STUDIES ON THE CONTROL AND PATHOPHYSIOLOGY OF BOVINE NEMATODIASES

by

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SUMMARY

Presented in this thesis are data from two parasitological studies on bovine parasitic gastroenteric and respiratory tract infections.

In the first of these the control and epidemiology of naturally acquired infections of Ostertagia ostertagi, Cooperia oncophora, Nematodirus battus and Dictyocaulus viviparus in treated and untreated calves were studied. Treatment was by an experimental pour-on formulation of the relatively new anthelmintic Ivermectin.

In the second the pathophysiological effects of an experimental daily trickle infection of O. ostertagi given to calves treated with a morantel tartrate sustained release device (MSRB) were compared with those of infected and clean control animals.

In Chapter 1 the General Introduction deals with the epidemiology, treatment and control of bovine helminthiasis in the United Kingdom and this is followed by the general Materials and Methods in Chapter 2.

Chapter 3, which begins with a revue of the recently developed anthelmintic Ivermectin, deals with the treatment and control of naturally acquired infections with gastrointestinal and respiratory tract nematode parasites. It is clear from the data presented that topically applied ivermectin can be successfully used to control bovine gastrointestinal parasites and that it is also extremely effective against the lungworm Dictyocaulus viviparus.

Increasingly, it would appear that Nematodirus battus, or at least the strain of this species on pasture at the Glasgow University Veterinary Hospital, is adapting to cattle and can now cause clinical disease in this host.

Radioisotopic methodology was a main feature of the second study, described in Chapter 4, which also highlights some of the problems associated with experimental infections designed to simulate a natural uptake of infective larvae under field conditions. While the daily infection dose level of 2,000 O. ostertagi L_3/calf/day was insufficient to cause clinical disease it was nevertheless enough to alter the physiological parameters under study which revealed some of the adverse effects of a subclinical infection of Ostertagia species. Although it was shown that the activity of the MSRB was probably greatest against the adult rather than the larval stages of the parasite, it remains to be determined at which parasitic stage the anthelmintic action of this device primarily occurs.

Finally the data obtained from both experiments is discussed in Chapter 5.

CHAPTER ONE

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Parasitic gastroenteritis (PGE) in cattle is recognised as an economically important disease in temperate climates throughout the world. Numerous works carried out in such zones have shown that the abomasal parasite, Ostertagia ostertagi, is primarily responsible for most of the pathogenic effects characteristic of bovine PGE; these are associated with a range of clinical signs e.g. lowered appetite, failure to gain weight or weight loss, and diarrhoea.

Ostertagia ostertagi, first described by Ostertag in 1890, has a direct life cycle typical of the trichostrongylids with a free living, or preparasitic, phase followed by a parasitic phase of development within the host.

A diagrammatic representation of the life cycle is given in Figure (1).

Fertilised eggs are passed to the exterior in the faeces and under suitable conditions of temperature and humidity (optimally temperature 18^{o}C and humidity 90%) develop and hatch as first stage larvae (L_1) which then moult to become second larval forms (L_2). Both the L_1 and L_2 larval stages are motile and use bacteria as their principal food source.

The L_2 then moults to the third infective larva (L_3). As the L_3 is confined within the retained cuticle of the L_2 it cannot feed and must survive on stored nutrients acquired by the earlier stages. Development from egg to infective larvae under favourable circumstances takes about 7 - 10 days.

Larval development and survival again depend principally on temperature and humidity with mild moist conditions being the

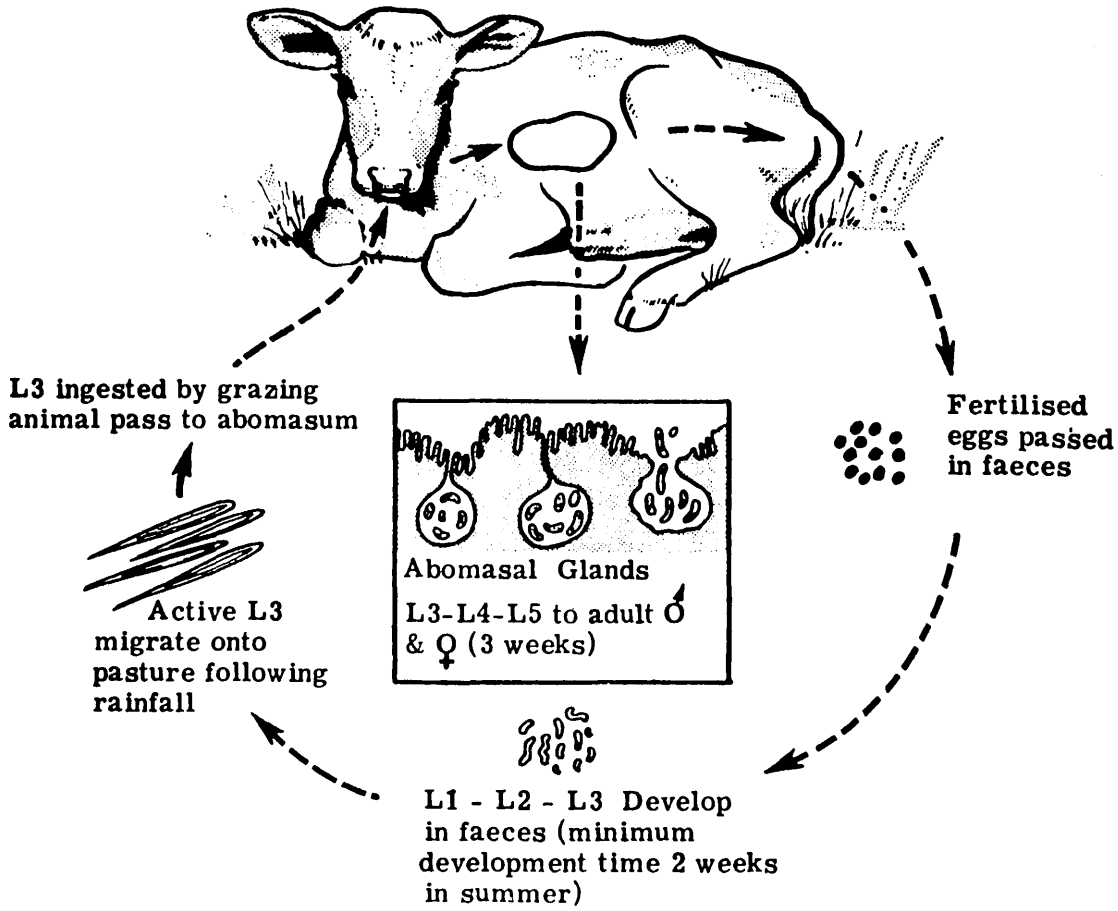


Figure 1. Life cycle of Ostertagia ostertagi

most favourable and a hot dry environment the most unsuitable. Development from egg to L_3 takes place within the large faecal masses produced by cattle and the process whereby the L_3 migrate away from these faecal pats on to the herbage and so become available to grazing cattle is usually referred to as translation (Michel and Parfitt, 1956).

Rose (1962) demonstrated the dependence of translation on rainfall. By monitoring L_3 populations around artificially watered faecal pats he showed that in the absence of wet conditions the L_3 remain congregated within or adjacent to the faeces. Since cattle are reluctant to eat the herbage close to faecal masses contact between the host and the parasite in dry conditions is therefore minimal.

Epidemiological studies in Western Europe have shown that there is an annual seasonal variation in the numbers of O. ostertagi L_3 on pasture and that this occurs in a regular manner. This has been demonstrated by workers in several countries. For example in the U.K. by Michel (1969a), Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart (1965b); in Germany by Burger, Eckert, Wetzel and Michael (1966); in France by Raynaud, Loudren and Jolivet (1971); in the Netherlands (Kloosterman, 1971); in Denmark (Henriksen, Jorgensen, Nansen, Sejrsen, Larsen and Klousen (1975) and in Switzerland (Eisenegger and Eckert, 1975).

A proportion of L_3 survive the winter on pasture and susceptible calves grazing, especially under intensive conditions, during their first season i.e. from May onwards can

ingest large numbers of larvae which then complete their development to the pathogenic L_5 and adult stages in three to four weeks. The large number of eggs produced by this first generation of adult worms hatch and develop to the infective stage by mid-July. If the calves continue to be set-stocked on the same pasture these larvae are ingested to produce a second generation of adults within a few weeks. Under these conditions ostertagiasis of a kind known as Type I can occur at any time from late July until the end of the autumn (Anderson et al, 1965b).

After ingestion of the L_3 by a susceptible host, the sheath is shed in the rumen and the larva moves to the abomasum where it penetrates a gastric gland to undergo further development to the fourth larval stage (L_4). After a period of about seven days the final moult to the fifth larval or (L_5) stage takes place and 18 days after initial infection the parasites emerge from the glands as sexually mature adults to lie on the mucosal surface. The main damage to the host occurs during the emergence of the L_5 from the gastric glands. During this period the parietal cells, which produce hydrochloric acid, are replaced by rapidly dividing, undifferentiated non-acid secreting cells (Ritchie, Anderson, Armour, Jarrett Jennings and Urquhart, 1966). Initially, these cellular changes occur in the parasitised gland, but as it becomes distended by the growing worm which increases from 1.3 to 8.0 mm in length, these changes spread to the surrounding non-parasitised glands, the end result being a thickened hyperplastic gastric mucosa. Macroscopically, the lesion is a raised nodule with a visible central orifice; in

heavy infections these nodules coalesce to produce an effect reminiscent of morocco leather. Due to incompletely formed cell junctions between the rapidly dividing and undifferentiated cells which now line the parasitised glands there is an increased permeability of the abomasal glandular epithelium to macromolecules such as pepsinogen and plasma proteins. In addition another recent theory suggests 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). The results of these changes are (a) leakage of pepsinogen into the circulation leading to elevated plasma pepsinogen levels and (b) loss of plasma proteins into the gut lumen eventually leading to hypoalbuminaemia and weight loss with diarrhoea. The aetiology of the latter is unknown but may be associated with the loss of acid conditions in the abomasum allowing the entry of massive numbers of bacteria into the small intestine (Jennings, Armour, Lawson and Roberts, 1966).

As the temperature falls in the autumn, many of the L_3 ingested through September to late November do not mature but become inhibited at the early fourth larval stage (EL_4) with up to 80% of such larvae becoming arrested in development (Anderson, Armour, Jennings, Ritchie and Urquhart, 1965a; Armour, Jennings and Urquhart, 1969 and Michel, 1969b). It has been clearly shown (Ross, 1965; Anderson *et al*, 1965a; Armour, Jennings and Urquhart, 1969; Smith, 1973 and Reid and Armour, 1972) in Western Europe and North America that larval inhibition coincides with

the exposure of L_3 to falling temperatures but the underlying mechanism is still unknown. Conversely, in Australia the phenomenon does not appear to be related to a temperature effect on infective larvae but is associated with dry, arid conditions (Hotson, 1967; Anderson, 1971). In Northern Nigeria (Hart, 1964) and South America (Furlong, Lovisi deAbreu and Verneque, 1985; Fiel, Steffan, Ambrustolo and Biondani, 1985) have also noted that larval inhibition appears to be related to a decrease in environmental humidity.

Such infections, acquired late in the year are generally asymptomatic or show only mild symptoms and this is sometimes referred to as the pre-Type II phase.

Maturation of these EL_4 takes place during winter and early spring and if large numbers of these develop at the same time then the clinical Type II disease ensues. The pathogenesis and clinical signs are broadly similar to those of Type I except that generally only a small number of calves in any one group are clinically affected. There are two possible explanations for this. It may be that the calves which develop Type II ostertagiasis have ingested much larger numbers of larvae or alternatively, that the sequential maturation of larval population as demonstrated by Michel (1974) may have been more regular in unaffected calves so that insufficient numbers of larvae developed at any one time to produce clinical disease.

DIAGNOSIS

A tentative diagnosis of ostertagiasis may be made on the basis of clinical signs such as inappetance, diarrhoea and weight loss.

The time or season of presentation is another feature of the disease and here a detailed history from the farmer is useful in obtaining information particularly about grazing and animal management. For example, in Europe Type I ostertagiasis occurs from July until September and Type II from March to May.

Faecal egg counts can be useful in the case of Type I ostertagiasis where a count of more than 1000 eggs per gram (epg) is usually found in clinical cases (Anderson et al, 1965b). For Type II ostertagiasis, however, faecal egg counts are of little diagnostic value. Estimation of plasma-pepsinogen levels is another aid to diagnosis. In endemic areas ostertagiasis in animals up to two years is reflected by a rise in plasma-pepsinogen level to more than 3.0 international units of tyrosine (Iu) compared with normal values of about 0.7 - 1.5 Iu.

In older cattle, elevated plasma pepsinogen levels do not necessarily correlate with large adult worm burdens, but may represent a leakage into plasma from a hypersensitive mucosa following heavy larval challenge (McKellar, 1984).

At post mortem the abomasal mucosa shows a characteristic appearance with circular greyish white nodules giving the so-called "morocco leather" effect due to hyperplasia and the loss of differentiation of the mucosal cells. Adult worms can also be seen on close inspection by the naked eye.

In clinical cases worm burdens are usually high with more than 40,000 parasites present but in animals which have been diarrhoeic for several days before necropsy a lower number of adult worms is often found (Anderson et al, 1965b; Anderson,

Hamsky and Titchen, 1976; 1981).

Herbage analysis can also help in determining the level of challenge to older animals in which a laboratory diagnosis is more difficult due to less reliable plasma pepsinogen and faecal egg count parameters.

With a larval count in excess of 1000 larvae per kilogram of dried herbage (L_3/kdh) the daily larval intake of grazing cows is in excess of 10,000. This level is probably sufficient to cause clinical disease in susceptible adult animals or to upset the normal functioning of the gastric mucosa in immune cows (Armour, 1985).

Recently, studies carried out in the Netherlands (Keus, Kloosterman, Van den Brink, 1981) have used the Elisa (Enzyme Linked Immunosorbent Assay) method in order to achieve a serological diagnosis. Good correlation has been demonstrated between antibody titre and clinical signs and also between plasma pepsinogen levels and worm populations in bovines suffering from Type I and Type II ostertagiasis.

Recent experimental work conducted by McKellar (1984) and Pitt, Fox, Gerrelli and Jacobs (1987), has demonstrated a marked rise in levels of gastrin in response to different patterns of infections with Ostertagia ostertagi and this could be of potential diagnostic value.

TREATMENT

For the efficient treatment of parasitic gastroenteritis in cattle (especially ostertagiasis) an anthelmintic should be at least 90% effective against the developing larval stages and adult worms (Armour and Bogan, 1982). Where treatment is

administered in the autumn or winter it is probably that a large number of larvae will be arrested in development so that it is necessary to use drugs which are effective against such inhibited stages.

Available drugs which meet some of these requirements originate from three main chemical groupings:

(1) The benzimidazoles (fenbendazole, oxfendazole, albendazole) and probenzimidazoles (thiabendazole, febantel, thiophionate).

(2) Imidazothiazoles (tetramisoles and pyrantel).

(3) Avermectins (Ivermectin).

The anthelmintic efficiency of the benzimidazole and probenzimidazole groups is high, but the most effective are the less - soluble compounds (fenbendazole, oxfendazole and albendazole). Such compounds dissolve slowly and effective concentrations are maintained for an extended period both in the gut and in plasma. This important feature of this group makes it highly effective not only against adult and developing larval stages but also against arrested larvae.

As the probenzimidazoles must be converted to benzimidazoles in the host, to obtain a good efficiency an increase in dosage rate of such compounds is recommended.

All the benzimidazoles and probenzimidazoles are given orally or intraruminally.

The Imidazothiazoles (e.g. levamisole) are effective against developing larvae and adult worms but not effective against arrested larvae. This anthelmintic can be administered subcutaneously or orally and is also active by topical

application.

Pyrantel tartrate and morantel tartrate have a high efficiency against adult worms and developing larval stages, but again efficiency is low against arrested larvae. These drugs are administered orally and recently a sustained release rumen bolus (Paratect, Pfizer Ltd., Sandwich, Kent) has been developed for use in cattle (Jones, 1981).

Studies with the newly found avermectins, especially the chemically modified derivative ivermectin, have shown them to have a wide spectrum of activity against arrested and developing larvae and adult worms (Armour, Bairden and Preston, 1980). This anthelmintic is given subcutaneously to cattle.

With Type I ostertagiasis a good response after treatment with the standard dosage rates of the modern benzimidazoles, the probenzimidazoles, levamisole or Ivermectin.

For the efficient treatment of Type II ostertagiasis it is essential that the drug used is effective against arrested larvae as are the modern benzimidazoles and Ivermectin.

CONTROL

Control of bovine ostertagiasis is aimed mainly at young stock in the herd and is achieved by a variety of methods. In any control system one of the most important objects is to limit contact between the host and the infective stages of the parasite, which in ostertagiasis are the third stage infective larvae. Thus determination of the seasonal pattern of L_3 on herbage (Michel, 1969b; Armour, 1974) has provided the basis for the application of most of the control methods available.

Three main systems are currently advocated: strategic anthelmintic therapy, grazing management and a combination of both. An example of the latter is the so-called Weybridge "Dose and Move" system (Michel, 1969) which is widely used in the Northern Hemisphere. It is based on two factors; firstly that although L_3 are rarely acquired in sufficient numbers by young cattle grazed in spring to cause clinical disease, considerable numbers of eggs may be deposited on the pasture in three to four weeks after treatment.

Secondly since it takes until mid-July for these newly deposited eggs to mature to L_3, a move early in July to pasture which had remained ungrazed by cattle since the previous autumn, should result in a low level of infection.

It is essential that prior to moving the animals are treated with an effective anthelmintic to remove the primary infection and they should then remain relatively worm-free for the rest of the grazing season.

The availability of helminth infective stages to grazing animals can also be affected by certain management practices. Accordingly systems used to combat bovine ostertagiasis include rotational grazing of cattle, sheep and a feed crop, rotational grazing of different age groups of cattle and alternate grazing of cattle with a different host species.

Good control of bovine ostertagiasis has been achieved using methods which involve either grazing mixed host species together (Arundel and Hamilton, 1975) or alternate grazing of different host species (Barger and Southcott, 1975; Southcott and Barger, 1975; Rutter, 1975). The success of such systems depends on the

host specificity of Ostertagia species. Rotational grazing of adult and young stock has also been shown to have a production benefit. Improved liveweight gains were reported when susceptible dairy calves were rotationally grazed on permanent pastures and followed by replacement heifers in their second or third grazing season (Leaver, 1970; Nagle, Brophy, Caffrey and O'Nuallian, 1980). However, this system is only suitable for farms with an excess of grazing land and to be successful it is important that careful management of the paddocks grazed by the young calves is followed. These animals are only allowed to graze the upper leafy parts of the grass and in this way avoid the bulk of L_3 which are concentrated in the lower quartile of the herbage (Crofton, 1954). This system also depends on the immunity of the heifers acquired by their previous exposure to challenge as age 'per se' does not guarantee immunity to infection (Bailey and Herlich, 1953; Armour, 1970; Selman, Reid, Armour and Jennings, 1976). Also even if heifers have acquired good resistance to Ostertagia ostertagi it is never absolute and weakening of such immunity can occur particularly around the time of calving (Michel, Lancaster and Hong, 1979). Some studies however have shown that rotational grazing systems involving only cattle can be less effective than set-stocking of cattle on pasture (Levine and Clark, 1961; Michel, 1969b). In this instance it is thought that pasture regrowth during the period when the paddock is rested, provides a luxuriant grass cover which encourages larval survival. In comparison the sparse herbage in set-stocked areas causes larval populations to be

exposed to the effects of adverse environmental conditions and therefore reduces larval survival.

In some European countries a method known as "zero grazing" (Borgsteede, 1977) is employed to prevent ostertagiasis. Here the animals are permanently housed and grass is harvested and fed to the stabled calves. In Borgsteede's trial of a partial zero grazing method, housing was limited from April to June by which time pasture infestation had declined to extremely low levels owing to the mortality of the overwintered L_3; although some infection occurred in the calves grazed after June and some contamination of the pasture took place, the levels of L_3 later in the season were very much lower in comparison with the control paddocks.

Prophylactic anthelmintic medication is by far the most popular method of control and is generally concentrated in the early part of the grazing season (Pott, Jones and Cornwell, 1974; Armour, 1978; Herd and Heider, 1980) to limit pasture contamination with eggs during periods when environmental conditions are optimal for development of the free-living larval stages i.e. spring and summer in temperate climates or autumn and winter in the subtropics. Using oral anthelmintic medication, calves going to pasture in early May normally require two treatments, i.e. at three and six weeks after turnout, whereas calves turned out in April require three treatments at intervals of three weeks. Where parenteral Ivermectin (Ivomec, Merck, Sharp and Dohme Ltd.) is used the interval between first and second treatments may be extended to five weeks due to the residual activity of this compound (Armour, Bairden, Batty,

Davison and Ross, 1985).

Various routes of administration have been evolved e.g. the administration of anthelmintic in feed (Crowley, Foreyt, Bliss and Todd, 1977) or drinking water (Downey and O'Shea, 1985) are both convenient methods of treatment particularly for animals under grazing conditions. They are labour-saving systems there being no need to handle animals for treatment. As the principle aim is to keep pasture free of contamination for the whole season it also eliminates the necessity to move calves to "clean" pasture at certain times, thus facilitating flexible grassland management (Downey and O'Shea, 1981; 1985). However, although the management problems associated with regular treatment can be overcome with these systems it has been shown to be difficult to regulate individual daily consumption of the anthelmintic at fixed concentrations.

An equally convenient and more consistent method of administering anthelmintics continuously is by means of intraruminal sustained release devices such as that described by Jones (1981). This device was designed to provide a sustained release of the anthelmintic morantel tartrate over a 90 day period. Given to calves twenty four hours prior to turn-out, this treatment largely prevents the development to patency of infections acquired from overwintered larvae and so prevents egg deposition during the spring.

Using these boli liveweight gain benefits over untreated controls have been achieved over one grazing season (Jones, 1981; Armour, Bairden, Duncan, Jones and Bliss, 1981; Jacobs, Fox,

Walker, Jones and Bliss, 1981; Entrocasso, Parkins, Armour, Bairden and McWilliam, 1986). Also a study on the use of a morantel sustained release bolus (MSRB) administered to dairy cows in the spring has shown a significant improvement in milk production, milk fat and protein content (Bliss, Jones and Conder, 1982).

