BOVINE PARASITIC GASTROENTERITIS AND BRONCHITIS:

CONTROL *vs* IMMUNITY

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DECLARATION

The studies described in this thesis are original, any collaboration having been acknowledged, and were carried out at the Department of Veterinary Parasitology, Glasgow University Veterinary School under the joint supervision of Prof. J. L. Duncan and Dr. K. Bairden.

DEDICATION

To my Parents with thanks

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SUMMARY

This thesis comprises a series of studies carried out (1) to review the literature on parasitic gastroenteritis and bronchitis with special emphasis on epidemiology, immunity and control methods (2) to determine the influence of anthelmintic prophylaxis in calves on their subsequent immunity to *Ostertagia, Cooperia* and *Dictyocaulus* as yearlings (3) to assess the parasitological status of adult dairy cattle and (4) to compare the efficacy of several techniques used in these studies.

In one to two year old cattle, the influence of chemoprophylaxis during their first seasons grazing on the subsequent development of immunity was investigated. Three first season chemoprophylactic programmes i.e. ivermectin at 3, 8 and 13 weeks post turnout and moxidectin injectable or moxidectin pour-on at turnout and 5 weeks post turnout were compared. The results, as judged by faecal egg counts and serum pepsinogen concentrations, suggested that these first season prophylactic treatments in calves had a deleterious influence on their apparent immune status during their second year when compared to non strategically treated animals. However, the necropsy results did not tend to support this observation. In terms of post challenge worm burdens, susceptibility to *Ostertagia*, *Cooperia* and *Dictyocaulus* was noted in all animals at the end of the first housing period and was considered to be a consequence of waning of immunity. During the second grazing season a marked susceptibility to *Dictyocaulus* was observed with the animals of all four groups requiring therapeutic treatment. At the end of the second grazing season all of the cattle had developed a significant immunity to *Cooperia* and *Dictyocaulus* while moderate to substantial numbers of *Ostertagia* EL4 were observed in all remaining animals irrespective of previous treatments.

In a second study, an abattoir survey of the *Ostertagia* worm burdens in adult dairy cattle was undertaken. Significant monthly and seasonal variations in the proportion of adult and L4 *Ostertagia* in the total worm burdens were observed. A significant positive correlation between total *Ostertagia* adult and L4

burdens was also found. In addition there was a clear monthly and seasonal variation in serum pepsinogen concentrations. However, there was no significant correlation between serum pepsinogen levels and worm burdens. When compared with a similar survey carried out in the West of Scotland in 1981 the results reported here indicate an increase in the magnitude of *Ostertagia* worm burdens which may indicate a change in the expected host/parasite relationship with a shift in the potential for disease to the older animal.

Of the traditional parasitological and biochemical techniques used for most of these studies two were evaluated and compared with alternatives. Pepsin digestion was compared with saline incubation in terms of efficiency and convenience for the recovery of *Ostertagia* L4 from bovine abomasa. Both methods were found to be equally efficient in recovering *Ostertagia* L4 although saline baermannisation proved to have advantages over the pepsin digestion technique in terms of convenience, recovering good quality worm specimens and requiring inexpensive materials.

A comparison of the original assay of Edwards *et al.* (1967) with the micro assay of Paynter (1992) for the estimation of serum pepsinogen concentrations showed that, overall, original method was superior in terms of low variation recorded both within day and among days. This was largely due to the lower margin of operator error due to the use of higher quantity of materials in the original method. With high pepsinogen activity samples both methods showed similar variations.

BOVINE PARASITIC GASTRO-ENTERITIS AND BRONCHITIS: A LITERATURE REVIEW

INTRODUCTION

Parasitic gastro-enteritis (PGE) in pastured livestock occurs throughout the world and infection with gastrointestinal nematodes almost invariably results in reduced weight gains, milk yield and carcass quality. Such losses have been associated with marked changes in feed intake and condition, gastrointestinal functions, protein metabolism and body composition (Fox 1993). The pathogenic and economic significance of infection with gastrointestinal nematodes have been well established and are universally accepted. In addition, bovine parasitic bronchitis is a major parasitic disease in many countries in Europe e.g. UK (Jarrett, McIntyre and Urquhart 1954) and The Netherlands (Saatkamp, Eysker and Verhoeff 1994). Calves in their first grazing season are most commonly affected clinically with older cattle more often acting as a reservoir of lungworm infection. The trend towards more intensive systems of animal production and the slow development of host immunity, in the case of gastrointestinal nematode infections, have combined to accentuate the problems of helminth diseases which, therefore, pose a major threat to the livestock industry.

