5	166.00	175.00	166.00	163.00	159.00
6	170.00	181.00	171.00	169.00	164.00

Replicate	Treatments	
	6	7
1	149.00	149.00
2	151.00	155.00
3	153.00	160.00
4	160.00	168.00
5	165.00	174.00
6	169.00	179.00

**ONE WAY ANALYSIS OF VARIANCE :**

=====

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	601.33	6.00	100.22	1.16
Experimental error	3034.00	35.00	86.69	
Total	3635.33	41.00		

Critical value for a 5% significance test is  $F_{.95}(6,35) = 2.42$

Since the calculated F-value is less than the critical F-value, then the data suggests that treatment effects are not different.

# Appendix S

## N. battus Combined Infection Study

### Individual and Group Mean Plasma Pepsinogen Levels

Group	Animal No.	Day					
		1	7	14	21	28	33
1	057	886	867	1,033	1,355	1,444	1,866
	058	769	799	967	1,425	1,543	1,768
	059	780	812	1,105	1,344	1,655	1,567
	060	655	804	1,209	1,657	1,766	1,731
Mean		733	821	1,079	1,445	1,602	1,733
SD		94	31	104	146	139	142
2	R41	657	724	865	789	812	863
	R42	688	693	733	766	800	874
	R43	798	688	756	793	769	756
	R44	754	758	790	864	813	799
Mean		724	716	786	803	799	823
SD		64	32	58	42	21	56
3	G32	655	699	680	679	711	720
	G33	674	701	658	690	763	801
	G34	658	745	679	733	658	854
	G35	673	678	744	756	810	832
Mean		665	706	690	715	736	802
SD		10	28	37	36	66	59
4	B66	763	860	1,055	1,164	1,766	2,002
	B67	654	844	1,141	1,355	1,566	1,777
	B68	721	831	1,090	1,422	1,488	1,657
	B69	704	845	978	1,344	1,697	1,888
Mean		711	845	1,066	1,321	1,629	1,831
SD		45	12	68	110	125	148
5	Y80	655	731	744	781	777	803
	Y81	678	689	732	663	739	864
	Y82	677	788	699	786	765	768
	Y83	733	769	738	755	855	867
Mean		686	744	728	746	784	826
SD		33	44	20	57	50	48



Appendix S (Cont'd)

Group	Animal No.	Day					
		1	7	14	21	28	33
6	P94	732	798	1,011	1,211	1,320	1,873
	P95	729	859	1,122	1,374	1,266	1,555
	P96	706	834	1,156	1,266	1,641	1,873
	P97	711	899	1,035	1,322	1,555	1,699
Mean		720	848	1,081	1,293	1,446	1,750
SD		13	42	69	70	181	154
7	Bk6	604	711	822	705	788	708
	Bk7	665	683	765	762	763	772
	Bk8	679	699	690	794	681	756
	Bk9	711	657	761	770	703	721
Mean		665	688	760	758	734	739
SD		45	23	54	38	50	30

**N. battus Combined Infection Study**  
**Statistical analysis of pepsinogen data**

ANIMAL DESIGNS... One Way anova then Range Test    Date... 30-Jul-91

Experiment title..... Appendix S  
 Number of treatments... 7

		Description:	No. of obsvs.
		-----	-----
Treatment 1	Gp1		6
Treatment 2	Gp2		6
Treatment 3	Gp3		6
Treatment 4	Gp4		6
Treatment 5	Gp5		6
Treatment 6	Gp6		6
Treatment 7	Gp7		6

Replicate	Treatments				
	1	2	3	4	5
1	733.00	724.00	665.00	711.00	686.00
2	821.00	716.00	706.00	845.00	744.00
3	1079.00	786.00	690.00	1066.00	728.00
4	1445.00	803.00	715.00	1321.00	746.00
5	1602.00	799.00	736.00	1629.00	784.00
6	1733.00	823.00	802.00	1831.00	826.00

Replicate	Treatments	
	6	7
1	720.00	665.00
2	848.00	688.00
3	1081.00	760.00
4	1293.00	758.00
5	1446.00	734.00
6	1750.00	739.00

ONE WAY ANALYSIS OF VARIANCE :  
 =====

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F Value
Treatment	2361164.95	6.00	393527.49	5.24
Experimental error	2627165.83	35.00	75061.88	
Total	4988330.79	41.00		

Critical value for a 5% significance test is  $F_{.95}(6,35) = 2.42$

Since the calculated F-value is greater than or equal to the critical F-value, then the data suggests that treatment effects are different.

NEWMAN KEULS MULTIPLE RANGE TEST :  
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K	Means	Ranges	L.S.R.	Treatment	St. Error
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	154.50			5	
2	155.67	2.89	10.98	4	3.801
3	157.67	3.48	13.23	1	
4	157.83	3.84	14.60	6	
5	160.33	4.11	15.62	3	
6	164.17	4.30	16.34	7	
7	165.17	4.46	16.95	2	

Treatment Significantly  
Comparison Different

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2 V 5	No
2 V 4	No
2 V 1	No
2 V 6	No
2 V 3	No
2 V 7	No
7 V 5	No
7 V 4	No
7 V 1	No
7 V 6	No
7 V 3	No
3 V 5	No
3 V 4	No
3 V 1	No
3 V 6	No
6 V 5	No
6 V 4	No
6 V 1	No
1 V 5	No
1 V 4	No
4 V 5	No