The intraruminal administration of oxfendazole also has been developed showing a comparable efficacy related to the oral device (Anderson and Laby, 1977; Bairden and Armour, 1983).

CHAPTER TWO

MATERIALS AND METHODS

MATERIALS AND METHODS

1. Animals

The calves used in the experiments 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. Weighing of all animals was carried out using a weigh crate suitable for small ruminants (Avery Scales Ltd., Glasgow).

In accordance with common practice supplementary feeding consisting of 1 to 3 kg of hay per day was given towards the end of the grazing season i.e. late September/early October.

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 calves were 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

The McMaster flotation technique devised by Gordon and Whitlock (1939) was used to detect the presence of trichostrongyle eggs in the faecal samples collected for

analysis. 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 2000 revolutions per minute (rpm) for two minutes. The supernatants from both tubes were then discarded and the remaining faecal mass broken up by rotary agitation (Whirlmixer, Scientific Industries Ltd.). One tube was then filled to its former level with saturated salt 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 gives 1 gram in 15 ml.

Volume under one square equals 0.15 ml.

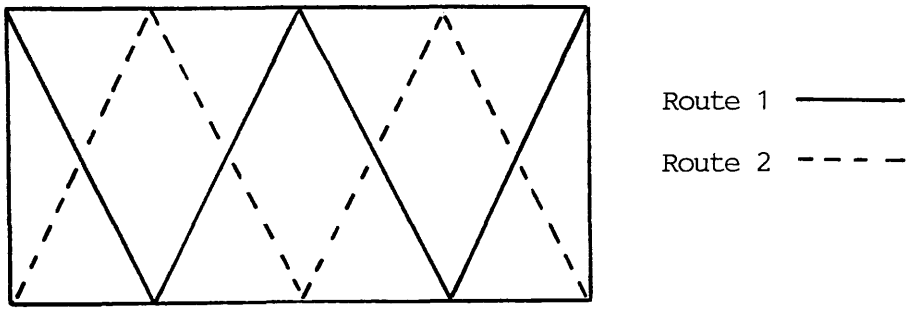
No. of eggs seen in one square x 100 = No. of eggs/gram and

No. of eggs seen in two squares x 50 = No. of eggs/gram.

The detection of D. viviparus larvae in faeces was achieved using the modified baerman method of Henriksen (1965) in which 10 grams of faeces was suspended in a gauze bag in warm water contained in a conical measure. After a minimum of 6 hours the supernatant was drawn off leaving a final volume of 10 ml and the

gauze plus faeces discarded. The numbers of larvae in 1 ml were determined and the results expressed as larvae per gram of faeces.

(b) Herbage samples were collected by crossing the experimental plot as shown in the following diagram:



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

Herbage was also collected from the immediate vicinity of faecal pats. From around twenty, reasonably fresh faecal deposits, ten plucks of grass were taken giving a total of 200 plucks per plot.

Initially both types of samples were treated as follows:-

The bag containing the grass was weighed then put into a small hand operated washing machine (Easy Pressure Washer, Classic Supplies Ltd., Leeds). Eight litres of lukewarm water were added, the bag tied and the machine turned through one

hundred revolutions. Before removing the bag and herbage from the machine a small incision was made in the bag and the washings allowed to pass through a coarse mesh sieve (aperture size 2 mm) into a bucket. The remaining herbage, still in the bag, was then removed and as much fluid recovered from it as possible by squeezing. The grass was then 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 transferred to a beaker. The washings from the randomly collected herbage were processed using a Baermann apparatus and those from the herbage taken near faeces were processed by the bile agar method of Jorgensen (1975). This latter method is used primarily for isolation of Dictyocaulus viviparus larvae from herbage and is based on the stimulating effect of bile on lungworm larvae and the ability of such larvae to migrate from agar gel.

The traditional Baermann technique, with a few modifications was employed as follows:-

The larval suspension was drawn through a coarse filter paper (Whatmans Grade 113, 18.5 cms) 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 the baermann filter funnel. After standing for at least six hours, 10 ml of fluid were withdrawn and the larvae in 1 ml differentiated and counted.

The near faeces sample was made up to a volume of 60 ml and preheated to 37°C in an incubator. To this was added 75 ml of the bile-agar mixture (60 ml 3% Difco-Bacto agar plus 15 ml ox bile) at a temperature of 48°C and after gentle mixing poured on to a wet 20 x 35 cm J-cloth (Johnson and Johnson, Slough) positioned on the base of a flat bottomed plastic tray. After setting for 15 - 30 minutes, the cloth was lifted, wound into a roll and immersed in warm water in a glass cylinder the roll being held in place by means of a plastic straw. The apparatus used is shown in Plate 1. Following incubation at 37°C for 12 hours, 10 ml of fluid was drawn off and the larvae in 1 ml differentiated and counted as before.

The criteria for larval identification were those detailed in Tehnical Bulletin No. 18 (Ministry of Agriculture Fisheries and Food) and used by Keith (1953) i.e. body length, prolongation of the second larval sheath beyond the tail of 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. They were then slaughtered 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. The large intestine and its contents were discarded as L_3 of Oesophagostomum radiatum were absent from all the pasture samples and Trichuris species eggs from all of the faecal samples

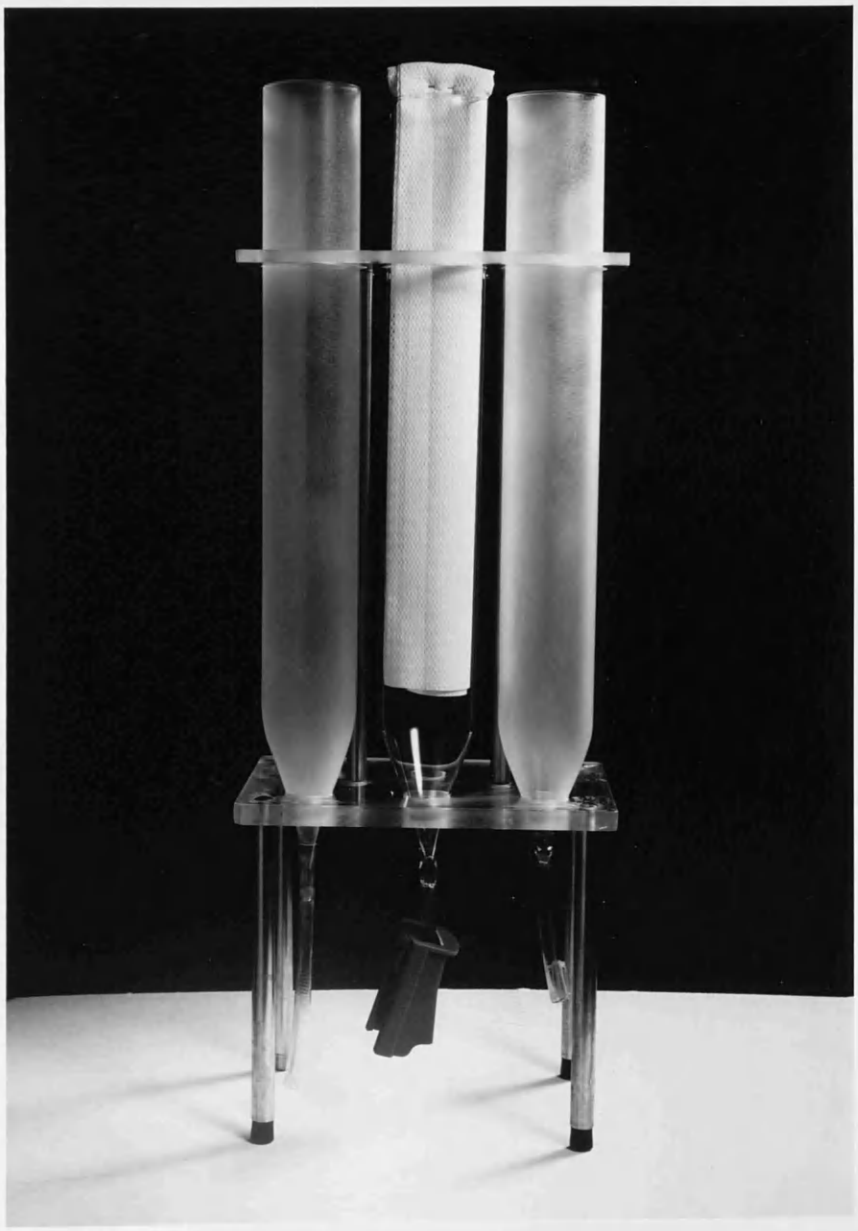


PLATE 1. Larval extraction apparatus used in the bile/agar technique

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) and duplicate samples of 200 ml withdrawn and formalised for subsequent examination. The abomasal mucosa was 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. After staining with iodine and decolourising using sodium thiosulphate 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_4) depending on bursal or vulvar development, the presence of a sheath projection and size respectively. In the case of O. ostertagi the EL_4 were considered as being arrested in development since this stage is reached in four days and the animals were retained free from further infection for a minimum of seven days. Appendix A gives details of the pepsin/HCl mixture and iodine/hypo solutions used.

A perfusion method similar to that of Inderbitzen (1976) was employed to recover lungworms. The procedure was as follows:-

The pericardial sac was opened to expose the pulmonary artery in which a 2 cm incision was made. A rubber tube was introduced pointing towards the tracheal bifurcation and fixed in situ by double ligatures. The remaining great vessels were tied off and water from a mains supply allowed to enter via the

pulmonary artery. The water entering the vascular system eventually ruptured the capillary network to return via the bronchial tract and out from the trachea. Twenty litres of washings were collected from each set of lungs and were filtered through a 37 micron aperture sieve. Parasites recovered in this way were left overnight at 4°C. The parasites were counted and differentiated on the basis of size and stage of development i.e. 4th larval stages : 1-2 cm and the absence of any definite internal structure: 5th larval stages: 3 - 5 cms and a noticeably increasing developmental pattern, particularly in the uterine and bursal region Adult; 5 - 10 cms presence of eggs in female and fully developed bursal region in male in mature parasites.

4. Blood Examination

(a) Plasma pepsinogen estimation

Increased plasma pepsinogen levels have been shown to be indicative of 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, Lancaster, Hong and Berret (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 3000 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-Ciocalteau 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. The detailed procedure used is given in Appendix B.

Statistical Methods

Where applicable worm burden results were analysed using unequal variance t-tests on the log transformed data.

CHAPTER THREE

THE EFFICACY OF A POUR-ON FORMULATION OF IVERMECTIN AGAINST NATURALLY ACQUIRED INFECTIONS OF BOVINE GASTROINTESTINAL AND RESPIRATORY TRACT NEMATODES

THE EFFICACY OF A POUR-ON FORMULATION OF IVERMECTIN AGAINST NATURALLY ACQUIRED INFECTIONS OF BOVINE GASTROINTESTINAL AND RESPIRATORY TRACT NEMATODES

A REVIEW OF THE RECENTLY DEVELOPED ANTHELMINTIC IVERMECTIN

In Europe normally for calves going to pasture in early May two treatments, three and six weeks after turnout are used whereas calves turned out in April require three treatments at intervals of three weeks. The advent of drugs such as the Avermectins has extended the intervals between treatments due to their residual activity.

Avermectins are produced by an actinomycete, Streptomyces avermitilis which was originally isolated from soil in Japan (Campbell, 1985).

Studies with one drug of this group, a chemically modified derivative B1 avermectin have shown it to be highly effective against early larval stages (arrested and developing larvae) as well as adults of the important cattle nematodes by either the subcutaneous or oral routes (Armour, Bairden and Preston, 1980).

A remarkable feature of ivermectin when given parenterally, is its persistent activity for at least two weeks after administration (Barth, 1983; Armour, Bairden, Batty, Davison and Ross, 1985). This persistent activity of Ivermectin should be of practical significance in many control programmes since it will relieve farmers of the need to move cattle to clean pasture immediately after treatment and also by reducing the frequency of treatment.

Ivermectin is absorbed systematically after oral or subcutaneous administration, but is absorbed to a greater degree when given subcutaneously the profile of the drug in plasma varying with the route of administration and the nature of the formulation. The effective dosage rate is extremely low (200 mcg/kg) and from studies with radio-labelled ivermectin in cattle it appears that the drug is mainly excreted in the faeces of treated animals with less than 2% passed in the urine (Campbell, Fisher, Stapley, Albers-Shonberg and Jacob, 1983). It has been proposed that this anthelmintic acts by potentiating the release and binding of gamma-aminobutyric acid (GABA) in certain nerve synapses. In nematodes, GABA acts as neurotransmitter sending signals between interneurones and motor neurones. The enhanced GABA binding results in an elimination of signal transmissions to the motor neurones resulting in paralysis of the nematodes (Campbell, 1985).

The activity of ivermectin is also high against a range of ectoparasites including mites, lice and warbles (Lee, Dooge and Preston, 1980; Lavigne and Smith, 1983; Steffan, Fiel, Ambrustolo and Biondoni, 1985) and would seem to be an ideal drug for the treatment of animals at housing in late autumn when ectoparasites and arrested larvae are often present concurrently (Marriner and Armour, 1986).

While the toxicity of ivermectin per se is low and it is safe for use in pregnant animals (Marriner and Armour, 1986) withdrawal times are long and it should not be used in lactating dairy cattle. Although it is difficult to detect the very low residues present after dosing these are sufficient to show

activity against lice at 30 days post-treatment (Armour and Bogan, 1982). Formulations of ivermectin for cattle are available as liquid for oral and subcutaneous administration and there is now a compound which can be applied to the skin although the latter is not yet commercially available.

Any method of treatment which makes the handling of animals easier is always of interest and a topically applied anthelmintic offers an attractive and practical route of administration. To date there is only one such anthelmintic compound, levamisole which is currently available as a topical preparation for pour-on application in cattle (ADAS, Anthelmintics for cattle, sheep, pigs and horses, 1983).

Some of the required characteristics of drugs used in pour-on formulations are outlined below and examples of advantages and disadvantages of their use are given.

There is little known specifically about the pharmacokinetics of topically applied anthelmintics but by analogy in general the following phases occur: Firstly, the tissue barrier must be passed by an appropriate diffusion mechanism after which distribution about the animal is via blood plasma. These drugs therefore require both a lipid solubility and a degree of aqueous solubility; the former to be able to pass through the epithelial layer etc., since cellular membranes are essentially comprised of phospholipid and the latter to be miscible in the plasma.

The greatest barrier to drug absorption is the outer stratum corneum layer of the skin. It is comprised of 10 - 20 layers of

dead, flattened, keratinised cells evolved from the underlying living epidermis. These cells also have a surrounding intracellular layer of lammellar lipids. Thus the choice of solvent/vehicle is critical when dealing with drugs to be applied topically.

Of several advantages perhaps the main value of pour-on application of drugs to animals is the convenience of the technique compared with other means of administration, in that animal handling is minimal and application simple. Another possible advantage could be an improvement in efficacy combined with reduced toxicity. For example, the efficacy of some anthelmintics is related not only to the concentration of the drug achieved in plasma or gut but also the duration of this concentration. This could be obtained by a slow absorption profile.

Disadvantages in the use of pour-on formulations include dermal absorption being influenced by factors such as thickness of the skin, the length of hair or coat (summer or winter), differences in breed or type of animal. Climatic conditions also pose potential problems and anthelmintic efficacy could vary considerably according to the climatic conditions at the time of application (Forsyth, Gibbon and Pryor, 1983).

The following study was set up to determine the efficacy of a new ivermectin pour-on formulation in a three, eight and thirteen week prophylactic dosing programme.

EXPERIMENTAL DESIGN

The experimental design is summarised in Table 1. Twenty-four castrated male Friesian calves aged 4 - 5 months, reared

under conditions which precluded infection with helminths, were used. The animals were ranked by weight into four groups each of six calves and were individually identified by means of colour coded and numbered ear tags. Because of the different grazing histories of the four paddocks, the principal calves were grazed "en masse" over the whole area for the first eighteen days of the study to ensure as uniform an initial contamination of pastures as possible. Thereafter the calves were set stocked in their respective paddocks for the remainder of the grazing season. At the start of set-stocking, parasite naive tracer calves were put into each paddock, allowed to graze for fourteen days then housed and necropsied two weeks later.

Groups 1 (Blue) and 3 (Red) were treated with ivermectin (Ivomec suspension L-640, 471-2232-E3812) at a dose rate of 200 mcg/kg bodyweight using a pour-on formulation while groups 2 (White) and 4 (Green) remained as untreated controls. The anthelmintic was applied from a measuring cylinder along the back of the calves which were treated at 21, 56, 91 days after turnout.

The animals were grazed from 7th May to 9th October, 1987 when they were housed. Three animals from each group were to be retained for further studies in 1988 and the remaining calves necropsied two weeks post-housing. At the end of the grazing season a further two parasite free tracer calves were introduced into each paddock, allowed to graze for two weeks, housed and necropsied two weeks later as before.

TABLE 1

Experimental Design

	Days 0 - 148*	Days 21, 56 and 91	Day 149
Group 1 (Treated)	Grazed on contaminated pasture	Ivermectin @ 1ml/10kg	Housing
Group 2 (Control)	"	Nil	"
Group 3 (Treated)	"	Ivermectin @ 1ml/10kg	"
Group 4 (Control)	"	Nil	"

* May - October 1987.

The calves were clinically examined each week, weighed and their respiratory rates noted. Faeces were collected weekly for examination for the presence of trichostrongyle eggs and D. viviparus larvae by the McMaster method (Gordon and Whitlock, 1939) and a modified Baermann (Henriksen, 1965). Blood samples, taken every fortnight, were processed for plasma pepsinogen levels by the method of Edwards, Jepson and Wood (1960). Each week pasture samples were taken near faecal pats and at fortnightly intervals random samples from each paddock were collected the former being processed by the bile/agar technique of Jorgensen (1975) and the latter by a method similar to that

described by Parfitt (1955).

At post mortem the worms present in the abomasum, small intestine and lungs were counted and identified by the methods outlined by Ritchie et al (1966) and Inderbitzen (1976).

RESULTS

Clinical

No adverse reactions to scheduled treatments were observed in the calves of groups 1 and 3. With the exception of animal No. R10 which required antibiotic therapy shortly after its first ivermectin treatment the general condition of these animals appeared normal throughout the experimental period. This compared with the untreated calves where by early June the majority of the animals of the control groups were diarrhoeic and as a result became slower and weaker. This was particularly noticeable during handling.

Several control calves had to be necropsied before the end of the study due mainly to severe nematodiriasis and parasitic gastroenteritis. These were; Calf No. G16 (4th June) replaced by No. G27; Calf Nos. G14 and W1 (19th and 22nd July) replaced by Nos. G29 and W32; Calf No. 28 (24th August) no replacement; Calf Nos. W32, W3, W5 and W4 (2nd, 4th, 19th and 23rd September) no replacements; Calf No. G27 (8th October) no replacement animal introduced.

In the treated Group 3 one calf (No. R10) was necropsied in extremis early in the trial (3rd June) with the main post mortem findings being nematodiriasis and a non-parasitic pneumonia. A replacement calf (R26), after being treated topically with ivermectin, was added to this group.

Following the death of a second calf (G16) due to extremely heavy Nematodirus battus infestations it was decided to treat all the animals in this group with Thiabendazole (MSD Ltd.) at 66 mg/kg bodyweight (18.75 ml/kg) when required. Where animals again developed diarrhoea and their condition deteriorated a further anthelmintic treatment with Thiabendazole at 66 mg or 110 mg/kg was given according to the severity of the clinical signs. In one instance (Animal No. G15) four times the recommended dose rate i.e. 220 mg/kg was used. TBZ was chosen because of its lack of effect on lungworm and moderate effect on Ostertagia and Cooperia thus causing minimal disturbance to the epidemiology of these infections.

In September, animal Nos. G15, G17 and W31 were given four times the recommended dose of thiabendazole i.e. 75 ml/50 kg. One animal (No. W5) was also treated with levamisole (ICI Ltd.) at 1 ml/10 kg.

Rehydration therapy was also given in the form of Ionalyte solution (Intervet) at a 1 : 16 dilution.

From August (week 12) several animals from the control group were seen to be coughing and on Week 16 lungworm larvae were detected on faecal examination.

Antibiotic therapy was given to several animals where necessary.

Respiratory Rates

Group mean respiratory rates are shown in Figure 2 and detailed in Appendix C.

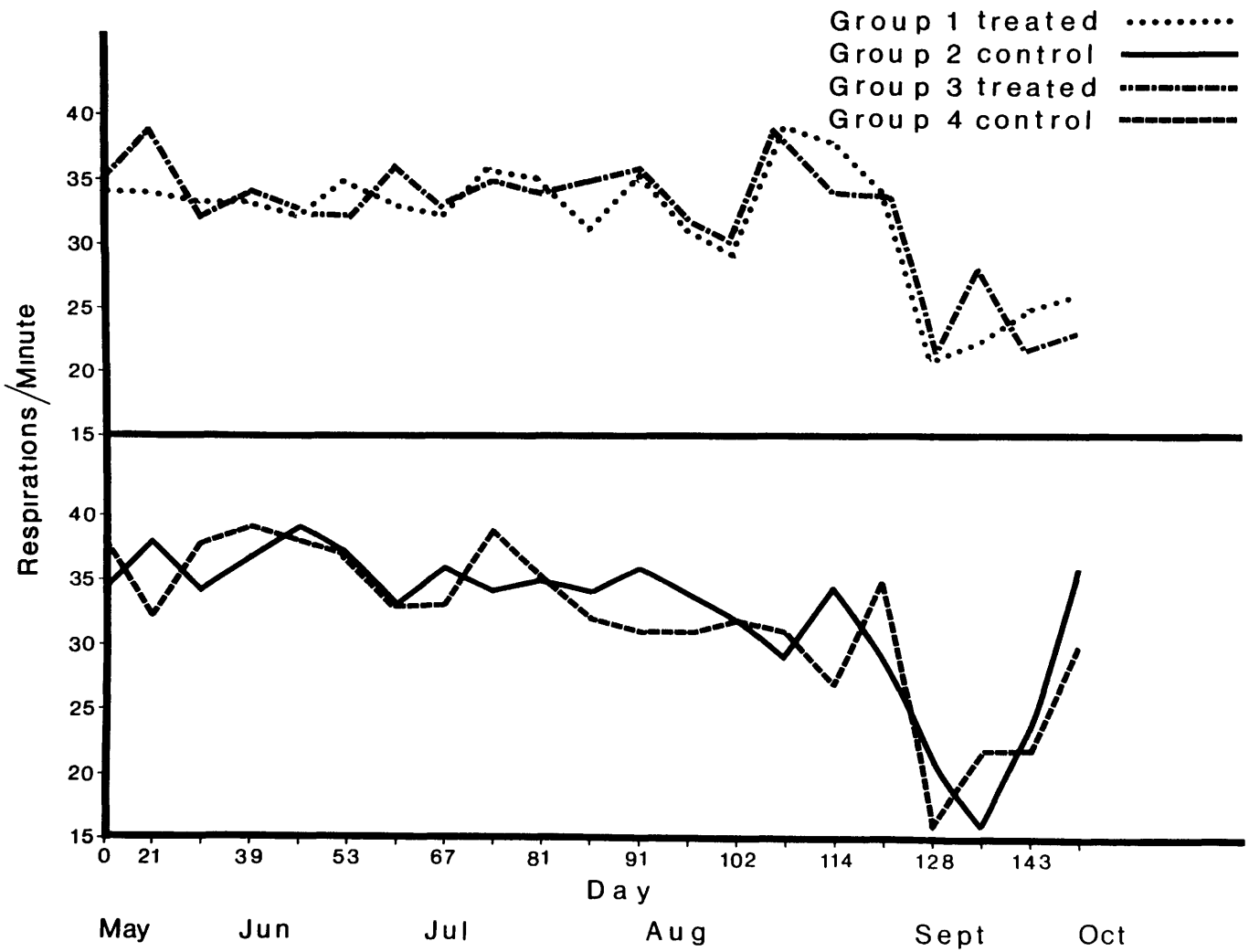


Figure 2. Group mean respiratory rates

Group mean respiratory rates at turnout were 34 rpm and 38 rpm for control Groups 2 and 4 respectively. Although control group mean increases in respiratory rate were small with a mean maximum of 39 rpm being recorded on Day 104 individual animals exhibited marked increases in respiration during the grazing period e.g. No. G18, 60 rpm on Day 104.

In the treated animals a similar pattern of respiration was observed with group mean increases to 42 rpm from initial levels of 34 and 35 rpm being recorded. Individual animal maximum values of 60 and 48 rpm were recorded from the animals of treatment Groups 1 and 3 respectively.

Liveweight

Group mean bodyweights are shown in Figure 3 and detailed in Appendix D. The mean liveweight for the control groups at the beginning of the trial were 125 kg (Group 2) and 124 kg (Group 4).

The animals of Group 2 gained weight until Week 6 after which very little weight gain occurred and on Week 15 a weight loss (to a Group mean of 149 kg) was recorded. This occurred coincidentally with the presence of diarrhoea and poor grazing conditions.

In the control replicate Group 4, mean bodyweight gains ranging from 12 to 56 kg were recorded until week 13 when a decrease to a final Group mean of 165 kg was recorded in Week 22.

The mean bodyweights of the two treatment groups at the beginning of the trial were 126 kg and 128 kg. Thereafter the calves of Groups 1 and 3 increased steadily in weight until Week 13. The mean bodyweights of both treated groups then

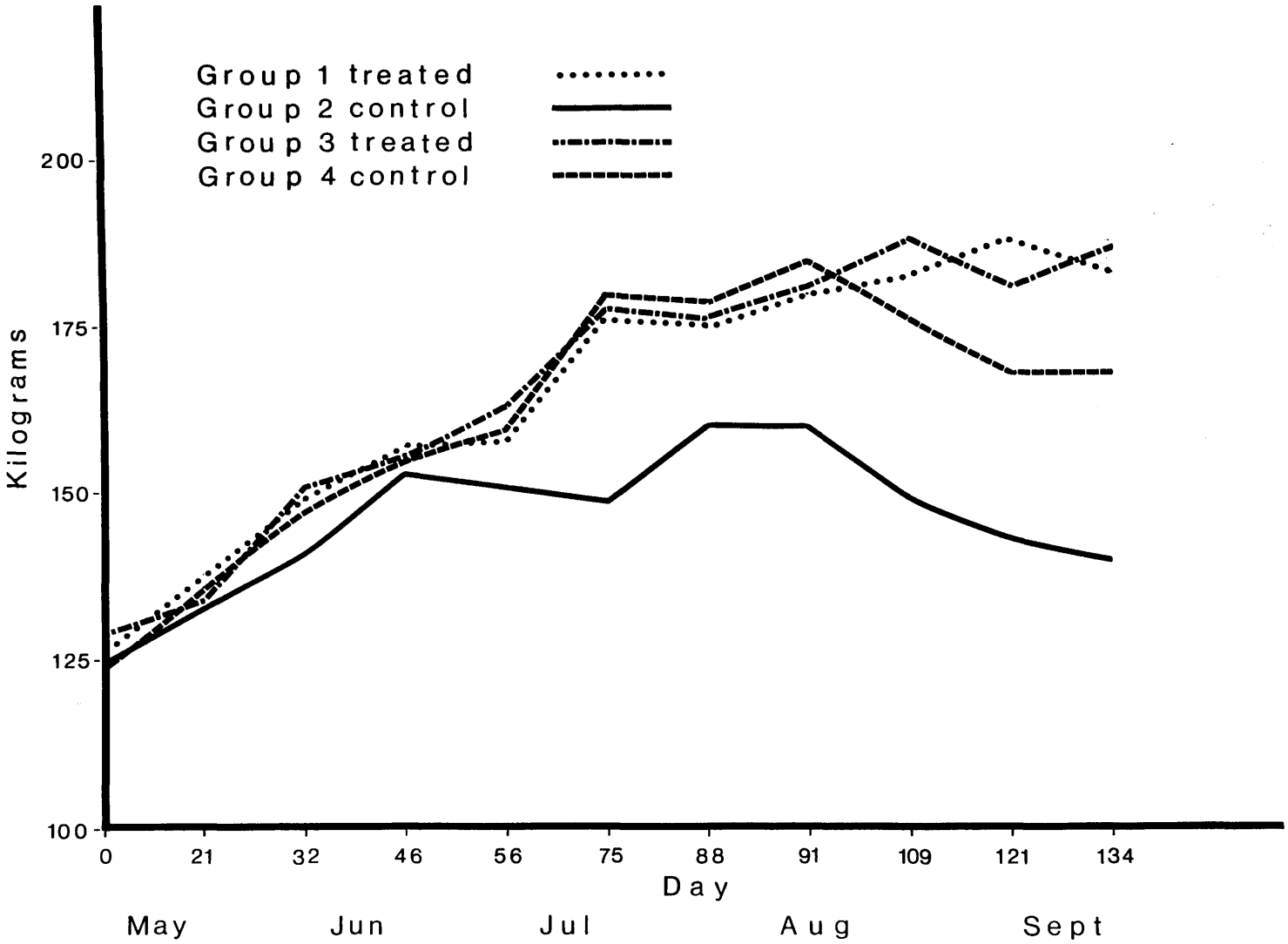


Figure 3. Group mean bodyweights

remained steady with a final mean of 188 kg being recorded on Week 22 for both groups.

Plasma Pepsinogen

Group mean plasma pepsinogen levels of all four groups are illustrated in Figure 4 and detailed in Appendix E.

Normal plasma pepsinogen levels were detected in all animals at the beginning of the trial with means of 557, 601, 575 and 571 mU Tyrosine being observed in Groups 1, 2, 3 and 4 respectively. In the control calves blood pepsinogen levels then increased steadily to reach maximum values of 7273 mU tyrosine on 24th August (Group 2, Week 15) and 5853 mU tyrosine on 21st September (Group 4, Week 19). Pepsinogen levels in individual calves ranged from 538 to 9386 mU tyrosine with the highest levels being seen in the animals of Group 2.

By contrast after an early increase to 1061 (Group 1, Week 3) and 1467 (Group 3, Week 6) mU tyrosine, the plasma pepsinogen levels of the treated calves stabilised at just over 1000 mU tyrosine for the remainder of the study.

Faecal Egg Counts

Replicate group mean trichostrongyle faecal egg counts, excluding N. battus, are shown in Figure 5 with N. battus data being illustrated in Figure 6. Appendices F and G detail individual results.

Positive trichostrongyle egg counts were obtained from the untreated control groups after 3 weeks of grazing. Egg output then increased steadily to a mean peak of 3350 epg in Group 2 calves in Week 18 (17th Sept.) and 800 epg in those of Group 4 on

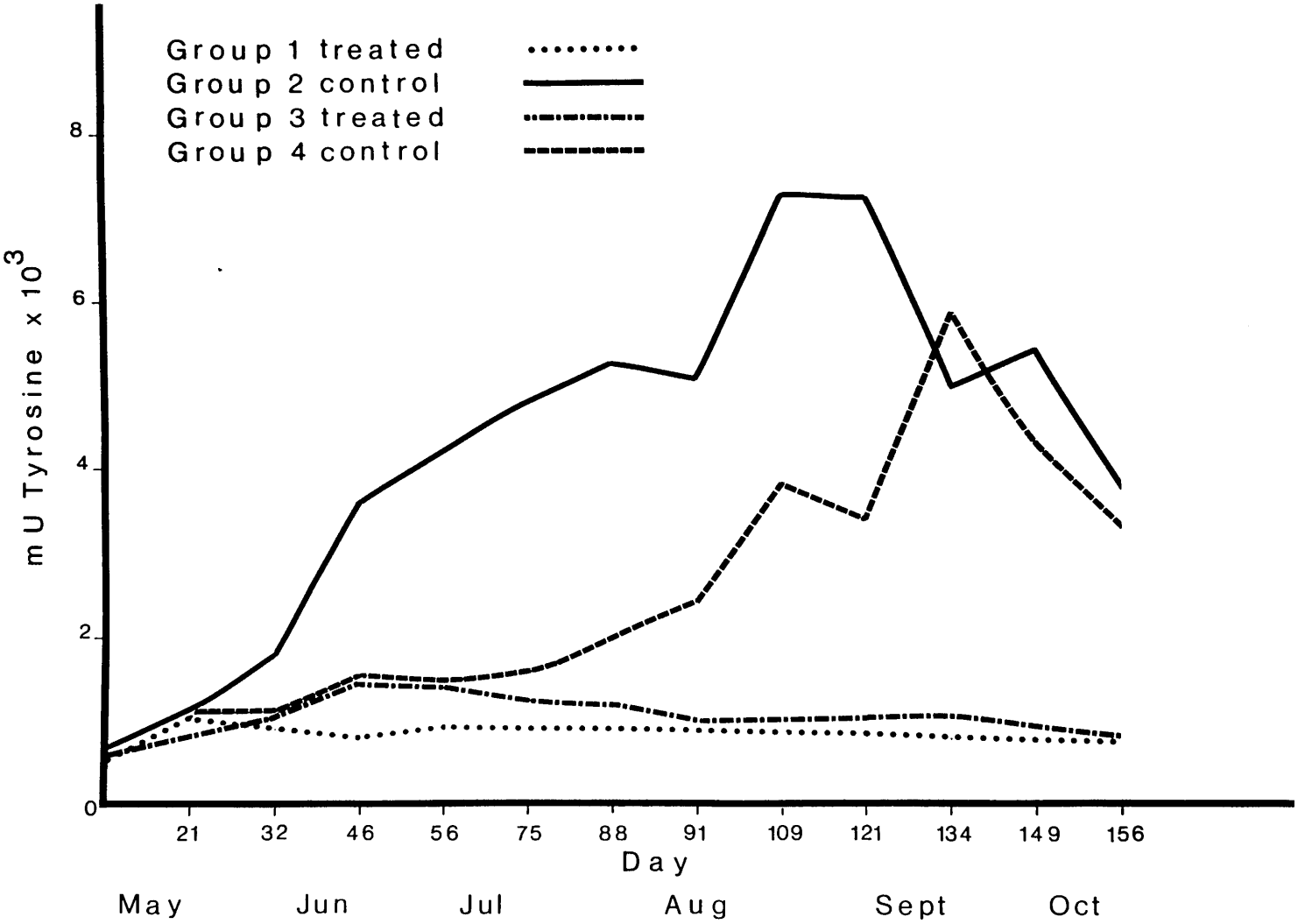


Figure 4. Group mean plasma pepsinogen levels

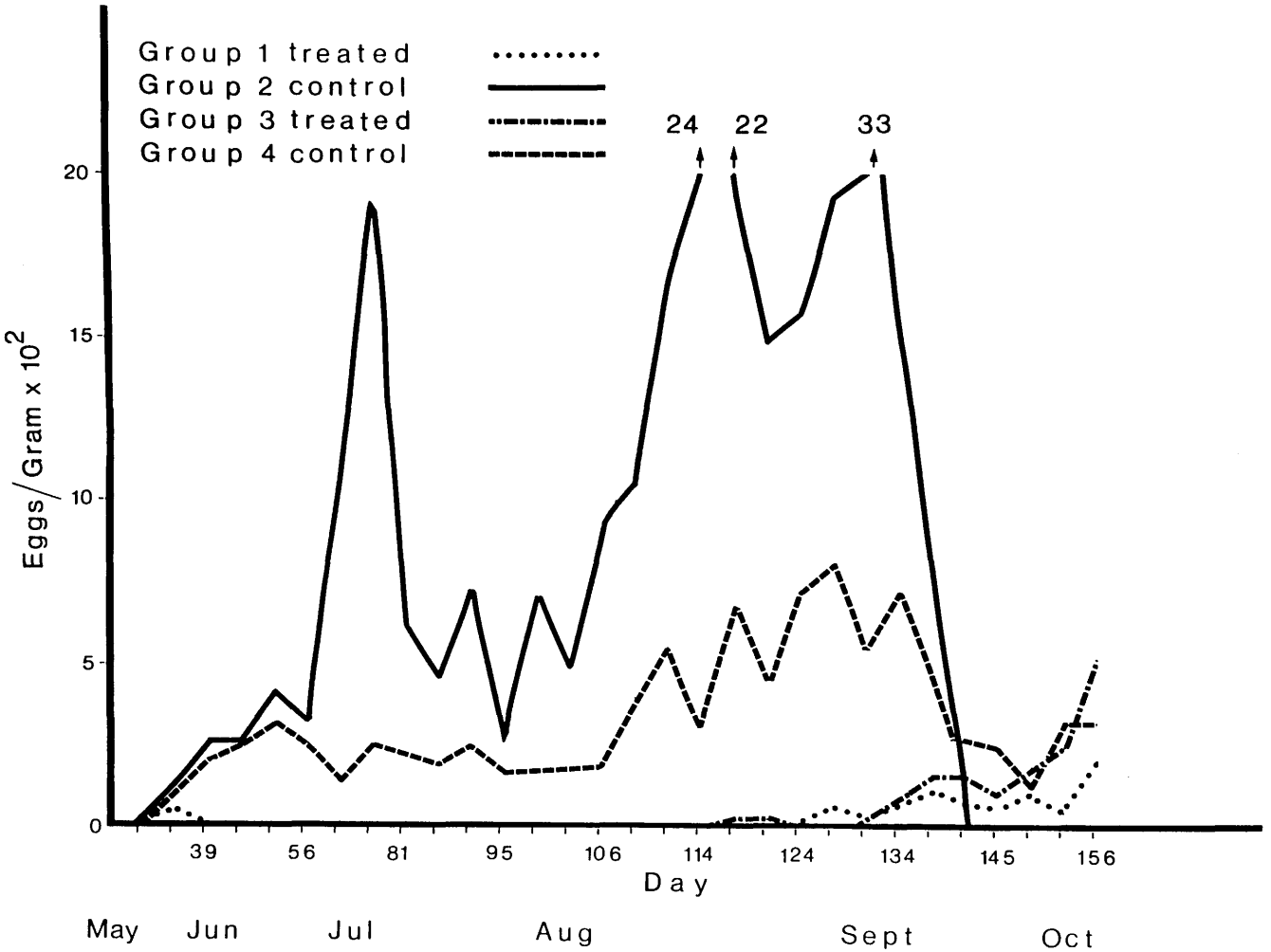


Figure 5. Group mean trichostrongyle egg counts (excluding N.battus)

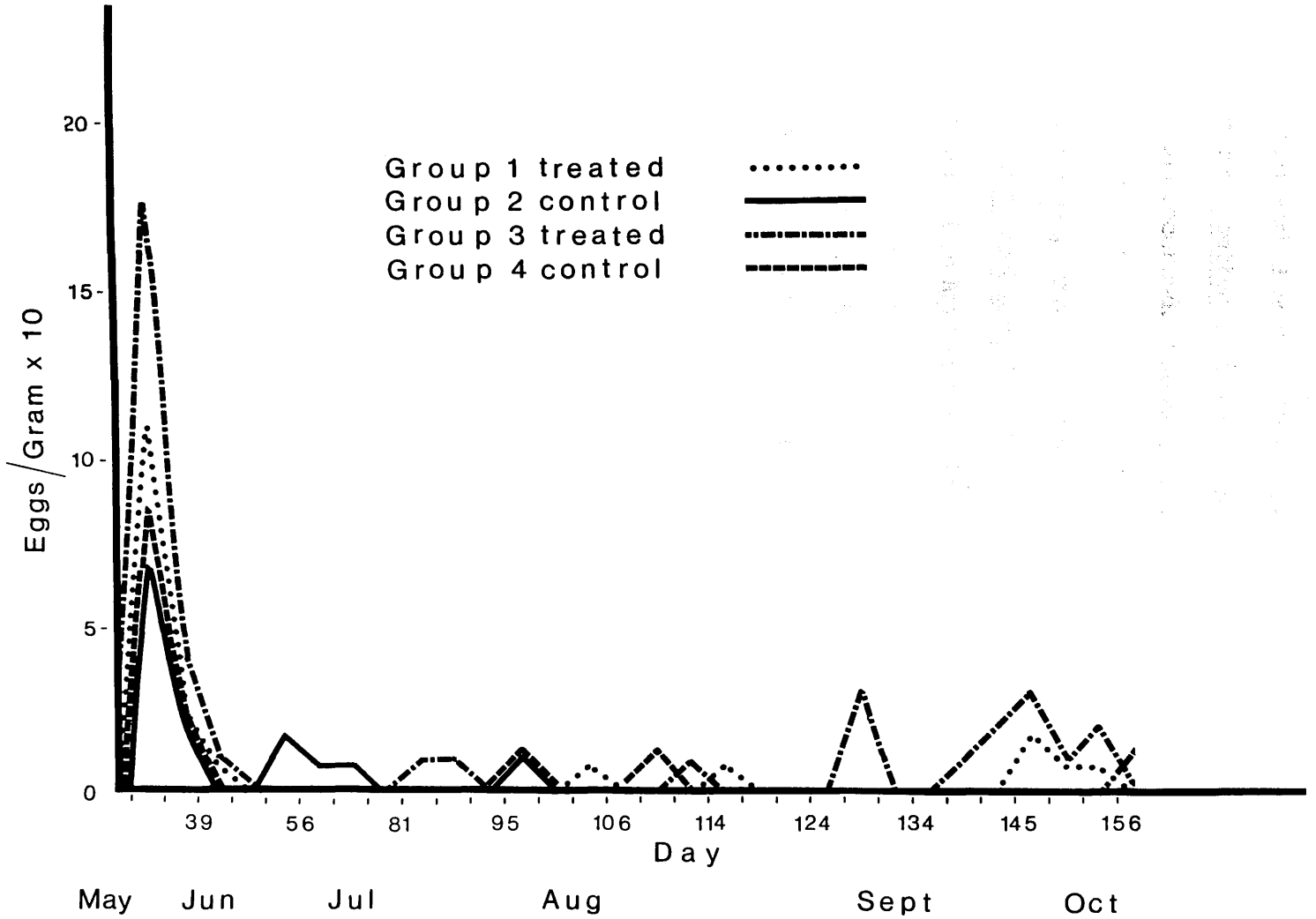


Figure 6. Group mean N. battus faecal egg counts

Week 18 (14th Sept.). Fluctuations in the faecal egg counts of both control groups occurred following salvage anthelmintic treatment with thiabendazole.

From Week 3 most of the animals in both control groups were excreting N. battus eggs with mean values of 67 and 83 N. battus epg being recorded. The majority of faecal samples thereafter were negative for N. battus although individual animals continued to excrete eggs throughout the season.

Mean D. viviparus faecal larval counts are shown in Figure 7 and detailed in Appendix H. Low numbers of D. viviparus first stage larvae were recovered from control animal faeces from the end of August and throughout September and October. Mean larval recoveries from Group 2 ranged from 2 to 228 and in Group 4 from 3 to 17 lpg.

In the treatment Groups 1 and 3 positive faecal egg counts from individual calves were recorded on Week 3 with group means of 42 and 17 epg being noted. Thereafter low egg counts were observed (mean 8 - 20 epg) until Week 16 (31st August). Egg output then increased and during the remainder of the experiment ranged from 40 to 433 epg with the maximum recovery being recorded on Week 22 (13th October).

By Week 3 faecal samples from most of the calves of both treated groups were positive for Nematodirus battus eggs with means of 108 and 175 epg being observed for Groups 1 and 3 respectively. Following scheduled treatment N. battus eggs were detected occasionally.

No D. viviparus L_1 were detected in the faeces of the ivermectin treated animals at any time throughout the grazing

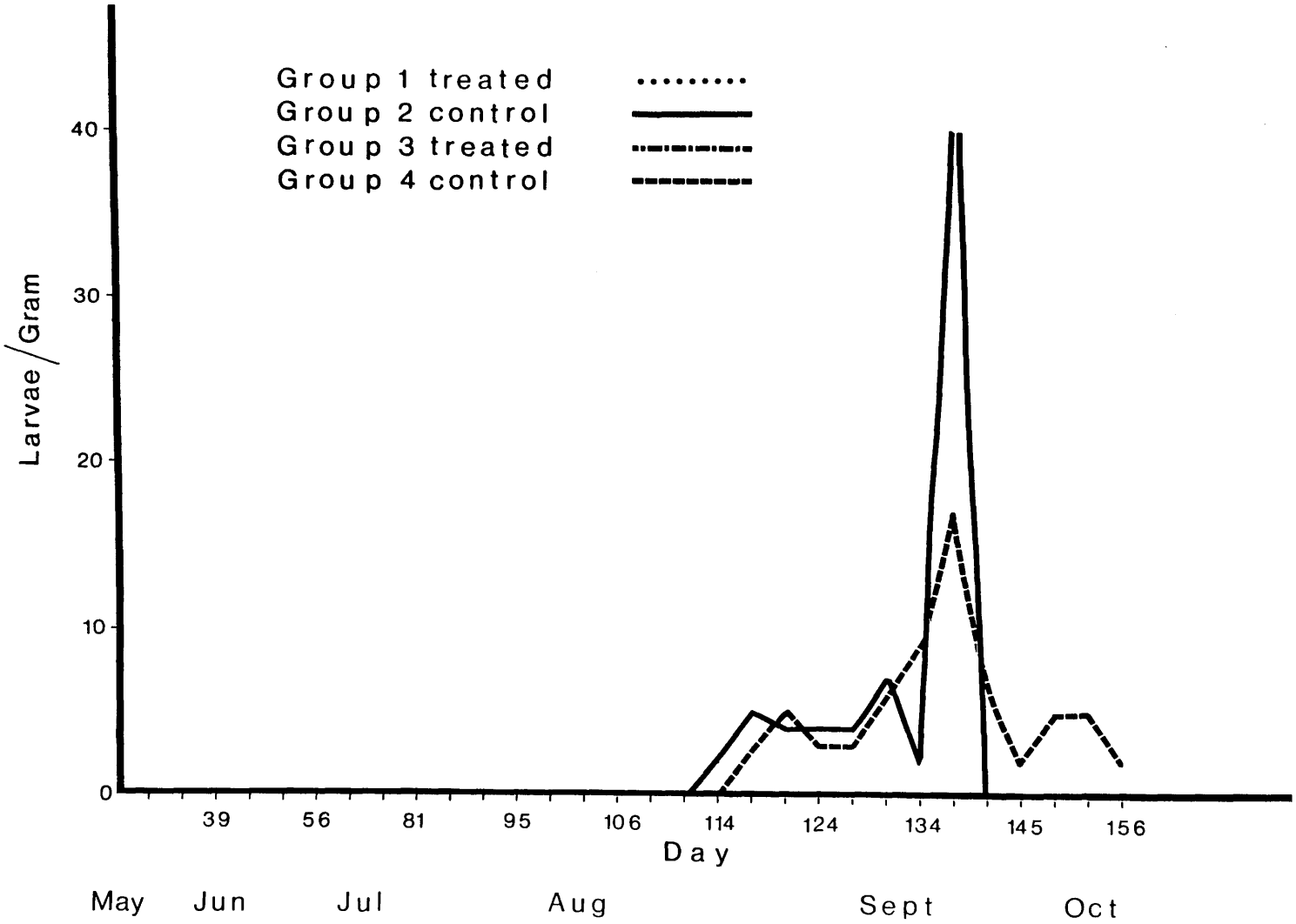


Figure 7. Group mean D.viviparus faecal larval counts

period.

Pasture Larval Counts

O. ostertagi, C. oncophora and N. battus larval recoveries expressed as L_3/kg dried herbage are shown in Figure 8 and tabulated in Appendices I1, 2 and 3.

O. ostertagi infective larvae were present on both control areas at turnout with recoveries of 2667 and 385 L_3 kdh being recorded from Paddocks 2 and 4 respectively. The highest recoveries were subsequently noted during late August/September with maximum numbers of 9941 and 9691 being recovered on Weeks 14 and 18 (Paddock 2 and 10,180 and 10,772 on Weeks 14 and 19 (Paddock 4).

Cooperia spp infective larvae were also detected from the day of turnout and as with O. ostertagi they were most prevalent during the latter part of the grazing season. Maximum recoveries of this genus were 6755 (Week 18 Paddock 2) and 5677 (Week 19 Paddock 4).

At turnout, infective larvae of N. battus were present with maximum counts of 8429 L_3/kdh being recorded on Week 3 (Paddock 2) and 3250 L_3/kdh on Week 17 (Paddock 4).

D. viviparus L_3/kdh recoveries from randomly collected herbage and herbage taken near faeces are shown in Figure 9 and this data is detailed in Appendix J. Larvae of D. viviparus were detected in samples from control Paddock 2 on the day of turnout (333 L_3/kdh) and from both control paddocks after 32 days (8th June). Subsequently D. viviparus larvae were intermittently present on both control areas throughout the second half of the

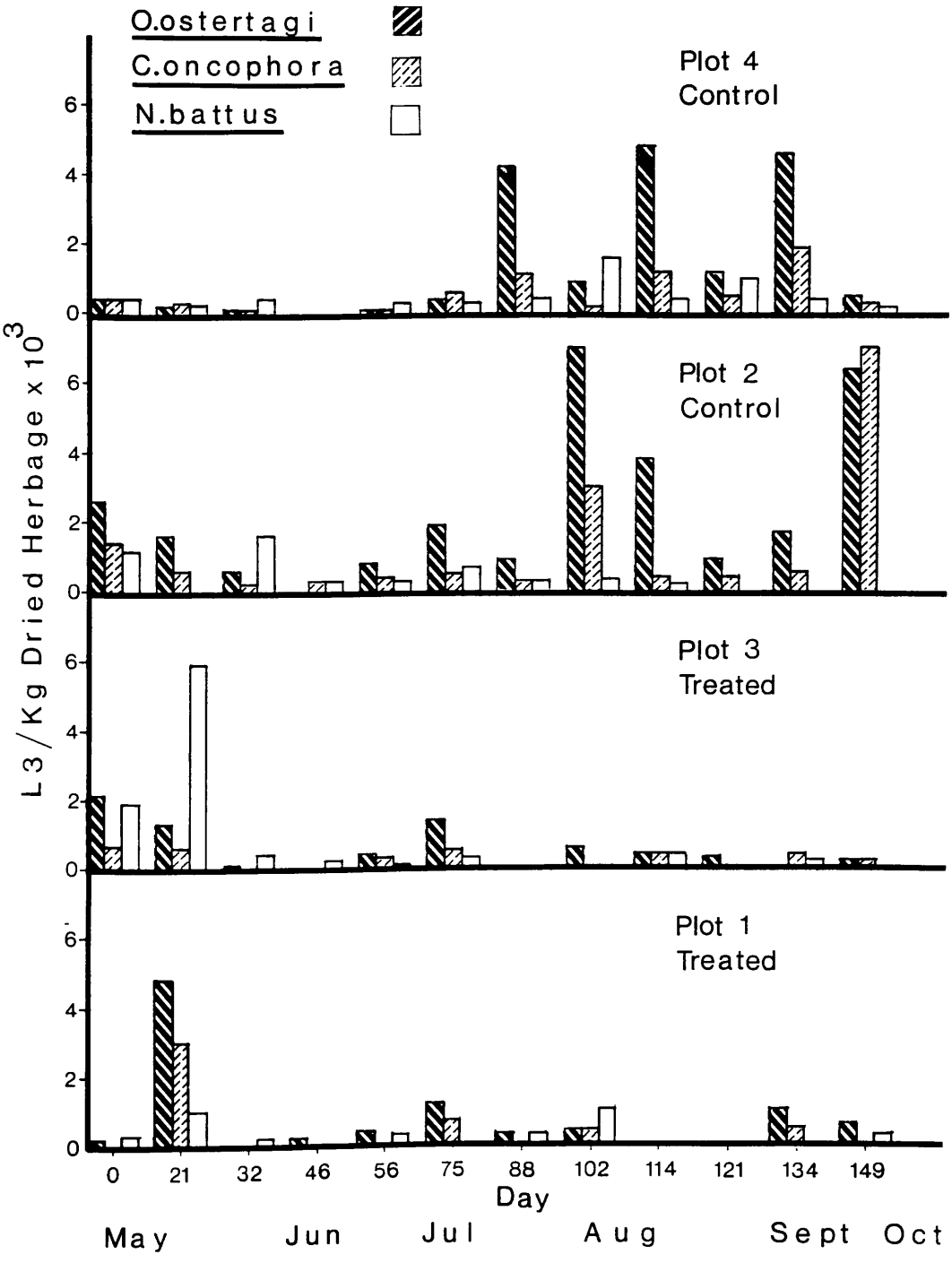


Figure 8. O.ostertagi, C.oncophora and N.battus larval recoveries from herbage

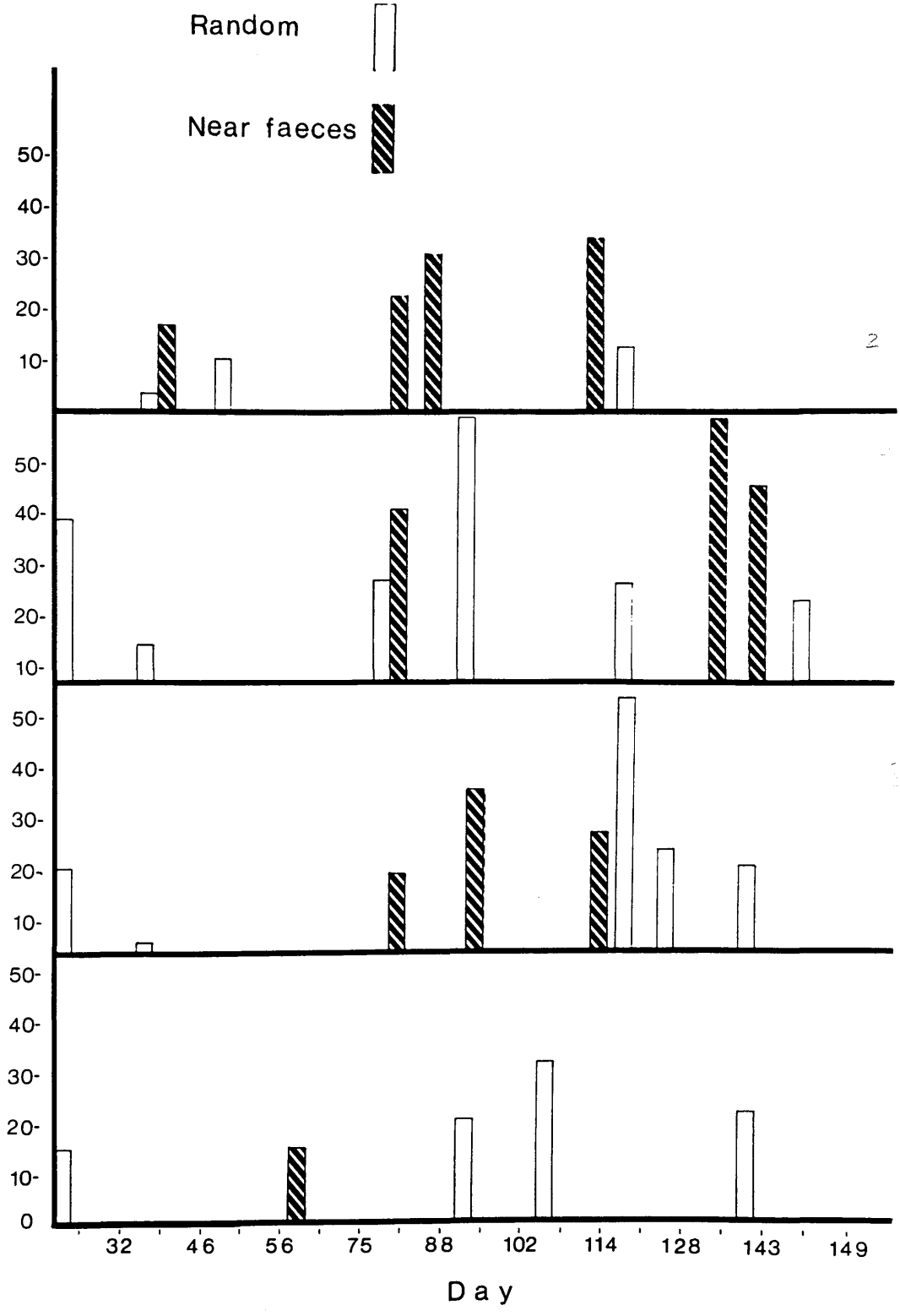


Figure 9. D.viviparus larval recoveries from random and near faecal pat herbage

grazing season with maximum recoveries of 587 on 14th September (Week 18 Plot 2) and 347 on 24th August (Week 15 Plot 4) being obtained.

Ostertagia spp. infective larvae were present on the day of turnout on both areas grazed by the treated groups. Recoveries of 4865 L_3/kdh on Paddock 1 (Week 3) and 2167 L_3/kdh on Paddock 3 (Week 1) were obtained. Subsequently larval recoveries from both areas decreased although on Weeks 10 (21st July) and 20 (28th September) higher levels of 2401 and 3502 L_3/kdh were observed on Paddock 1.

With the exception of a recovery of 2973 L_3/kdh on Week 3 (28th May) Cooperia spp larval numbers were subsequently low throughout the grazing season on both areas grazed by the treated calves.

Infective larvae of N. battus were present from the start of grazing on Paddocks 1 and 3 (322 and 1833 L_3/kdh respectively) but a higher recovery of this species was noted from Paddock 3 with a maximum recovery of 5851 L_3/kdh being seen at the end of May. Thereafter the numbers of this species gradually decreased although low numbers remained throughout the experiment.

Low numbers of Dictyocaulus viviparus infective larvae were present on both areas at turnout. Increased numbers of this species were present in August (Week 12) with recoveries of 204 and 322 L_3/kdh being obtained from Paddocks 1 and 3. Occasional positive samplings were observed thereafter with the maximum level of 310 L_3/kdh being seen on Plot 1 in Week 14 and 322 L_3 kdh on Plot 3 in Week 12. The maximum recovery of D. viviparus from Plot 3 was 510 L_3/kdh in Week 16 from the

randomly collected sample.

Post Mortem Data

Abomasal pH plus abomasal and lung lesion scores at post mortem of the spring and autumn tracer calves are given in Table 2 with worm burden data being detailed in Table 2a. Lesion scoring and pH data from the principal calves is shown in Table 3 with the corresponding worm burden results being given in Table 3a. Both scoring systems were based on a range of 1 to 5 with 1 - 2 representing light to moderate, 3 - 4 moderate to heavy and 5 severe infestation.

Tracer Worm Burdens

Ostertagia worm burdens of the spring tracer calves were low to moderate with totals of 1400, 10200, 2300 and 600 being found in the animals grazed on Paddocks 1, 2, 3 and 4 respectively.

Both Cooperia and N. battus were present in all but one spring tracer (no N. battus recovered from the Paddock 2 animal) with the former species ranging from 200 to 8000 and the latter from 1400 to 8600.

D. viviparus was recovered from one animal at post mortem i.e. No. Y73 which had grazed on control Paddock 2 and which harboured only one adult parasite.

In the autumn when two tracer animals were introduced into each paddock much higher recoveries of Ostertagia spp were found in the calves which had been put on to the plots which had been grazed by the control calves with mean recoveries of 64900 and 42400.

TABLE 2

Individual pH, abomasum and lung lesion scores of spring tracer calves at post mortem

	Animal No.	Date of Necropsy	Abomasal pH	Abomasal lesion score	Lung lesion score
Paddock 1	Y72	6/6/87	3.6	0	0
Paddock 3 (treated)	Y74	24/6/87	2.9	2	0
Paddock 2	Y73	24/6/87	2.9	3	0
Paddock 4 (controls)	Y75	24/6/87	2.9	1	0
Communal tracer	T2	10/6/87	3.7	2	0

Individual pH, abomasum and lung lesion scores of autumn tracer calves at post mortem

	Animal No.	Date of Necropsy	Abomasal pH	Abomasal lesion score	Lung lesion score
Paddock 1 (treated)	B34	19/11/87	2.4	2	1
	B35	19/11/87	2.6	1	0
Paddock 3 (treated)	R88	19/11/87	2.7	1	0
	R89	19/11/87	3.6	1	0
Paddock 2 (control)	W36	23/11/87	2.6	3	1
	W37	19/11/87	3.1	3	1
Paddock 4 (controls)	G40	19/11/87	2.5	3	2
	G41	23/11/87	2.8	3	1

TABLE 2A

Individual worm burdens of spring tracer calves

	Animal No.	Ostertagia ostertagi Adult	DL_4*	L_4*	Total	Cooperia oncophora Adult	DL_4*	L_4	Total
(Treated)									
Group 1	Y72	300	300	800	1,400	400	-	-	400
Group 3	Y74	2,300	-	-	2,300	200	-	-	200
(Controls)									
Group 2	Y73	10,000	-	200	10,200	5,400	2,600	-	8,000
Group 4	Y75	600	-	-	600	400	-	-	400
Communal tracer	T2	32,200	-	400	32,600	200	-	-	200

Individual worm burdens of autumn tracer calves

	Animal No.	Ostertagia ostertagi Adult	DL_4	L_4	Total	Cooperia oncophora Adult	DL_4	L_4	Total
(Treated)									
Group 1	B34	-	-	400	400	-	-	-	-
	B35	-	-	-	-	-	-	-	-
	Mean			200	200				0
Group 3	R88	-	-	200	200	-	-	1,000	1,000
	R89	-	-	-	-	-	-	-	-
	Mean			100	100			500	500
(Controls)									
Group 2	W36	8,400	3,000	27,200	38,600	1,000	-	20,400	21,40
	W37	5,200	6,200	79,800	91,200	600	200	67,000	67,80
	Mean	6,800	4,600	53,500	64,900	800	100	43,700	44,60
Group 4	G40	4,600	11,200	53,600	69,400	4,000	-	41,400	45,40
	G41	1,000	-	14,400	15,400	1,800	-	28,800	29,90
	Mean	2,800	5,600	34,000	42,400	2,900		35,100	37,10

* DL_4 = Developing fourth stage larve. L_4 = Early fourth stage larvae

TABLE 2A (Cont'd)

Individual worm burdens of spring tracer calves (Cont'd)

	Animal	Nematodirus battus				D.viviparus
	No.	Adult	DL_4*	L_4*	Total	Total
(Treated)						
Group 1	Y72	1,400	-	-	1,400	-
Group 3	Y74	8,600	-	-	8,600	-
(Controls)						
Group 2	Y73	-	-	-	-	1
Group 4	Y75	1,100	500	-	1,600	
Communal tracer	T2	11,500	-	-	11,500	4

Individual worm burdens of autumn tracer calves

	Animal	Nematodirus battus				D.viviparus
	No.	Adult	DL_4	L_4	Total	Total
(Treated)						
Group 1	B34	-	-	-	-	84
	B35	800	-	-	800	9
	Mean	400			400	47
Group 3	R88	200	-	-	200	5
	R89	-	-	-	-	97
	Mean	100			100	51
(Controls)						
Group 2	W36	2,200	-	-	2,200	38
	W37	1,800	-	-	1,800	378
	Mean	2,000			2,000	208
Group 4	G40	6,600	-	-	6,600	283
	G41	5,000	-	-	5,000	451
	Mean	5,800			5,800	367

* DL_4 = Developing fourth stage larve. L_4 = Early fourth stage larvae

TABLE 3

Individual pH, abomasum and lung lesion scores at post mortem

	Animal No.	Date of Necropsy	pH	Abomasal lesion score	Lung lesion score
Group 1 (Treated)	B20	29/10/87	3.2	1	1
	B21	29/10/87	3.2	1	1
	B24	29/10/87	2.8	2	1
Group 3 (Treated)	R10	3/6/87	N/D	1	1
	R7	29/10/87	3.8	0	1
	R12	29/10/87	3.9	1	0
	*R26	29/10/87	3.5	1	1
Group 2 (Control)	W1	22/7/87	5.1	3	N/D
	W2	24/8/87	7.2	3	N/D
	*W32	2/9/87	7.2	3	N/D
	W3	4/9/87	6.9	3	2
	W5	19/9/87	N/D	3	2
	W4	23/9/87	6.1	3	2
Group 4 (Control)	G16	4/6/87	5.3	2	N/D
	G14	19/7/87	3.6	1	N/D
	*G29	11/9/87	N/D	3	2
	*G27	8/10/87	6.4	2	2

* Replacement animals

TABLE 3A

Individual worm burdens of principal calves

	Animal No.	Date	Ostertagia ostertagi Adult	DL_4**	L_4**	Total
Group 1 (Treated)	B20	29/10/87	400	-	-	400
	B21	29/10/87	600	400	4,200	5,200
	B24	29/10/87	2,400	-	600	3,000
	Mean		1,133	133	1,600	2,867
Group 3 (Treated)	R10	3/6/87	1,400	-	-	1,400
	R12	29/10/87	1,000	-	-	1,000
	R7	29/10/87	-	-	-	-
	*R26	29/10/87	1,800	-	400	2,200
	Mean		1,050		133	1,150
Group 2 (Control)	W1	22/7/87	21,800	3,600	9,300	34,700
	W2	24/8/87	165,800	-	200	166,000
	*W32	2/9/87	8,400	600	-	9,000
	W3	4/9/87	329,200	14,800	13,000	357,000
	W5	19/9/87	160,680	5,200	25,200	191,080
	W4	23/9/87	120,800	21,400	48,200	190,400
	Mean		134,447	8,433	15,983	158,030
Group 4 (Control)	G16	4/6/87	19,300	200	-	19,500
	G14	19/7/87	6,400	200	400	7,000
	*G29	11/9/87	-	-	-	-
	*G27	8/10/87	85,600	24,000	53,200	162,800
	Mean		27,825	6,100	13,400	47,325

* Replacement animals. ** DL_4 = Developing fourth stage larvae.

L_4 = Early fourth stage larvae

Groups 1 - 3 T - 4.080174 DOF 4.199363 (sig $p<0.05$)
Groups 2 - 3 T - 6.82486 DOF 6.340334 (sig $p<0.05$)

TABLE 3A (Cont'd)

Individual worm burdens of principal calves

	Animal No.	Date	Cooperia oncophora Adult	DL_4**	L_4	Total
Group 1 (Treated)	B20	29/10/87	5,800	400	800	7,000
	B21	29/10/87	2,800	500	-	3,300
	B24	29/10/87	4,200	200	800	5,200
	Mean		4,267	367	533	5,167
Group 3 (Treated)	R10	3/6/87	-	-	-	-
	R12	29/10/87	15,000	-	3,400	18,400
	R7	29/10/87	10,000	-	7,800	17,800
	*R26	29/10/87	18,000	-	4,000	22,000
	Mean		10,750		3,800	14,550
Group 2 (Control)	W1	22/7/87	20,200	400	100	20,700
	W2	24/8/87	15,200	-	600	15,800
	*W32	2/9/87	1,100	-	-	1,100
	W3	4/9/87	2,400	-	5,200	7,600
	W5	19/9/87	1,500	1,500	20,200	23,200
	W4	23/9/87	18,100	1,300	16,500	35,900
	Mean		9,750	533	7,100	17,383
Group 4 (Control)	G16	4/6/87	2,600	-	-	2,600
	G14	19/7/87	800	-	200	1,000
	*G29	11/9/87	-	-	-	-
	*G27	8/10/87	300	-	11,400	11,700
	Mean		925	0	2,900	3,825

* Replacement animals. ** DL_4 = Developing fourth stage larvae.

L_4 = Early fourth stage larvae

Groups 1 - 2 T - 5.977073 DOF 2.358881 (sig $p<0.05$)

TABLE 3A (Cont'd)

Individual worm burdens of principal calves

	Animal No.	Date	Nematodirus battus				D.viviparus
			Adult	DL_4**	L_4**	Total	Total
Group 1 (Treated)	B20	29/10/87	-	-	-	-	11
	B21	29/10/87	200	-	-	200	17
	B24	29/10/87	-	-	-	-	5
	Mean		67			67	11
Group 3 (Treated)	R10	3/6/87	10,800	-	-	10,800	-
	R12	29/10/87	-	-	-	-	5
	R7	29/10/87	-	-	-	-	14
	*R26	29/10/87	-	-	-	-	8
	Mean		2,700			2,700	7
Group 2 (Control)	W1	22/7/87	400	-	-	400	5
	W2	24/8/87	-	-	-	-	-
	*W32	2/9/87	600	-	-	600	-
	W3	4/9/87	-	-	-	-	82
	W5	19/9/87	-	-	-	-	102
	W4	23/9/87	-	-	-	-	53
	Mean		167	0	0	167	40
Group 4 (Control)	G16	4/6/87	35,200	200	-	35,400	5
	G14	19/7/87	2,800	1,000	-	3,800	2
	*G29	11/9/87	-	-	-	-	52
	*G27	8/10/87	-	-	-	-	80
	Mean		9,500	300	0	9,800	35

* Replacement animals. ** DL_4 = Developing fourth stage larvae.

L_4 = Early fourth stage larvae.

Mean C. oncophora worm burdens of these animals were high (44600 Group 2 and 37100 Group 4) with moderate recoveries of N. battus being recorded (mean 2000 Group 2, 5800 Group 4).

All tracer animals had lungworm burdens at necropsy with mean recoveries of 208 and 367 from the animals grazed on control Paddocks 2 and 4 compared with means of 47 and 51 from those grazed on the treated areas i.e. Paddocks 1 and 3.

Principal Calf Worm Burdens

Ostertagia worm burdens in the control calves were substantial with means of 158030 and 47325 being found in Groups 2 and 4 respectively. Lower Cooperia burdens were found with the higher burdens being recovered from the animals of Group 2 which had a mean count of 17383; a mean of 3825 was recovered from Group 4 calves. N. battus populations were low to moderate with 167 and 9800 of this species being present in the animals of Groups 2 and 4 respectively.

With two exceptions, positive lungworm counts were recorded from all the control calves at post mortem with the maximum recovery of 102 being seen in the calves of Group 2.

Worm burdens of the treated calves were generally lower than the controls with Ostertagia means of 2867 and 1150 from Groups 1 and 3 which also harboured mean Cooperia burdens of 5167 and 14550. Two of the treated calves necropsied had N. battus burdens with total recoveries of 200 and 10800 of this species being present in animal numbers B21 and R10 respectively.

With the exception of calf No. R10 which was necropsied early in the season, all treated animals had lungworm burdens which ranged from 5 to 17.

DISCUSSION

The results of this trial, primarily designed to evaluate the control of Ostertagia, Cooperia and Dictyocaulus infections by topically applied ivermectin, were complicated by heavy Nematodirus battus infections acquired by the permanent calves of all groups within three weeks of turnout in the Spring. Since all the experimental paddocks had been grazed by calves in 1986, the N. battus infective larvae on the pastures in the Spring of 1987 (Figure 8) undoubtedly originated from eggs excreted by the 1986 calves. The infections were sufficiently heavy to cause clinical nematodiriasis in many of the controls (> 35,000 worms in G16) and at least one of the ivermectin treated calves (> 10,000 in R10); the latter indicating the marginal nature of ivermectin efficacy against the genus Nematodirus. It was also the first time that clinical N. battus infections have been recorded in calves. Before discussing the other parasites it is also worth mentioning that once again evidence of some N. battus larvae hatching within the same grazing season in which they were deposited was observed (Figure 8) and these resulted in moderate burdens in autumn tracer calves (Table 2A).

Despite the impact of nematodiriasis resulting in the death of several calves, including one treated calf, the need for thiabendazole therapy in all the controls and the introduction of four replacements during the trial, several positive benefits of topical ivermectin prophylactic treatments can be identified.

Firstly, and most significantly, excellent control of ostertagiasis was achieved. This is evident from the improved

liveweight gains from the two treated groups as shown in Figure 3 although the precise maximum benefit cannot be readily determined due to the death of seven original control calves by September. However, if one simply considers the total weight gain/hectare of the 11 surviving calves in the two treated groups which was 818 kg against that of the five surviving controls which was 181 kg the evidence of benefit is clear. The trichostrongyle faecal egg counts, which included Ostertagia eggs, were also markedly reduced (Figure 5) in the treated calves and this resulted in much lower levels of infective larvae of this genus on the pastures grazed by the treated calves in the second half of the grazing season. In contrast, high faecal egg counts were present in the control calves (despite salvage therapy with thiabendazole) and high numbers of Ostertagia infective larvae were present on the pastures in the second half of the grazing season. These results also correlated well with the plasma pepsinogen levels which were low in the treated calves and high in the controls and with the abomasal lesion scores and pH of the permanent calves which were also low in the treated calves and high in the controls. The low numbers of infective larvae of Ostertagia which developed on the plots grazed by the treated calves were reflected in relatively low numbers of Ostertagia spp in the permanent calves at post mortem in the autumn and extremely low numbers in the autumn tracer calves which were indeed lower than in the spring tracer calves which had grazed on the same areas.

Secondly, very good control of Cooperia spp, mainly C. oncophora was achieved. While most of the weight advantages

of treated over control animals could be ascribed to the prevention of the more pathogenic Ostertagia infections by the prophylactic ivermectin treatments, the good control of Cooperia must have also contributed to this effect. The trichostrongyle faecal egg counts of the treated calves, which included Cooperia spp, were negligible compared with those of the controls and resulted in much lower levels of Cooperia infective larvae on the pastures in the second half of the grazing season compared to the control plots; also, very low numbers of C. oncophora worms were present in the autumn tracer calves compared to those grazing on the control plots. As with Ostertagia spp the numbers of C. oncophora in the autumn tracers were actually lower than in the spring tracers. The one conflicting result in relation to Cooperia was the similarity of worm burdens in the permament calves of both control and treated groups suggesting that the control of Cooperia spp had not been effective. One interpretation of this data is that the acquisition of immunity to Cooperia, which develops much more rapidly than that against Ostertagia, had been partially delayed in the treated calves whereas in the controls an acquired immunity was already functional. This interpretation is supported by two results; first, the worm burdens in the autumn tracer calves in the control plots were actually greater than in the permanents (see Tables 2a and 3a) demonstrating the immunity which was acquired by the latter; second, within the treated calves the heaviest Cooperia burden was in one of the replacements which had been exposed to infection for a much shorter period than its cohorts

and therefore had acquired a lower immunity. A similar result was obtained in other grazing trials designed to examine the effects of a prophylactic anthelmintic regimen with a benzimidazole anthelmintic in the spring (Armour, Bairden, Oakley and Rowlands, 1988, in press) and there is no doubt that the tracer burdens and pasture counts at the end of the trial are the best parameters for assessing the effectiveness of the control programme.

Thirdly, on initial examination the control of lungworm infections appeared to have been highly successful with no lungworm larvae being detected in the faeces of the treated calves throughout the grazing season, whereas all the control calves became positive during August and September. Furthermore, coughing was widespread in the controls during the autumn although only one calf had a significantly increased respiratory rate and the lungworm challenge did not appear to attain proportions capable of causing severe parasitic bronchitis. However, the lungworm pasture larval counts and the lungworm burdens of the permanent and control calves suggest that prophylaxis was nothing like as absolute as the results of the faecal examinations suggest. Thus, from Figure 9 it can be seen that lungworm larvae were present both around recently excreted faeces and randomly throughout the pastures grazed by both control and treated calves. The technique used to examine around the faecal pats was the bile agar technique usually considered more sensitive than the Baerman technique, which was used for the randomly collected grass samples for logistic reasons, yet positive samples were recovered just as frequently by the latter

technique. Higher levels of larvae were certainly recovered from the control plots as can be seen from Figure 9 particularly from the plot grazed by control group 2 and this was reflected in the higher burdens found in the control autumn tracers, these being 5 times as numerous as those found in the autumn tracers from the paddocks grazed by the treated calves. The source of the lungworm larvae on the plots grazed by the treated calves is once again intriguing. There is a school of thought that these larvae originate by field to field tranmission and in this case the neighbouring plots were those grazed by the control calves. This hypothesis is difficult to accept as lungworm larvae were first detected on the same date around freshly passed faeces on both control plot 2 (Figure 9) and treated calf plot 3; furthermore, on the treated calf plot 1 and control plot 4, larvae were actually detected much earlier. Of course these data may simply reflect the inadequacy of the techniques used for pasture sampling and this technical inadequacy may also account for the apparently negative faecal larval counts of the treated calves throughout the year and indeed in the control calves prior to August. It is certainly interesting that using 10 gms of faeces in the Baerman apparatus no lungworm were recovered from the faeces of the permanent treated calves yet at post mortem they harboured between 5 and 17 lungworms.

Another possibility is that lungworm larvae recovered from the pastures originated from persistent larval populations in the root mat although it is difficult to explain why these should so frequently occur around faeces. Clearly, there is a great deal

of epidemiological study required in relation to bovine lungworm infections.

In summary, treatment of calves at 3, 8 and 13 weeks after turnout to graze in the spring of 1987 with topical ivermectin proved to be highly effective in controlling Ostertagia and Cooperia infection. Lower levels of larval populations accumulated in the pastures grazed by the treated calves and lower worm burdens were present at post mortem. The prevention of lungworm infection was not quite so successful and low burdens were acquired by the treated calves.

CHAPTER FOUR

THE PATHOPHYSIOLOGY OF A TRICKLE INFECTION OF *O.ostertagi* IN CALVES

THE PATHOPHYSIOLOGY OF A TRICKLE INFECTION OF O.ostertagi IN CALVES

INTRODUCTION

Gastrointestinal parasites can have a wide range of pathophysiological effects on the host. In ruminants studies on gastrointestinal nematode parasites have revealed reduction in voluntary feed intake, failure to gain weight or weight loss and usually diarrhoea. Furthermore there are a number of characteristic changes in blood constituents e.g. hypoalbuminaemia, a depression in the serum total protein concentration and increases in the concentration of pepsinogen in the blood. In bovine abomasal parasitism caused by O. ostertagi the main damage occurs as the early fifth stage larvae emerge from the gastric glands. As a result of the loss of functional parietal cells, acid secretion is impaired and pH values are elevated (Ritchie et al, 1966). The presence of adult O. ostertagi in the abomasum may also stimulate zymogen cells to increase pepsinogen production either directly or through excretory/secretory mediators (McKellar, Duncan, Armour, Lindsay and McWilliam, 1987). This would effectively increase the concentration gradient between the abomasum and blood thus promoting leakage.

A useful means of studying these pathophysiological effects of helminth parasitism is by the use of radioisotopic methods and the following study examines the parasitological and pathophysiological effects on young calves of a controlled trickle infection with O. ostertagi infective third stage larvae (L_3).

EXPERIMENTAL DESIGN

There were three principal groups:-

Group 1. The two calves of this group were designated as clean controls and were maintained free of parasites.

Group 2. Four calves were given a morantel sustained release bolus (MSRB, Paratect, Pfizer Ltd.) prior to the administration per os of 2,000 O. ostertagi L_3 daily for six weeks. Two were used for radioisotopic investigation and two necropsied, one on Day 21 and one on Day 42 after initial infection.

Group 3. Four calves were treated in the same manner as those in Group 2 but without prior MSRB administration. Again two calves were used for radioisotopic study and two for necropsy at three and six weeks post infection.

The calves were weighed weekly when blood and faecal samples were also collected for biochemical and parasitological analyses.

The six principal calves (i.e. excluding those necropsied at three and six weeks) were slaughtered in Week 12 of the experiment.

SPECIAL MATERIALS AND METHODS

Feed Allowance

The calves were individually given a complete ration twice a day (Superstar Cubes, Hamlyn Milling). The daily feed allowance of 3.0 kg/day was calculated to supply sufficient metabolisable energy (ME) to allow for a daily liveweight gain of about 0.35 kg (Ministry of Agriculture, Fisheries and Food, Book 433, 1984).

Larval Infections

The O. ostertagi larvae were harvested from faecal cultures no more than ten days prior to oral inoculation of the calves, and were maintained at room temperature (17°C) until required.

Radioisotopic Techniques

Radioisotopic studies using $^{51}CrCl_3$-albumin, ^{125}I-albumin and tritiated water (TOH) were conducted with two calves from each of the treatment groups (Clean, MSRB and infected) on Days 35-47 and 70-82 following the first infection.

Radioisotopes

Radioisotopes were obtained from Amersham International, England.

Tritiated water (^3H_2O) was diluted to the required concentration with isotonic saline prior to injection. ^{51}Chromium chloride solution was supplied in sterile isotonic saline ready for injection as required. ^{125}I sodium iodide was supplied carrier-free in NaOH solution and the bovine albumin was labelled by the method of McFarlane (1958), prior to injection of the ^{125}I-albumin.

Injection of radioisotopes

The injections of the radio-labelled substances preparations were given by intrajugular catheter. At the beginning of the study period each calf received 100 MBq ^3H_2O and four days later 74 MBq of $^{51}CrCl_3$ and 15 MBq of ^{125}I-bovine albumin. The calves were also dosed per os with 10 ml of 0.75% KI solution for three days prior to the ^{125}I-albumin injection and during the subsequent recording period in order to saturate the thyroid with iodine and thus ensure urinary excretion of ^{125}I from catabolised

^{125}I albumin.

Blood Sampling and Plasma Preparation

Following the injection of the tritiated water, heparinized blood samples were taken at 3, 4, 5, 6 and 7 hours after injection. One ml. duplicate plasma samples taken from these blood samples were treated with 10% trichloroacetic acid and centrifuged. 1.0 ml of the clear protein-free supernatant solutions were treated with 8 ml of liquid scintillation fluid (Unisolve 1, Koch-Light Ltd).

Blood samples were collected 15, 30, 60, and 360 minutes following the $^{51}CrCl_3$ and ^{125}I-albumin injection and then twice daily for five days thereafter. Daily samples were taken for the remainder of the experimental period. Each blood sample was centrifuged and duplicate 1 ml plasma samples were taken and diluted with 9 ml 0.02 N NaOH for posterior radioactivity measurements.

Collection and preparation of urine and faeces samples

The calves were restrained in metabolism crates during the investigations and fitted with faecal bags. The total daily faecal output of each calf was thoroughly mixed. From each daily mixed faecal samples eight weighed counting vials were filled with approximately 10 grams of faeces. The vials were then reweighed and the exact weight of faeces ascertained.

Total daily urine output was also collected and weighed. Duplicate 1 ml volumes of the urine were put onto weighed vials which were reweighed to ascertain the weight of urine in the samples and 9 ml of 0.02 N NaOH was added.

Radioactivity Assay

(a) ^{3}H Assay

Samples were assayed in a liquid scintillation spectrometer (United Technologis Packard).

(b) ^{51}Cr and ^{125}I Assay

Plasma, urine and faecal samples were assayed in a solid scintillation multi-channel spectrometer (United Technologis Packard).

Calculation and Expression of Results

Total body water

The ^{3}H radioactivity of the plasma samples (counts/min/ml) were plotted against time on semi-log paper. The biological half life (T 1/2) of the injected $^{3}H_{2}O$ was calculated from this graph. Total body water was determined by the dilution principle and the body water turnover rate in litres/day was calculated as follows.

$$\text{Water turnover (l/day)} = \frac{0.6923 \times \text{TBW (litres)}}{\text{T 1/2 (days)}}$$

Calculation of plasma volume and catabolic rate of albumin % per day

The ^{125}I radioactivity of duplicate 1 ml plasma samples collected at 15, 30 and 60 minutes after injection were plotted against time on semi-log paper. Extrapolation of this time to time = 0 gives the ^{125}I radioactivity of the plasma after mixing (at equilibrium). Plasma volume (Vp) was calculated as follows:-

$$\text{Vp (ml)} = \frac{\text{Total injected } ^{125}I \text{ radioactivity}}{^{125}I \text{ radioactivity counts/ml/plasma at equilibrium}}$$

The ^{125}I radioactivity of all plasma samples as a percentage of the ten minutes value were plotted against time on semi-log paper. From this graph the ^{125}I radioactivity of the total intravascular albumin pool at the beginning of each 24 hour period was ascertained. Daily catabolic rate of albumin was then calculated:-

$$\frac{\text{Total excreted radioactivity in urine and faeces/day}}{\text{Total radioactivity in the intravascular albumin pool at the start of the 24 hour period.}} \times 100$$

Gastrointestinal plasma protein loss

Plasma protein loss (ml/day) into the gastrointestinal tract was calculated each day as follows:-

$$\text{Pp ml/day} = \frac{\text{Total daily } ^{51}Cr \text{ radioactivity in faeces (c/min)}}{\text{Radioactivity of plasma at the start of the 24 hour period (counts/min/ml)}}$$

RESULTS

Plasma Pepsinogen Values

Plasma pepsinogen levels of the six principal calves are shown in Figure 10 and detailed in Appendix K.

From initial levels ranging from 479 to 669 mU Tyrosine the infected control values reached a significantly elevated mean of 2,811 mU on Day 71. This compared with 1,017 mU for the MSRB and 674 mU for the clean control animals. At necropsy the clean control and bolus calves had normal pepsinogen levels (542 Group 1 and 746 Group 2) while the infected control mean of 1,443 was still slightly elevated.

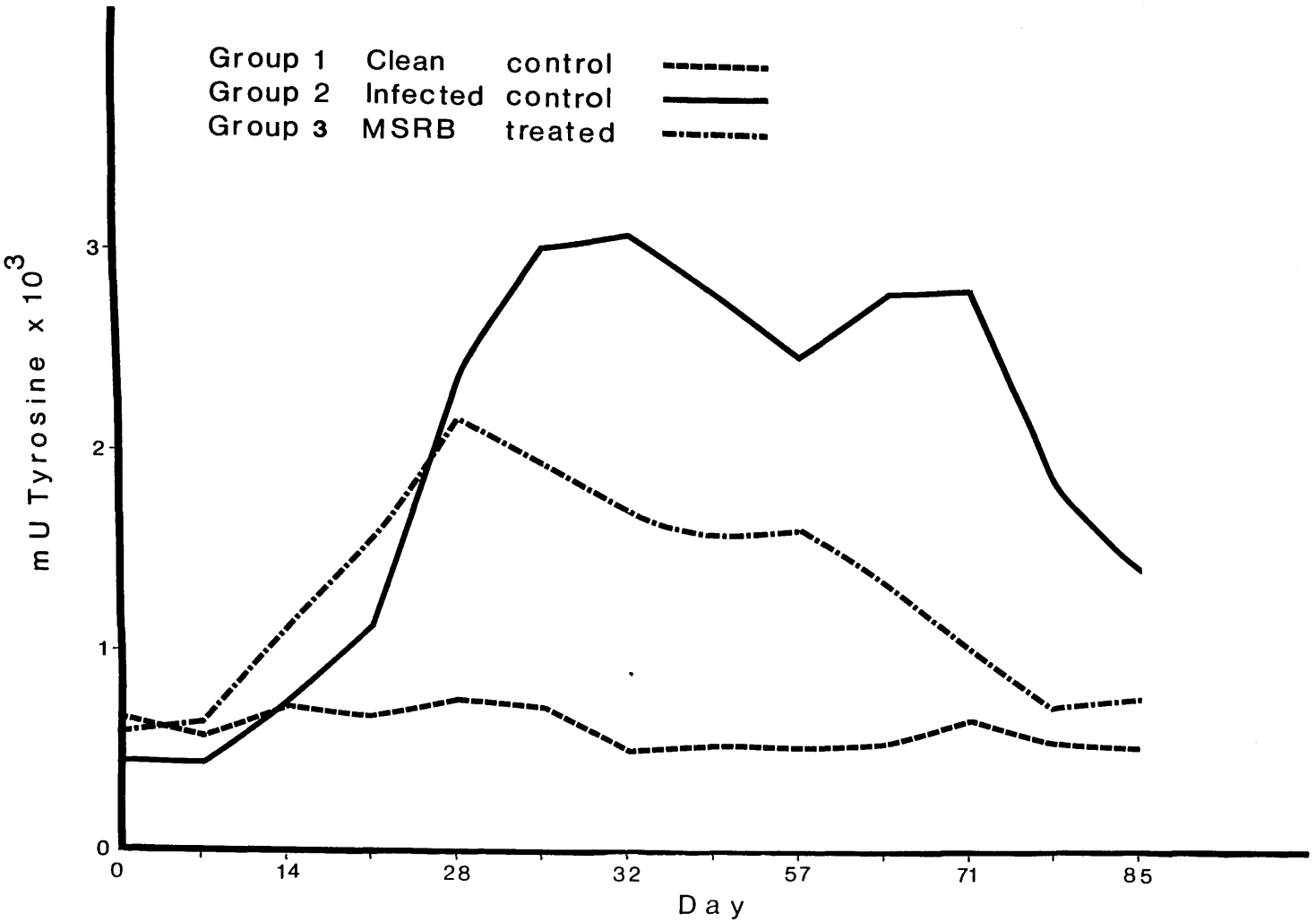


Figure 10. Group mean plasma pepsinogen levels of the radioisotope study calves

Radioisotopic Studies

Details of the pathophysiological changes are given in Appendix L.

Plasma volume measurements for both investigation periods, Days 35 - 47 and Days 70 - 82, revealed no significant differences between treatment groups.

During Days 35-47 total body water as a percentage of bodyweight was increased for one infected calf and tritiated water half life was increased for both infected calves implying water retention. Elevated faecal clearances of both ^{125}I and ^{51}Cr isotopes were shown by the infected control calves compared to the clean controls and this increased albumin loss to the gastrointestinal tract was reflected in an increase of approximately 30% in the albumin catabolic rate and a reduced ^{125}I-albumin biological half life. MSRB treated calves showed only slight increases in faecal clearance of ^{125}I and albumin catabolic rate compared to the clean controls.

During Days 70 - 82 body water measurements were normal for all six calves and no significant differences were recorded. In one infected control calf, No. 03, an elevated faecal clearance of ^{51}Cr persisted; this animal also showed an increased albumin catabolic rate compared to the clean controls. This indicated a continued loss of albumin into the gastrointestinal tract suggestive of a persisting infection and correlated well with a continued elevation of plasma pepsinogen levels and a moderate faecal egg output. The other infected calf, No. 04, was apparently recovering from infection at this time as is shown by

a reduced faecal clearance of ^{51}Cr indicating termination of the albumin leak to the gastrointestinal tract. Albumin catabolic rate was particularly low at this time and was correlated with a greatly extended ^{125}I-albumin half life. This is indicative of post infection hypocatabolism of albumin).

No adverse pathophysiological differences were observed when comparing the MSRB treated calves to the clean control animals during the second investigation period.

Parasitological Data

Weekly faecal egg counts (illustrated in Figure 11 and detailed in Appendix M) showed that the faeces of the clean controls were negative throughout the trial. In the MSRB group only occasional positive counts were recorded with a mean maximum of 25 epg (Day 35) compared with the infected control calves which had a mean count of 475 epg by day 28 rising to a mean maximum of 1,050 (Day 42). By the end of the study (Day 85) the mean faecal egg counts of the clean control, infected control and MSRB calves were 0, 275 and 0 respectively.

Worm burden data are given in Table 5.

The results confirm that the clean controls were free from infection. The establishment of O. ostertagi larvae in the infected control calves slaughtered 21 and 42 days after the initial infection was 85% and 40% with total burdens of 35,600 (4,400 L_4) and 33,750 (800 L_4) being recorded respectively, whereas in the MSRB group the corresponding values were 40% and 19% with total worm burdens of 17,000 (2,200 L_4) and 16,000 (200 L_4) being obtained. By Day 85 only a mean total of 4,650 worms were present in the infected control and 200 in the MSRB

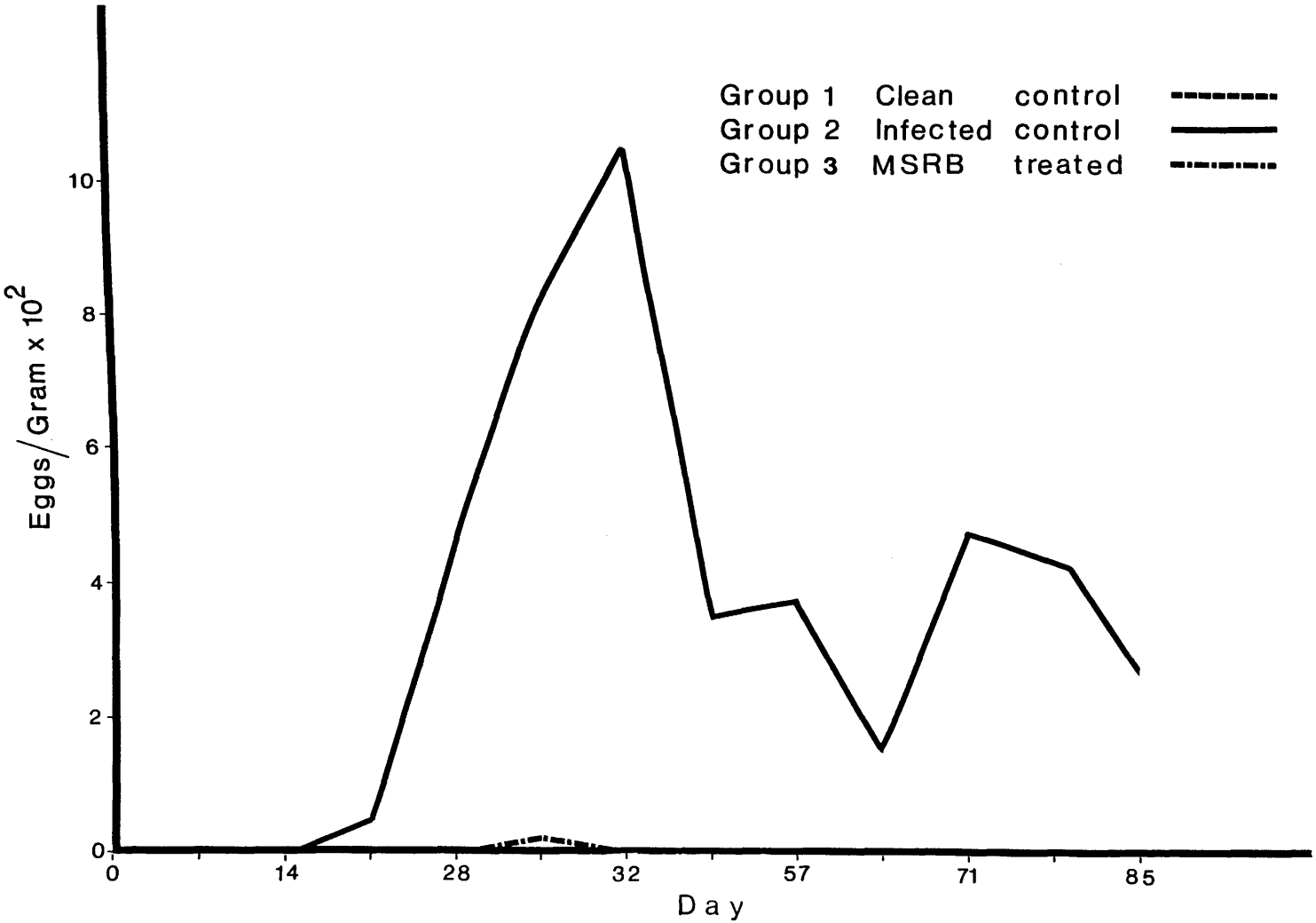


Figure 11. Group mean faecal egg counts of the radioisotope study calves

bolused group. Very few fourth stage larvae were present at this time and the abomasal pH was higher in the infected control calves.

DISCUSSION

The results indicated that the level of infection induced in this trial produced pathophysiological changes in the infected control calves. Prior administration of an MSRB markedly reduced these changes. There were no differences in appetite or mean liveweight gain between treatment groups but it is likely that the liveweight changes recorded for the infected calves may have included some retained water.

The daily liveweight gains for calves in the infected control, MSRB and clean control treatment groups were below those calculated for, and probably reflected both changes in gut fill and the stresses associated with housing in metabolism crates.

The pathophysiological measurements gave some indication of the adverse effects of O. ostertagi on calf metabolism. Alterations in pathophysiological values were greatest during the expected period of emergence of adult worms from the abomasal mucosa (Days 21 - 63). During this time plasma pepsinogen values of the infected control calves became elevated in relation to the values for the clean controls indicating leakage of pepsinogen from the abomasum. Although a similar pattern of pepsinogen values was noted in the MSRB calves it was of a lesser magnitude.

That leakage of plasma protein into the abomasa of infected control calves also occurred at this time was evidenced by increased faecal clearances of both ^{125}I and ^{51}Cr, increased

albumin catabolic rates and reduced half life of the radioactive albumin compared to the clean control calves. Loss of albumin in this manner leads to increased rates of synthesis but in severe infections, where this cannot match increased losses, hypoalbuminaemia results (Mulligan, Dalton and Anderson, 1963; Dargie, 1979). Pathophysiological measurements made after the completion of worm emergence (Days 70 - 82) revealed differing responses of the two infected control calves. One calf continued to show evidence of leakage of macromolecules between the blood and the abomasum while the other infected calf was apparently recovering from infection showing normal values for most of the parameters measured.

The particularly low value for albumin catabolic rate recorded for the former calf implies the occurrence of post infection hypocatabolism of albumin. Visual appraisal of the abomasa of the infected control calves revealed little damage due to O. ostertagi but the increased pH of the abomasal contents implied a continued impairment of digestive function. The increased half life of the tritiated water and the slight increase in total body water may indicate a degree of water retention by the infected controls during Days 35 - 47. This contrasts with the data obtained by Halliday, Dalton, Anderson and Mulligan (1965) where there was no evidence of increased water retention in calves suffering from Type I ostertagiasis. Holmes and Bremner (1971) also found that in sheep infected with O. circumcincta a reduction in body water turnover was principally associated with anorexia induced by parasitic infection but as the calves in this experiment did not become

inappetant the cause of reduced body water turnover is unknown.

The MSRB treated calves showed only slight alterations in pathophysiological parameters measured compared to the clean control animals and these were largely confined to the first investigation period (Days 35 - 47). The alterations to the protein metabolism of the infected controls and to a lesser extent the MSRB treated calves, as shown by the physiological study, occurred without alterations in apparent protein digestion. The higher abomasal pH of the infected calves implies that abomasal protein digestion was reduced, and it may be that a compensatory increase in digestive function occurred in the small intestine (Steel, 1978).

Several interesting parasitological results emerge from the data obtained during this study both from the infected control and bolus group calves. Firstly, at three weeks there was an 85% establishment of the total larval inoculum up to that time in the infected control animal and an almost 40% establishment in the bolused calf necropsied at this time. Such a high percentage establishment in the infected control calf is well in excess of that expected as a single inoculum of 50,000 third stage larvae would be expected to produce approximately 10,000 parasites i.e. a 20% establishment (Bairden, personal communication).

The worm recovery data from the 21 and 42 day necropsies is particularly interesting. Thus, after a total larval inoculum of 42,000 L_3 by Day 21, the infected control animal had a worm burden of 35,600 of which 4,400 were fourth stage larvae (L_4) while the corresponding MSRB calf had approximately 50% fewer

parasites i.e. a total burden of 17,000 of which the L_4 component was 2,200. This could suggest a larvacidal activity against the ingested stages, an efficacy against developing and adult parasites or more likely a combination of both. By Day 42, however, despite the cumulative daily infection level now totalling 84,000 L_3, both the control and MSRB animal worm populations appeared to have remained static with 33,700 and 16,000 worms being present at necropsy in the infected control and bolused animals respectively. There could be several explanations for this e.g. perhaps no larval establishment occurred after Day 21; a turnover of parasite population took place during Days 21 to 42; or, finally, that a combination of these factors was responsible.

The ratio of L_4 stages present in the infected control and MSRB animals at Day 42 compared to Day 21 had, however, altered with L_4 ratios of 2:1 and 4:1 being recorded for these animals respectively. This would point to an enhanced activity of the anthelmintic against ingested larvae and its continuing effect against the emerging and adult stages of the parasite.

Finally at Day 85 when the remaining animals were necropsied only 200 parasites remained in the MSRB calves while the control level was much higher at 4,000. The reduction in parasite population of the control calves could only have been due to worm expulsion by the host but the almost negative count of the MSRB calf indicates an extremely good effect against the developing and adult stages of the parasite. Whether this was due solely to specific activity against a particular stage of development or to an accumulation of morantel tartrate within the parasite during

development is not known.

It is also worth noting that despite a relatively high adult population during the first six weeks of infection, as evidenced by the early necropsy data, the maximum faecal egg count recorded from the bolused calves was 25 epg and that eggs were only detected on one occasion throughout the twelve week period in this group. This must have been due to either a suppression of egg laying or an almost absolute effect of the anthelmintic against emerging and not yet fully patent adult females. This would also account for the good epidemiological record of prophylaxis using the MSRB which has been shown to reduce pasture contamination during the grazing season.

The results of this study tend to support the view that the bolus acts against the adult stages of the parasite and not only ingested third stage larvae as is commonly thought to be the case (Pfizer, PBUK 16).

Although the small number of animals per group in this study must make any conclusion tentative it has been shown that the level of infection used in this trial produced pathophysiological changes in infected control calves without acute clinical disease occurring. Prior administration of an MSRB reduced these changes by lowering the establishment of infection and promoting the expulsion of adult worms. Further work is necessary, however, to determine exactly the stage or stages against which the bolus is most active.

TABLE 4

Experimental Design

	Animal no.	Radioisotopic studies	Necropsy
Group 1 (clean controls)	B1	+	Week 12
	B2	+	Week 12
Group 2 (MSRB treated)	Y66	-	Week 3
	Y65	-	Week 6
	Y63	+	Week 12
	Y64	+	Week 12
Group 3 (Infected controls)	O6	-	Week 3
	O5	-	Week 6
	O3	+	Week 12
	O4	+	Week 12

TABLE 5

O.ostertagi worm burdens plus abomasal pH and lesion scores at necropsy

	Animal no.	Necropsy	pH	Abomasal score	Adults	DL_4	L_4	Total
Infected control	06	Week 3	2.6	2	17,200	14,000	4,400	35,600
MSRB treated	Y66	"	2.5	2+	7,200	7,600	2,200	17,000
Infected control	05	Week 6	3.2	2+	30,550	2,400	800	33,750
MSRB treated	Y65	"	2.9	2	12,400	3,400	200	16,000
Isotope Calves								
Clean control	B1	Week 12	3.3	0	0	0	0	0
	B2	"	3.6	0	0	0	0	0
	Mean		-	-	0	0	0	0
MSRB treated	Y63	"	2.3	2	0	0	200	200
	Y64	"	2.6	2	0	0	200	200
	Mean		-	-	0	0	200	200
Infected control	03	"	3.7	2	1,500	0	1,000	2,500
	04	"	4.2	2	6,600	0	200	6,800
	Mean		-	-	4,050	0	600	4,650

CHAPTER FIVE

GENERAL DISCUSSION

GENERAL DISCUSSION

The results detailed in Chapter three of this thesis clearly show that a useful degree of control of bovine parasitic gastroenteritis is possible by the strategic use of a pour-on preparation of ivermectin. It is also obvious, however, from this data that parasite control was not absolute since patent infections of Nematodirus battus infection developed not only in the permanent calves of the control groups but also in at least one treated animal within three weeks of turnout in the spring. This demonstrates that, although this species is usually regarded as a parasite of lambs, young cattle not only play an important role in its epidemiology by pasture contamination but also may succumb to clinical nematodiriasis if faced with a sufficiently heavy challenge. In one of the treated calves the N. battus worm burden exceeded 10,000 indicating the occasional marginal nature of efficacy of the drug against this genus. It is of interest that it was reported previously that using a dose rate of 200 ug/kg ivermectin subcutaneously, that adult Nematodirus helvetianus appeared to be the least sensitive species tested (Campbell et al, 1983; Armour et al, 1980).

It is significant that hatching of N. battus larvae occurred within the same grazing season in which they were deposited and that the resulting infection was reflected in moderate infections of N. battus in the autumn tracer calves. The hatching in autumn of N. battus eggs has been described previously on experimentally contaminated plots (Gibson and Everett, 1981) and under field conditions (Rodgers, 1983; McKellar, Bairden, Duncan and Armour, 1983; Bairden and Armour, 1987).

Presumably the N. battus contamination on this grazing originated during a cattle/sheep integrated grazing study carried out from 1982-85. During this period cattle and sheep were alternated annually with each other and the levels of herbage contamination monitored. Apparently successful over the first two years the system seemed to break down in the third and fourth years when the faecal egg counts and worm burdens of both host species began to increase. It was also noted at this time that eggs of N. battus were being detected more regularly in the faeces of the calves. It is of interest, in reverse, to note that Jacobs (1987) reported that sheep may successfully passage N. helvetianus, one of the cattle species of Nematodirus.

Despite the complications with N. battus infections several positive benefits of topical ivermectin prophylactic treatments can be identified.

No adverse reaction to ivermectin treatments were observed and the treated animals appeared normal throughout the experimental period. The better liveweight gains of these animals also indicated non impairment of digestion. These results also show a good correlation with the plasma pepsinogen levels which were low in the treated calves and high in the controls. That faecal egg counts were also markedly reduced in the treated calves indicated lower worm burdens resulting in much lower levels of infective larvae being present on the pasture grazed by the treated calves in the second half of the grazing season. In contrast high faecal egg counts were observed in the control calves and correspondingly increased numbers of

Ostertagia and Cooperia infective larvae were recovered from the pastures grazed by the untreated calves in the second half of the grazing season.

The low numbers of Ostertagia infective larvae which were recovered from the plots grazed by the treated calves was reflected in relatively low numbers of Ostertagia species in the permanent calves at post mortem in the autumn. Such low burdens could, of course, also be the result of a "selfcure" phenomenon but the very low numbers of Ostertagia species present in the autumn tracer calves (which were in fact lower than those of the spring tracers) indicated that a lower pasture contamination rate was the primary reason.

The very much lower levels of Cooperia infective larvae present on the pastures in the second half of the grazing season compared to the control plots was reflected in the low numbers of C. oncophora worms found in the autumn tracer calves compared to those grazed on the control plots. As with Ostertagia species the numbers of C. oncophora in the autumn tracers calves were actually lower than in the spring tracers.

The one conflicting result in relation to Cooperia was the similarity of worm burdens in the permanent calves of both control and treated groups suggesting a poor efficacy of ivermectin against this species. However, another interpretation of this data could be that the acquisition of immunity to Cooperia which develops much more rapidly than against Ostertagia (Klosterman, Albers and Van den Brink, 1984), had been delayed in the treated calves whereas in the controls an acquired immunity was already functional. This hypothesis is supported by the fact

that the worm burdens in the autumn tracer calves in the control plots were greater than in the permanent calves demonstrating that an immunity had been acquired by the latter. Also, in the treated group, the heaviest Cooperia burden was found in a replacement animal which had been exposed to infection for a much shorter period than its cohorts and had therefore acquired a lower immunity.

The control of lungworm infection using the 3, 8 and 13 week dosing strategy was very successful with no lungworm larvae being detected in the faeces of the treated calves throughout the grazing season. This confirms the findings of Armour et al, 1987 and again demonstrates the susceptibility of D. viviparus to ivermectin. In contrast, evidence of patent lungworm infection was detected by faecal examination in all the control calves during August and September. Although coughing was widespread in the controls during autumn only one calf had a significantly increased respiratory rate and the lungworm challenge did not appear to attain proportions capable of causing severe parasitic bronchitis.

However, the lungworm pasture larval counts and the lungworm burdens of both the treated calves and their respective autumn tracers suggest that prophylaxis was not as absolute as the results of the faecal examination indicated and this contrasts with the results obtained by Armour et al (1987) when few or no D. viviparus were present in the treated groups at necropsy.

The patterns of D. viviparus larval recovery from herbage are difficult to explain but illustrate one of the main puzzles

associated with the epidemiology of bovine husk. Lungworm larvae were present both around recently excreted faeces and randomly throughout the pastures grazed by both control and treated calves throughout the grazing season. Certainly, higher levels of larvae were recovered from the control plots and the pasture infectivity was reflected in the higher burdens in the autumn tracers grazed on these plots. These were five times greater than the autumn tracer worm burdens from the paddocks formerly grazed by the treated calves.

The apparently good control of lungworm in the treated animals raises the question of the source of infection on the pasture. Overwintered larvae have been considered as a source of renewed herbage infestation with D. viviparus (Baxter and Allen, 1977) and also the possibility that a reservoir of such infective larvae could persist in soil from the previous grazing season (Nelson, 1977; Duncan, Armour, Bairden, Urquhart and Jorgensen, 1979). It has also been suggested that earthworms might carry larvae from the soil to the surface where they may be ingested by susceptible cattle (Oakley, 1981). Finally Robinson (1962) and Jorgensen (1982) consider that the transfer of lungworm larvae may be due to Pilobolus fungi. Lungworm larvae have been found on the sporangia of these fungi and can be transported considerable distances when the latter discharge their fungal spores.

It is clear from a comparison of the data presented in Figures 8 and 9 that D. viviparus larvae were recovered on all plots from herbage samples taken both randomly and near faecal pats on many occasions prior to larvae being detected in the

faeces. This occurred despite the fact that no D. viviparus larvae were seen in the faeces of any treated animal during the grazing season. As has been mentioned these lungworm larvae may have originated from persistent larval populations or by field to field transmission but, if this was the case, it is difficult to explain why they should so frequently be found around faeces.

It is an intriguing suggestion that earthworms, regularly found "dining" under faecal pats, deposit larvae ingested in other areas causing a "pat to pat" transmission phenomenon. Whatever the mode of contamination, however, it is clear that there is still a great deal of epidemiological study required in relation to bovine lungworm infections.

From the data presented in Chapter four several interesting areas for discussion emerge and although the numbers of animals per group were low some tentative conclusions can be drawn.

Low level trickle infections of calves to simulate a natural uptake of gastrointestinal nematode larvae has been shown to be successful in the study of the pathogenicity of Cooperia and other helminth parasites (Armour, Bairden, Holmes, Parkins, Ploeger, Salman and McWilliam, 1987; Jeffcoate, Holmes, Fishwick, Boyd, Bairden and Armour, 1987). When such studies also involve total daily faecal and urine collection for metabolic and radioisotope studies, careful consideration must be given to the number of larvae administered as a balance between noticeable pathological effect and clinical disease with concurrent weakness and diarrhoea must be struck. If the latter supervenes then practical aspects such as faecal collection can

prove to be very difficult.

Although the results of the radioisotope study confirm that the level of infection induced in this trial was responsible for pathophysiological changes in the infected control calves and the prior administration of an MSRB markedly reduced these changes, there were no differences in appetite or mean liveweight gain between treatment groups. It is possible that increased water retention was responsible for part of the liveweight gain of the infected calves as this parameter has been shown to be higher in parasitised ruminants than in parasite free animals (Halliday, Dalton, Anderson and Mulligan, 1968; Entrocasso et al, 1986; Abbott, Parkins and Holmes, 1986).

The alterations to the protein metabolism of the infected controls and to a lesser extent, the MSRB treated calves, occurred without apparent reduction in protein digestion. It is perhaps worth noting in this context the work of Steel (1978) which demonstrated that the small intestine is able to compensate for abomasal dysfunction in ovine ostertagiasis by enhanced digestion and absorption occurring lower down the gastrointestinal tract. Another possibility is that part of the lost protein on reaching the large intestine is degraded by bacteria to ammonia which after being absorbed is transported to the liver. Then some of the ammonia is converted to uric acid and excreted in the urine while part may be resynthesised to amino acids which would then be available for protein synthesis (Topps, 1983; Entrocasso et al, 1986)

The pathophysiological measurements gave some indication of the adverse effects of O. ostertagi on calf metabolism with

changes in pathophysiological values being greatest during the expected period of emergence of adult worms from the abomasal mucosa (Days 21 - 63). During this time plasma pepsinogen values of the infected control calves became elevated in relation to the values for the clean controls indicating leakage of pepsinogen from the abomasum. At this time leakage of plasma protein into the abomasa also occurred as was evidenced by increased faecal clearances of both ^{125}I-albumin and ^{51}CrCl3 albumin, increased albumin catabolic rates and a reduced half life of the radioactive albumin. In ostertagiasis, hypercatabolism of protein due to an increase in loss of albumin in the abomasum has been described by Mulligan et al, 1963; Halliday et al, 1968; Holmes and McLean, 1971; Steel, Symons and Jones, 1980; Yakoob, Holmes, Parkins and Armour, 1983; Yakoob, Holmes and Armour, 1983. This loss of albumin leads to increased rates of synthesis but in severe infections, where this cannot match the increased losses, hypoalbuminaemia results.

The MSRB treated calves showed only slight alterations in the pathophysiological parameters measured compared to the clean control animals and these were largely confined to the first investigation period (Days 35 - 47).

From the three and six week necropsy worm burdens it is clear that the Paratect bolus did not prevent the establishment of considerable worm burdens during the early period of infection. Considering the generally good performance of the bolus in the field this is somewhat surprising, but perhaps the more pertinent question to arise is that of when the anthelmintic

action of the bolus comes into effect. Obviously as indicated above it is not totally effective at time of infection (i.e. on ingestion of infective larvae) nor can its effect be absolute at time of parasite emergence from the abomasal mucosa. Taking these two factors together with the generally held view of a poor efficacy of morantel tartrate against larval stages in the mucosa this leaves the problem largely unsolved and, as with a definitive epidemiological study into bovine husk, this would seem to be an area worthy of more detailed study.

APPENDIX A

PEPSIN/HCL

Dissolve 100 grams of pepsin powder in 8 litres of luke warm water. Add 300 ml concentrated HCL slowly and stir well. Make final volume up to 10 litres. Store at $4^{o}C$.

IODINE SOLUTION

Dissolve 907 grams of potassium iodide in 650 ml boiling water. Add 510 grams Iodine crystals and make up to 1 litre.

SODIUM THIOSULPHATE SOLUTION

Dissolve 100 grams of sodium thiosulphate in 5 litres of water.

APPENDIX B

PLASMA PEPSINOGEN ESTIMATION

Reaction

Plasma is incubated with bovine serum albumin substrate (B.S.A.) at pH 2 for 24 hours and the phenolic amino acids liberated (tyrosine) are estimated using Folin-Ciocalteu's reagent. Corrections are made for the normal (i.e. non-incubated) content of tyrosine-like substances and also the release of these substances from the BSA substrate during the incubation period (i.e. corrected for any acid-hydrolysis which may take place during the 24 hr incubation).

Reagents

2% Bovine Serum Albumin (BSA)

2N Hydrochloric Acid (NCl)

4% Trichloroacetic Acid (TCA)

0.25 N Sodium Hydroxide (NaOH)

Folin-Ciocalteu's Reagent (Diluted 1 + 2 v/v with distilled water immediately before use)

Stock Standard Tyrosine 1.812g tyrosine in 1000 ml 0.1N HCl (10 u mols/ml)

Working Tyrosine Standards

(1) 10 ml Stock Standard diluated to 1000 ml (2.0 ml contains 0.2 u mols)

(2) 20 ml Stock Standard diluated to 1000 ml (2.0 ml contains 0.4 u mols)

Procedure

(In 30 ml universal bottle or suitable sealable container).

Plasma or Serum Tests

Two universals for each test, one labelled "Control" and the other "Test".

2.5 ml acidified BSA and 0.5 ml plasma (serum) in both universals. "Test" universal incubated for 24 hrs at 37°C, the "Control", immediately after the addition of plasma, is precipitated by the addition of 5.0 ml of 4% TCA.

BSA Blanks (in duplicate i.e. 4 universals)

2.5 ml acidified BSA and 0.5 ml distilled water in each universal. Two universals (Incubated-Blanks) are incubated for 24 hrs at 37°C. The other two universals (Unincubated-Blanks) are immediately precipitated with 5.0 ml 4% TCA.

Allow precipitated "Controls" and "Unincubated-Blanks" to stand for ten minutes after mixing, to ensure efficient floculation of the precipitate, and then filter through a Whatman No.44 filter paper.

After the 24 hr incubation period is completed, 5.0 ml of 4% TCA is added to each universal and "undigested" BSA is precipitated and filtered through a Whatman No.44 filter paper.

N.B. Filtrates are stable and can be stored in the refrigerator at this stage until the incubated samples are also available.

Treatment of Filtrates

Pipette 2.0 ml of each filtrate into suitably labelled flasks (50 ml conicals) containing 20 ml of 0.25N NaOH.

Set up flasks (in duplicate) containing 2.0 ml of each working tyrosine standard (i.e. 0.2 u mols and 0.4 u mols).

Set up Reagent Blank containing 2.0 ml distilled water with 20 ml 0.25N NaOH.

To all flasks add 3.0 ml diluted Folin-Ciocalteu's reagent.

Allow to stand for 30 minutes, then read the blud colour in a spectrophotometer at 725 mu. (Colour is fairly stable but is good practice to keep a standard time interval between addition of Folin-Ciocalteu's reagent and reading the resultant colour).

Calculation of Results

1. Subtract 'Reagent Blank' from all spectrophotometer readings.
2. From "Tyrosine Standards" calculate a factor for the conversion of all spectrophotometer readings to u mols tyrosine and convert all readings into equivalent "u mols tyrosine".
3. If incubated BSA and plasma ("Test").....A

   Non-Incubated BSA and plasma ("Controls").....B

   A-B = total release of tyrosine on incubation.
4. Incubated BSA alone ("Incubated BSA-Bland").....C

   Non-incubated BSA alone (Non-Incubated BSA-Blank...D

   C-D = Tyrosine released from BSA substrate due to incubation alone (**no pepsinogen**)
5. (A-B) - (C-D) = tyrosine in u mols released on incubation due to action of activated pepsinogen in **0.125 ml plasma (serum) in 24 hrs.**
6. Calculate tyrosine in **u mols** released by **1000 ml** plasma (serum) per **minute** = **International Units** or x 1000 = milli Units Tyrosine. [Multiply (5.) x factor 5.56].

APPENDIX C

Individual and Group Mean Respiratory Rates

	Animal No.	DAY 0	21	32	39	46	53	56	67	75	81	88	91	95	102	104	106
Group 1 (Treated)	B19	28	36	32	32	36	36	36	28	32	36	32	28	28	28	60	32
	B20	36	36	36	36	32	36	32	32	36	40	28	36	32	28	32	38
	B21	32	28	40	32	28	40	32	32	36	32	32	36	33	30	36	20
	B22	36	32	32	32	32	32	32	40	40	36	36	32	30	28	36	24
	B23	40	36	28	36	32	32	28	32	36	32	28	36	32	30	40	32
	B24	32	36	28	28	32	36	36	28	36	32	28	36	32	28	48	40
	Mean	34	34	33	33	32	35	33	32	36	35	31	34	31	29	42	31
Group 2 (Control)	W1	32	36	40	44	44	36	36	32	36	–	–	–	–	–	–	–
	W2	36	40	44	36	48	36	28	32	36	36	32	36	34	32	30	48
	W3	32	36	36	36	36	36	36	36	40	36	28	32	34	36	40	24
	W4	40	40	40	40	36	40	32	32	40	32	36	36	33	30	44	24
	W5	36	40	40	32	40	40	32	32	44	36	36	36	34	32	40	44
	W6	28	36	36	32	32	32	32	32	36	36	36	40	35	30	36	40
	Mean	34	38	34	37	39	37	33	33	39	35	34	36	34	32	38	36
Group 3 (Treated)	R7	36	40	32	36	36	32	36	32	32	32	36	32	31	30	40	36
	R8	36	36	32	32	28	28	40	32	36	36	36	36	34	32	36	36
	R9	32	36	28	32	32	36	32	36	36	32	36	32	31	30	36	32
	R10	36	44	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	R11	36	40	36	36	36	32	36	28	36	36	36	36	30	28	48	28
	R12	32	40	32	32	28	32	36	36	36	36	32	40	34	28	48	28
	Mean	35	39	32	34	32	32	36	33	35	34	35	35	32	30	42	32
Group 4 (Control)	G13	44	32	36	36	32	36	32	40	36	36	32	32	32	32	48	52
	G14	36	36	40	36	40	40	32	32	–	–	–	–	–	–	–	–
	G15	36	36	36	40	36	36	36	32	32	40	28	32	31	30	40	36
	G16	44	32	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	G17	32	28	36	36	36	36	32	36	32	32	32	32	32	32	28	28
	G18	32	28	40	36	44	36	33	40	36	32	36	28	30	32	60	40
	Mean	38	32	38	39	38	37	33	36	34	35	32	31	31	32	38	39

APPENDIX C (Cont'd)

Individual and group mean respiratory rates

	Animal no.	DAY 109	112	114	117	121	124	128
Group 1 (Treated)	B19	48	48	52	28	32	28	20
	B20	36	36	24	32	36	32	20
	B21	32	32	36	24	48	32	16
	B22	36	28	36	28	32	32	20
	B23	52	28	36	28	28	28	24
	B24	32	32	44	24	28	28	24
	Mean	39	34	38	27	34	30	21
Group 2 (Control)	W1	-	-	-	-	-	-	-
	W2	-	-	-	-	-	-	-
	W3	28	28	36	24	-	-	-
	W4	28	28	36	24	32	16	24
	W5	28	36	40	32	28	24	20
	W6	32	32	36	24	28	20	18
	Mean	29	31	37	26	29	20	21
Group 3 (Treated)	R7	48	40	44	36	28	28	36
	R8	36	42	36	36	40	28	16
	R9	28	40	28	28	44	32	20
	R10	-	-	-	-	-	-	-
	R11	52	32	36	32	28	28	20
	R12	28	24	28	28	32	28	12
	Mean	38	36	34	32	34	29	21
Group 4 (Control)	G13	32	32	24	20	32	28	24
	G14	-	-	-	-	-	-	-
	G15	32	36	28	28	36	24	12
	G16	-	-	-	-	-	-	-
	G17	32	24	28	26	32	24	16
	G18	28	36	28	28	40	20	12
	Mean	31	32	27	26	35	24	16

APPENDIX C (Cont'd)

Individual and group mean respiratory rates

	Animal no.	DAY 131	134	138	143	145	149	152
Group 1 (Treated)	B19	20	24	28	20	20	24	24
	B20	20	24	28	20	20	32	24
	B21	24	20	24	28	24	24	24
	B22	24	24	20	24	28	28	28
	B23	20	20	24	24	20	24	32
	B24	24	20	24	32	24	24	32
	Mean	22	22	25	25	23	26	27
Group 2 (Control)	W1	-	-	-	-	-	-	-
	W2	-	-	-	-	-	-	-
	W3	-	-	-	-	-	-	-
	W4	16	16	-	-	-	-	-
	W5	24	-	-	-	-	-	-
	W6	16	16	20	24	28	36	20
	Mean	19	16	20	24	28	36	20
Group 3 (Treated)	R7	28	32	20	24	24	24	28
	R8	32	24	24	24	28	20	28
	R9	20	36	20	24	20	24	28
	R10	-	-	-	-	-	-	-
	R11	28	24	24	20	20	24	24
	R12	28	24	16	20	23	24	16
	Mean	27	28	21	22	23	23	25
Group 4 (Control)	G13	20	20	20	24	24	28	20
	G14	-	-	-	-	-	-	-
	G15	16	24	12	20	20	32	20
	G16	-	-	-	-	-	-	-
	G17	24	24	24	20	24	28	16
	G18	16	20	20	24	24	32	20
	Mean	19	22	19	22	23	30	19

APPENDIX D

Individual and Group mean bodyweights (kg)

	Animal no.	DAY 0	21	32	46	56	75	88
Group 1 (Treated)	B19	90	100	110	110	115	125	125
	B20	160	180	200	210	205	225	225
	B21	120	125	135	145	140	155	160
	B22	130	150	155	160	160	180	175
	B23	120	130	145	150	155	180	170
	B24	135	140	150	165	170	190	195
	Mean	126	138	149	157	158	176	175
Group 2 (Control)	W1	95	110	105	115	110	115	-
	W2	120	115	110	115	110	115	110
	W3	115	125	145	160	160	165	160
	W4	135	140	145	170	170	165	170
	W5	125	140	155	165	165	135	150
	W6	160	170	185	190	190	200	208
	Mean	125	133	141	153	151	149	160
Group 3 (Treated)	R7	110	120	130	120	140	160	155
	R8	120	140	160	175	180	190	190
	R9	150	150	175	185	185	205	205
	R10	120	130	-	-	-	-	-
	R11	120	115	125	130	135	140	140
	R12	145	150	165	170	175	195	185
	Mean	128	134	151	156	163	178	175
Group 4 (Control)	G13	140	160	180	195	195	220	215
	G14	100	120	120	130	130	-	-
	G15	110	120	135	145	150	170	170
	G16	130	130	-	-	-	-	-
	G17	145	165	180	185	190	195	195
	G18	120	120	120	120	130	135	135
	Mean	124	136	147	155	159	180	179

APPENDIX D (Cont'd)

Individual and Group mean bodyweights (kg)

	Animal no.	DAY 91	109	121	134	149	156
Group 1 (Treated)	B19	130	130	130	135	125	135
	B20	230	225	225	215	215	225
	B21	165	170	160	170	155	165
	B22	185	185	180	175	170	180
	B23	167	169	180	180	180	200
	B24	200	215	250	220	210	220
	Mean	180	182	188	183	176	188
Group 2 (Control)	W1	-	-	-	-	-	-
	W2	110	86	-	-	-	-
	W3	160	155	-	-	-	-
	W4	170	155	135	130	-	-
	W5	160	165	130	-	-	-
	W6	200	185	165	150	160	160
	Mean	160	149	143	140	160	160
Group 3 (Treated)	R7	165	175	170	175	165	185
	R8	200	205	205	210	200	204
	R9	210	215	205	210	200	210
	R10	-	-	-	-	-	-
	R11	145	145	145	145	135	145
	R12	185	200	180	195	185	195
	Mean	181	188	181	187	177	188
Group 4 (Control)	G13	220	205	190	190	175	175
	G14	-	-	-	-	-	-
	G15	180	165	140	140	130	140
	G16	-	-	-	-	-	-
	G17	200	195	200	205	200	205
	G18	140	140	140	135	130	140
	Mean	185	176	168	168	159	165

APPENDIX E

Individual and group mean plasma pepsinogen estimation

	Animal no.	DAY 0	21	32	46	56	75	88
Group 1 (Treated)	B19	603	1009	504	735	826	1025	885
	B20	564	787	1088	1016	1036	832	702
	B21	524	1219	1128	1120	1250	1084	1229
	B22	564	1572	1235	1160	1072	991	1296
	B23	551	851	628	613	597	670	518
	B24	538	930	NS	800	840	924	885
	Mean	557	1061	917	907	937	921	919
Group 2 (Control)	W1	604	474	588	1399	1442	2027	-
	W2	603	996	778	1320	1675	1949	1899
	W3	564	1677	3075	5184	6603	8275	9386
	W4	642	631	1088	2234	2645	5364	5538
	W5	577	1809	3157	7583	9120	5474	2849
	W6	617	1074	2155	3899	4243	5871	6673
	Mean	601	1110	1807	3603	4288	4827	5269
Group 3 (Treated)	R7	565	695	711	1055	1212	1110	1019
	R8	577	1140	1264	1373	1537	1761	1401
	R9	551	984	1264	1854	1611	1308	1481
	R10	538	683	-	-	-	-	-
	R11	655	1077	764	1441	1099	1045	1205
	R12	565	1311	1345	1615	1596	1123	903
	Mean	575	982	1069	1467	1411	1269	1202
Group 4 (Control)	G13	603	1061	1169	1786	2054	1840	2411
	G14	551	997	1332	1812	1684	-	-
	G15	577	1141	940	1307	1107	1549	2526
	G16	590	1034	-	-	-	-	-
	G17	551	1179	1141	1919	1607	1549	1441
	G18	551	853	1016	1016	1084	1469	1625
	Mean	571	1044	1120	1568	1507	1602	2001

APPENDIX E (Cont'd)

Individual and group mean plasma pepsinogen estimation

	Animal no.	DAY 91	109	121	134	149	156
Group 1 (Treated)	B19	867	773	596	688	832	700
	B20	675	643	563	564	631	590
	B21	983	864	908	1076	997	1191
	B22	1302	1239	1513	1170	772	811
	B23	637	734	522	457	382	492
	B24	828	916	987	1002	1069	832
	Mean	882	862	848	826	781	769
Group 2 (Control)	W1	-	-	-	-	-	-
	W2	2108	2883	-	-	-	-
	W3	8483	11223	-	-	-	-
	W4	5565	7247	5953	2524	-	-
	W5	3773	6302	6575	-	-	-
	W6	6013	8711	8655	7398	5410	3801
	Mean	5188	7273	7061	4961	5410	3801
Group 3 (Treated)	R7	905	799	923	905	832	762
	R8	1238	1602	1466	1351	1269	995
	R9	1239	1187	1130	1400	1175	811
	R10	-	-	-	-	-	-
	R11	868	915	898	997	678	688
	R12	803	747	652	841	666	603
	Mean	1011	1050	1014	1099	924	772
Group 4 (Control)	G13	2199	5564	7127	8332	6771	7477
	G14	-	-	-	-	-	-
	G15	2723	4347	4014	6138	4582	2110
	G16	-	-	-	-	-	-
	G17	1367	2016	1980	2017	2290	1742
	G18	1904	3402	612	6926	3541	2024
	Mean	2448	3832	3433	5853	4283	3338

APPENDIX F + APPENDIX G (Figs. in Brackets)

Individual and group mean faecal egg counts (*N. battus*)

	Animal no.	DAY 0	21	32	39	46
Group (Treated)	B19	0(0)	100(350)	350 (0)	0(0)	0(0)
	B20	0(0)	0(0)	0(100)	0(0)	0(0)
	B21	0(0)	0(0)	0(0)	0(0)	0(0)
	B22	0(0)	0(100)	0(0)	0(0)	0(0)
	B23	0(0)	100(0)	0(50)	0(0)	0(0)
	B24	0(0)	50(200)	0(0)	0(0)	0(0)
	Mean	0(0)	42(108)	58(25)	0(8)	0(0)
Group 2 (Control)	W1	0(0)	0(0)	0(50)	0(0)	0(0)
	W2	0(0)	0(350)	200(0)	0(0)	0(0)
	W3	0(0)	100(50)	150(0)	400(0)	350(0)
	W4	0(0)	50(0)	150(0)	50(0)	150(0)
	W5	0(0)	0(0)	100(50)	550(0)	850(0)
	W6	0(0)	0(0)	200(100)	600(0)	250(0)
	Mean	0(0)	25(67)	133(33)	267(0)	267(0)
Group 3 (Treated)	R7	0(0)	0(100)	0(0)	0(0)	0(0)
	R8	0(0)	0(0)	0(50)	0(0)	0(0)
	R9	0(0)	0(0)	0(0)	0(0)	0(0)
	R10	0(0)	0(50)	-	-	-
	R11	0(0)	50(600)	0(0)	0(50)	0(0)
	R12	0(0)	50(300)	0(150)	0(0)	0(0)
	Mean	0(0)	17(175)	0(40)	0(10)	0(0)
Group 4 (Control)	G13	0(0)	0(0)	0(0)	300(0)	100(0)
	G14	0(0)	0(150)	400(0)	0(0)	100(0)
	G15	0(0)	150(200)	0(0)	0(0)	250(0)
	G16	0(0)	50(100)	-	-	-
	G17	0(0)	50(0)	0(0)	300(50)	450(0)
	G18	0(0)	150(50)	150(0)	450(0)	350(0)
	Mean	0(0)	67(83)	110(0)	210(10)	250(0)

APPENDIX F + APPENDIX G (Figs. in Brackets) (Cont'd)

Individual and group mean faecal egg counts (N. battus)

	Animal no.	DAY 53	56	67	75	81
Group (Treated)	B19	0(0)	0(0)	0(0)	0(0)	0(0)
	B20	0(0)	0(0)	0(0)	0(0)	0(0)
	B21	0(0)	0(0)	0(0)	0(0)	0(0)
	B22	0(0)	0(0)	0(0)	50(0)	0(0)
	B23	0(0)	50(0)	0(0)	0(0)	0(0)
	B24	0(0)	0(0)	0(0)	0(0)	0(0)
	Mean	0(0)	8(0)	0(0)	8(0)	0(0)
Group 2 (Control)	W1	0(0)	0(0)	950(50)	2400(0)	-
	W2	100(50)	150(0)	2100(0)	2800(0)	0(0)
	W3	1000(50)	550(50)	350(0)	2000(0)	1100(0)
	W4	50(0)	150(0)	250(0)	850(0)	1400(0)
	W5	400(0)	700(0)	1750(0)	1750(0)	0(0)
	W6	900(0)	400(0)	900(0)	1700(0)	600(0)
	Mean	408(17)	325(8)	1050(8)	1917(0)	620(0)
Group 3 (Treated)	R7	0(0)	0(0)	50(0)	0(0)	0(0)
	R8	0(0)	0(0)	0(0)	0(0)	0(0)
	R9	0(0)	0(0)	0(0)	0(0)	50(0)
	R10	-	-	-	-	-
	R11	50(0)	0(0)	0(0)	50(0)	0(0)
	R12	0(0)	0(0)	50(0)	0(0)	50(50)
	Mean	10(0)	0(0)	20(0)	10(0)	20(10)
Group 4 (Control)	G13	100(0)	50(0)	0(0)	200(0)	200(0)
	G14	350(0)	250(0)	0(0)	-	-
	G15	1100(0)	850(0)	250(0)	350(0)	250(0)
	G16	-	-	-	-	
	G17	50(0)	50(0)	250(0)	200(0)	400(0)
	G18	0(0)	100(0)	200(0)	250(0)	50(0)
	Mean	320(0)	260(0)	140(0)	250(0)	225(0)

APPENDIX F + APPENDIX G (Figs. in Brackets) (Cont'd)

Individual and group mean faecal egg counts (N.battus)

	Animal no.	DAY 88	91	95	102	104
Group 1 (Treated)	B19	0(0)	0(0)	0(0)	0(0)	0(0)
	B20	0(0)	0(0)	0(0)	50(0)	0(50)
	B21	0(0)	0(0)	0(0)	0(0)	0(0)
	B22	0(0)	0(0)	0(0)	0(0)	0(0)
	B23	0(0)	100(0)	0(0)	0(0)	0(0)
	B24	0(0)	0(0)	0(0)	0(0)	0(0)
	Mean	0(0)	17(0)	0(0)	8(0)	0(8)
Group 2 (Control)	W1	-	-	-	-	-
	W2	0(0)	0(0)	100(0)	0(0)	0(0)
	W3	800(0)	800(0)	700(0)	1350(0)	800(0)
	W4	1350(0)	2000(0)	200(50)	1150(0)	850(0)
	W5	0(0)	0(0)	0(0)	150(0)	200(0)
	W6	150(0)	800(0)	350(0)	900(0)	600(0)
	Mean	460(0)	720(0)	270(10)	710(0)	490(0)
Group 3 (Treated)	R7	0(0)	0(0)	0(0)	0(0)	0(0)
	R8	0(0)	0(0)	0(0)	0(0)	0(0)
	R9	0(0)	0(0)	0(0)	0(0)	0(0)
	R10	-	-	-	-	-
	R11	0(0)	0(0)	0(0)	0(0)	0(0)
	R12	50(50)	100(0)	50(0)	0(0)	0(0)
	Mean	10(10)	20(0)	10(0)	0(0)	0(0)
Group 4 (Control)	G13	300(0)	100(0)	150(0)	0(0)	150(0)
	G14	-	-	-	-	-
	G15	150(0)	250(0)	150(0)	300(0)	200(0)
	G16	-	-	-	-	-
	G17	150(0)	300(0)	250(0)	300(0)	200(0)
	G18	150(0)	350(0)	100(50)	100(0)	150(0)
	Mean	188(0)	250(0)	163(13)	175(0)	175(0)

APPENDIX F + APPENDIX G (Figs. in Brackets) (Cont'd)

Individual and group mean faecal egg counts (N.battus)

	Animal no.	DAY 106	109	112	114	117
Group 1 (Treated)	B19	0(0)	0(0)	0(0)	0(0)	0(0)
	B20	0(0)	0(0)	0(0)	0(0)	0(0)
	B21	0(0)	0(0)	0(0)	0(0)	0(0)
	B22	0(0)	0(0)	0(0)	0(0)	0(0)
	B23	0(0)	0(0)	0(0)	0(0)	50(0)
	B24	0(0)	0(0)	0(0)	0(50)	50(0)
	Mean	0(0)	0(0)	0(0)	0(8)	17(0)
Group 2 (Control)	W1	-	-	-	-	-
	W2	600(0)	1200(0)	-	-	-
	W3	2250(0)	1400(0)	3800(0)	4700(0)	3750(0)
	W4	1000(0)	2000(0)	2100(0)	2650(0)	3200(0)
	W5	200(0)	200(0)	100(0)	700(0)	800(0)
	W6	250(0)	450(0)	250(0)	1600(0)	1200(0)
	Mean	920(0)	1050(0)	1688(0)	2413(0)	2263(0)
Group 3 (Treated)	R7	0(0)	0(0)	0(50)	0(0)	0(0)
	R8	0(0)	0(0)	0(0)	50(0)	0(0)
	R9	0(0)	0(0)	0(0)	0(0)	0(0)
	R10	-	-	-	-	-
	R11	0(0)	0(0)	0(0)	0(0)	0(0)
	R12	0(0)	50(0)	0(0)	50(0)	150(0)
	Mean	0(0)	10(0)	0(10)	20(0)	30(0)
Group 4 (Control)	G13	0(0)	450(0)	700(0)	650(0)	1150(0)
	G14	-	-	-	-	-
	G15	550(0)	850(50)	1050(0)	450(0)	450(0)
	G16	-	-	-	-	-
	G17	200(0)	250(0)	100(0)	0(0)	350(0)
	G18	0(0)	50(0)	350(0)	150(0)	750(0)
	Mean	188(0)	400(13)	550(0)	313(0)	675(0)

APPENDIX F + APPENDIX G (Figs. in Brackets) (Cont'd)

Individual and group mean faecal egg counts (*N. battus*)

	Animal no.	DAY 121	124	128	131	134	138
Group 1 (Treated)	B19	0(0)	0(0)	50(0)	50(0)	150(0)	200(0)
	B20	0(0)	0(0)	150(0)	50(0)	50(0)	250(0)
	B21	0(0)	0(0)	0(0)	50(0)	200(0)	100(0)
	B22	0(0)	0(0)	50(0)	0(0)	50(0)	100(0)
	B23	0(0)	0(0)	100(0)	50(0)	0(0)	0(0)
	B24	0(0)	100(0)	100(0)	0(0)	0(0)	0(0)
	Mean	0(0)	17(0)	75(0)	33(0)	75(0)	108(0)
Group 2 (Control)	W1	-	-	-	-	-	-
	W2	-	-	-	-	-	-
	W3	-	-	-	-	-	-
	W4	2450(0)	1850(0)	1800(0)	3800(0)	3000(0)	-
	W5	750(0)	2100(0)	2900(0)	1700(0)	-	-
	W6	1250(0)	1050(0)	1100(0)	4550(0)	0(0)	800(0)
	Mean	1483(0)	1667(0)	1933(0)	3350(0)	1500(0)	800(0)
Group 3 (Treated)	R7	50(0)	0(0)	50(0)	0(0)	0(0)	100(0)
	R8	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	R9	50(0)	0(0)	0(0)	0(0)	50(0)	0(0)
	R10	-	-	-	-	-	-
	R11	50(0)	50(0)	0(0)	0(0)	0(0)	0(0)
	R12	0(0)	0(0)	300(150)	200(0)	250(0)	700(50)
	Mean	30(0)	10(0)	70(30)	40(0)	60(0)	160(10)
Group 4 (Control)	G13	650(0)	900(0)	2250(0)	1400(0)	900(0)	1150(0)
	G14	-	-	-	-	-	-
	G15	550(0)	550(0)	100(0)	350(0)	150(0)	400(0)
	G16	-	-	-	-	-	-
	G17	400(0)	700(0)	500(0)	200(0)	300(0)	0(0)
	G18	150(0)	750(0)	350(0)	200(0)	1550(0)	100(0)
	Mean	438(0)	725(0)	800(0)	538(0)	725(0)	113(0)

APPENDIX F + APPENDIX G (Figs. in Brackets) (Cont'd)

Individual and group mean faecal egg counts (*N. battus*)

	Animal no.	DAY 143	145	149	152	156
Group 1	B19	50(0)	250(0)	150(0)	200(0)	650(0)
	B20	100(0)	0(0)	150(0)	50(0)	250(0)
	B21	50(0)	0(0)	0(0)	0(0)	0(0)
	B22	0(0)	0(0)	50(0)	0(0)	50(0)
	B23	200(0)	150(100)	150(50)	0(0)	0(0)
	B24	50(0)	0(0)	150(0)	50(50)	300(0)
	Mean	75(0)	67(17)	108(8)	50(8)	208(0)
Group 2 (Control)	W1	-	-	-	-	-
	W2	-	-	-	-	-
	W3	-	-	-	-	-
	W4	-	-	-	-	-
	W5	-	-	-	-	-
	W6	0(0)	0(0)	0(0)	0(0)	50(0)
	Mean	0(0)	0(0)	0(0)	0(0)	50(0)
Group 3 (Treated)	R7	100(0)	150(0)	50(0)	0(0)	100(0)
	R8	0(0)	0(0)	0(0)	150(0)	0(0)
	R9	0(0)	50(0)	0(0)	0(0)	150(0)
	R10	-	-	-	-	-
	R11	0(0)	100(0)	500(0)	500(0)	1225(0)
	R12	700(100)	250(150)	400(50)	600(100)	1150(0)
	Mean	160(20)	110(30)	190(10)	250(20)	525(0)
Group 4 (Control)	G13	1150(0)	750(0)	450(0)	850(0)	1150(0)
	G14	-	-	-	-	-
	G15	400(0)	0(0)	0(0)	0(0)	0(0)
	G16	-	-	-	-	-
	G17	0(0)	250(0)	100(0)	450(0)	150(50)
	G18	100(0)	0(0)	0(0)	0(0)	0(0)
	Mean	275(0)	250(0)	138(0)	325(0)	325(13)

APPENDIX H

Individual and group mean *Dictyocaulus viviparus* larval counts from faeces

	Animal no.	DAY 114	117	121	124	128	131
Group 1 (Treated)	B19	0	0	0	0	0	0
	B20	0	0	0	0	0	0
	B21	0	0	0	0	0	0
	B22	0	0	0	0	0	0
	B23	0	0	0	0	0	0
	B24	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
Group 2 (Control)	W1	-	-	-	-	-	-
	W2	-	-	-	-	-	-
	W3	5	6	-	-	-	-
	W4	0	5	4	4	4	11
	W5	0	3	1	2	2	9
	W6	2	6	7	5	5	2
	Mean	2	5	4	4	4	7
Group 3 (Treated)	R7	0	0	0	0	0	0
	R8	0	0	0	0	0	0
	R9	0	0	0	0	0	0
	R10	-	-	-	-	-	-
	R11	0	0	0	0	0	0
	R12	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
Group 4 (Control)	G13	0	3	4	1	1	15
	G14	-	-	-	-	-	-
	G15	0	1	3	0	0	9
	G16	-	-	-	-	-	-
	G17	0	0	5	8	8	0
	G18	0	8	7	3	3	0
	Mean	0	3	5	3	3	6

APPENDIX H (Cont'd)

Individual and group mean Dictyocaulus viviparus larval counts from faeces

	Animal no.	DAY 134	138	143	145	149	152	156
Group 1 (Treated)	B19	0	0	0	0	0	0	0
	B20	0	0	0	0	0	0	0
	B21	0	0	0	0	0	0	0
	B22	0	0	0	0	0	0	0
	B23	0	0	0	0	0	0	0
	B24	0	0	0	0	0	0	0
	Mean	0	0	0	0	0	0	0
Group 2 (Control)	W1	-	-	-	-	-	-	-
	W2	-	-	-	-	-	-	-
	W3	-	-	-	-	-	-	-
	W4	4	-	-	-	-	-	-
	W5	-	-	-	-	-	-	-
	W6	0	228	0	0	0	0	0
	Mean	2	228	0	0	0	0	0
Group 3 (Treated)	R7	0	0	0	0	0	0	0
	R8	0	0	0	0	0	0	0
	R9	0	0	0	0	0	0	0
	R10	-	-	-	-	-	-	-
	R11	0	0	0	0	0	0	0
	R12	0	0	0	0	0	0	0
	Mean	0	0	0	0	0	0	0
Group 4 (Control)	G13	13	15	8	6	18	20	8
	G14	-	-	-	-	-	-	-
	G15	8	11	14	0	0	0	0
	G16	-	-	-	-	-	-	-
	G17	3	2	0	0	1	0	0
	G18	12	40	0	0	0	0	0
	Mean	9	17	6	2	5	5	2

APPENDIX I1

Ostertagia ostertagi L3/Kg dried herbage

Day No.	Plot 1	Plot 2	Plot 3	Plot 4
0	161	2667	2167	385
21	4865	1571	1277	105
32	0	1035	235	571
39	0	567	150	175
46	213	0	0	0
53	0	508	465	0
56	734	1087	682	98
67	333	7778	600	1636
75	2401	3934	1887	1613
81	0	8372	943	313
88	204	4242	0	4242
95	0	1234	0	346
102	943	9941	533	10180
109	0	4177	0	174
114	660	1328	340	8112
121	1579	2404	201	2167
128	0	9691	222	1818
134	1437	4561	1190	10772
143	3502	2117	290	4700
149	570	6353	194	1231

APPENDIX I2

Cooperia oncophora L3/Kg dried herbage

Day No.	Plot 1 (Treated)	Plot 2 (Control)	Plot 3 (Treated)	Plot 4 (Control)
0	0	1333	667	385
21	2973	571	532	210
32	0	343	60	427
39	0	189	0	0
46	0	208	0	0
53	0	678	0	0
56	0	746	233	98
67	0	4444	0	909
75	645	417	629	1277
81	0	3256	187	0
88	208	731	0	1264
95	0	617	0	173
102	310	3388	0	1593
109	0	1044	0	0
114	330	382	340	2978
121	526	1248	0	1000
128	0	6755	222	909
134	788	1634	342	5677
143	1167	488	290	4700
149	360	6353	194	821

APPENDIX I3

Nematodirus battus L3/Kg dried herbage

Day No.	Plot 1 (Treated)	Plot 2 (Control)	Plot 3 (Treated)	Plot 4 (Control)
0	322	1167	1833	385
21	1081	8429	5851	210
32	185	2421	944	1104
39	455	4725	1500	175
46	0	208	1044	167
53	0	339	3256	0
56	597	220	116	196
67	0	0	0	0
75	0	625	233	282
81	0	0	0	0
88	204	731	0	862
95	0	0	0	692
102	1564	217	0	1609
109	0	1044	476	347
114	0	191	340	393
121	0	0	213	3250
128	0	1175	0	455
134	355	1581	171	1526
143	194	488	0	522
149	285	0	0	141

APPENDIX J

Dictyocaulus viviparus L3/Kg dried herbage

Day No.	Plot 1 (Treated)		Plot 2 (Control)	
	Random	Near faeces	Random	Near faeces
0	161	-	333	-
21	0	-	0	-
32	0	0	87	0
39	-	0	-	0
46	0	0	0	0
53	-	0	-	0
56	0	161	0	0
67	-	0	-	0
75	0	0	208	339
81	-	0	-	0
88	204	0	555	0
95	-	0	-	0
102	310	0	0	0
109	-	0	-	0
114	0	0	191	0
121	0	0	0	0
128	-	0	-	587
134	216	0	0	395
143	-	0	-	163
199	0	0	0	0

APPENDIX J (Cont'd)

Dictyocaulus viviparus L3/Kg dried herbage

	Plot 3 (Treated)		Plot 4 (Control)	
Day No.	Random	Near faeces	Random	Near faeces
0	167	-	0	-
21	0	-	0	-
32	30	0	35	178
39	-	0	-	0
46	0	0	109	0
53	-	0	-	0
56	0	0	0	0
67	-	0	-	0
75	0	164	0	238
81	-	0	-	313
88	0	322	0	0
95	-	0	-	0
102	0	0	0	0
109	-	238	-	347
114	510	0	131	0
121	201	0	0	0
128	-	0	-	0
134	171	0	0	0
143	-	0	-	0
199	0	0	0	0

APPENDIX K

Individual and group mean plasma pepsinogen estimation

	Animal no.	DAY 0	7	14	21	28	35	42
Group 1 (Clean control)	B1	797	626	765	732	809	737	594
	B2	541	541	668	641	740	543	425
	Mean	669	584	717	687	775	734	510
Group 2 (MSRB treated)	Y63	478	566	1,118	1,497	2,148	1,921	1,714
	Y64	650	749	1,142	1,608	2,161	1,647	1,688
	Mean	564	658	1,130	1,553	2,155	1,784	1,701
Group 3 (Infected control)	03	453	466	813	1,011	2,201	2,674	2,991
	04	504	466	740	1,229	2,473	3,362	3,148
	Mean	479	466	777	1,120	2,337	3,018	3,070
(Infected control)								
Necropsy Week 3	06	798	910	1,422	1,229	-	-	-
Necropsy Week 6	05	515	602	996	1,191	2,052	2,522	2,613
	Mean	657	756	1,209	1,210	-	-	-
(MSRB treated)								
Necropsy Week 3	Y66	502	699	1,215	1,252	-	-	-
Necropsy Week 6	Y65	533	587	753	1,127	1,607	2,240	1,688
	Mean	518	643	984	1,190	-	-	-

APPENDIX K (Cont'd)

Individual and group mean plasma pepsinogen estimation

	Animal no.	49	57	63	71	78	85
Group 1 (Clean control)	B1	596	586	567	808	601	560
	B2	493	462	529	539	509	523
	Mean	545	524	548	674	555	542
Group 2 (MSRB treated)	Y63	1,609	1,722	1,270	1,064	668	727
	Y64	1,558	1,522	1,435	969	814	764
	Mean	1,584	1,622	1,353	1,017	741	746
Group 3 (Infected control)	03	2,993	2,275	3,656	3,577	2,480	1,917
	04	2,583	2,683	1,895	2,045	1,185	968
	Mean	2,788	2,479	2,776	2,811	1,833	1,443
(Infected control)							
Necropsy Week 3	06	-	-	-	-	-	-
Necropsy Week 6	05	-	-	-	-	-	-
	Mean	-	-	-	-	-	-
(MSRB treated)							
Necropsy Week 3	Y66	-	-	-	-	-	-
Necropsy Week 6	Y65	-	-	-	-	-	-
	Mean	-	-	-	-	-	-

APPENDIX L

Pathophysiological changes in calves infected with Ostertagia ostertagi, infected, after prior administration of a MSRB or maintained as clean controls

Days 35-47		Clean B1	Clean B2	MSRB Y63	MSRB Y64	Infected O3	Infected O4
Plasma volume	Vp (ml/kg)	50.2	47.6	42.9	45.4	46.5	45.3
Faecal Clearance	^{125}I-albumin (ml/day)	37.4	39.5	41.5	41.0	46.9	58.9
Faecal Clearance	^{51}Cr-albumin (ml/day)	272.8	321.1	341.4	307.8	432.3	437.1
Catabolic Rate of Albumin	%	4.5	5.2	5.2	5.5	6.8	6.1
Albumin Tl/2 Disappearance	^{125}I-albumin (days)	27.8	20.0	23.0	27.6	18.7	18.4
Water turn over	L/day	0.29	0.30	0.35	0.29	0.28	0.22
Total body water	% bodyweight	73.3	74.1	74.0	72.5	84.6	73.4
TOH Disappearance	Tl/2 (days)	170	168	144	168	207	227
Days 63-82							
Plasma volume	Vp (ml/kg)	46.4	47.1	41.6	47.3	51.5	47.9
Faecal Clearance	^{125}I-albumin (ml/day)	46.3	30.6	28.9	23.7	42.6	54.1
Faecal Clearance	^{51}Cr-albumin (ml/day)	345.6	324.0	222.8	279.4	495.4	292.6
Catabolic Rate of Albumin	%	5.4	7.2	5.7	4.8	6.7	4.8
Albumin Tl/2 Disappearance	^{125}I-albumin (days)	12.4	16.0	19.4	20.6	16.3	35.8
Total body water	% bodyweight	65.1	72.4	70.6	62.7	69.0	69.7
TOH Disappearance	Tl/2 (days)	252	145	161	129	103	129
Water turn over	L/day	0.17	0.34	0.30	0.33	0.46	0.37

APPENDIX M

Individual and group mean faecal egg counts

	Animal no.	DAY 0	7	14	21	28	35	42
Group 1 (clean control)	B1	0	0	0	0	0	0	0
	B2	0	0	0	0	0	0	0
	Mean	0	0	0	0	0	0	0
Group 2 (MSRB treated)	Y63	0	0	0	0	0	0	0
	Y64	0	0	0	0	0	50	0
	Mean	0	0	0	0	0	25	0
Group 3 (Infected control)	03	0	0	0	0	600	900	1,650
	04	0	0	0	100	350	650	450
	Mean	0	0	0	50	475	775	1,050
(Infected control)								
Necropsy Week 3	06	0	0	0	50	-	-	-
Necropsy Week 6	05	0	0	0	50	150	300	250
	Mean	0	0	0	50			
(MSRB treated)								
Necropsy Week 3	Y66	0	0	0	-	-	-	-
Necropsy Week 6	Y65	0	0	0	0	50	0	0
	Mean	0	0	0	0			

APPENDIX M (Cont'd)

Individual and group mean faecal egg counts

	Animal no.	49	57	63	71	78	85
Group 1 (clean control)	B1	0	0	0	0	0	0
	B2	0	0	0	0	0	0
	Mean	0	0	0	0	0	0
Group 2 (MSRB treated)	Y63	0	0	0	0	0	0
	Y64	NS	0	0	0	0	0
	Mean	0	0	0	0	0	0
Group 3 (Infected control)	O3	650	600	200	700	700	300
	O4	150	150	100	250	150	250
	Mean	350	375	150	475	425	275
(Infected control)							
Necropsy Week 3	O6	-	-	-	-	-	-
Necropsy Week 6	O5	-	-	-	-	-	-
	Mean						
(MSRB treated)							
Necropsy Week 3	Y66	-	-	-	-	-	-
Necropsy Week 6	Y65	-	-	-	-	-	-
	Mean						

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