AETIOLOGY

A survey of the literature reveals the role of climate in determining the dominant parasite species responsible for bovine PGE. For example, in areas with a temperate climate, infection with *Ostertagia* and *Cooperia* species is the commonest cause of PGE in cattle (Armour 1967). By contrast, PGE in tropical areas is associated with *Haemonchus* and *Cooperia* (Fabiyi 1986). In some tropical areas of Asia including Burma, India, Indo-China, Indonesia, Malaysia, Philippines and Sri Lanka, *Mecistocirrus* tends to replace *Haemonchus* as the most important abomasal nematode of cattle and buffalo (Fernando 1965 and Fabiyi 1986).

In the United Kingdom, as in most countries in the northern hemisphere and in those areas of the southern hemisphere with winter rainfall, the single most

important cause of PGE in cattle is infection with *Ostertagia ostertagi* (Armour and Ogbourne 1982). However, some other genera of nematodes such as *Trichostrongylus, Cooperia* and *Nematodirus* have also been implicated, either alone or in combination, as a cause of bovine PGE (Bairden 1991 and Coop, Sykes and Angus 1979).

Ostertagia ostertagi was first described and named *Strongylus convolutus* by Ostertag (1890). It was then renamed by Stiles (1892) as *Strongylus ostertagi* with its current appellation being given by Ransome (1907).

Parasitic bronchitis in cattle is caused by infection with *Dictyocaulus viviparus* (Bloch 1782) which is localised in the respiratory tract. This disease is most prevalent in dairy and dairy cross calves in temperate regions.

LIFE CYCLES

Most gastrointestinal parasites and lungworms of ruminants pass through two distinct environments during the course of their life cycles; these are the external, including pasture and soil, and internal namely the gastrointestinal and pulmonary tracts.

LIFE CYCLE OF *O. ostertagi*

Free Living Phase

O. ostertagi can be taken as being representative of most members of the superfamily Trichostrongyloidea in that fertilised eggs, passed in the faeces, develop within the micro climate of the faecal pat through the first and second to the infective third larval stage. This process involves several different stages of development. Initially, depending on suitable temperature, moisture and oxygen tension the eggs hatch into first stage larvae (L1). These larvae undergo further development to become second stage larvae (L2) after the first moult. Similarly third stage larvae (L3) are produced after further development but the second moult is incomplete and the cuticle of the L2 is retained. These infective L3 are thus more resistant to adverse environmental conditions. In contrast to infective

L3 which are non-feeders, L1 and L2 are active and feed on bacteria in faeces. When moist conditions prevail, the infective larvae migrate from the faecal pat onto herbage and this process is known as translation (Michel and Parfitt 1956). Translation is greatly influenced by several environmental factors including temperature, humidity and the action of invertebrates (Armour 1980). Also animal management regimes, for example stocking rate, can have an effect on grass density which in turn affects faecal degradation and thus larval translation (Henriksen, Jorgensen, Nansen, Serjsen, Larsen and Klausen 1976).

Parasitic Phase

The phase of the life cycle which occurs within the host commences following ingestion of the third stage larvae. In the rumen the L3 exsheath and pass to the abomasum. Here they enter the gastric glands and moult to become fourth stage larvae (L4) in approximately four days (Ritchie, Anderson, Armour, Jarrett, Jennings and Urquhart 1966). A further period of growth in the gastric gland is followed by the final moult to the young adult stage which then emerges to lie on the mucosal surface (Armour 1970). Development from L3 to the adult stage seldom takes more than three weeks (Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart 1965a; Ritchie *et al.* 1966 and Murray, Jennings and Armour 1970) but this development can be extended for up to 6 months if larvae undergo hypobiosis at the early L4 stage.

LIFE CYCLE OF *D.viviparus*

Free Living Phase

Lungworm eggs hatch shortly after being laid by adult worms in the bronchi and first stage larvae are excreted in the faeces. As in *Ostertagia*, the L1 develop in faeces to become infective L3. As lungworm larvae are comparatively sluggish, translation of infective larvae to herbage is largely dependent on their close association with the fungus *Pilobolus* and the subsequent discharge of the fungal sporangia (Eysker and De Leeuw 1988) although Michel and Parfitt (1956)

have demonstrated translation by the disintegration of faeces by rain and other mechanical means.

Parasitic Phase

Jarrett, McIntyre and Jennings (1957) described this part of the life cycle of *D. viviparus*. The infective larvae are ingested and subsequently penetrate the small intestinal wall. By the third day of infection they reach the mesenteric lymph nodes and the third moult occurs in the lymphatic system. Fourth stage larvae then reach the lungs via the pulmonary arteries where the final moult takes place. Final development to the adult stage takes place in the lung but the parasite can be arrested in development at the L5 stage. The prepatent period of *D. viviparus* is three to four weeks.

EPIDEMIOLOGY

Control or prevention of bovine parasitism whether by anthelmintic treatment, pasture management or a combination of both requires a thorough understanding of the bionomics of the free living and parasitic stages of the parasites involved.

EPIDEMIOLOGY OF BOVINE PGE

Current knowledge of the epidemiology of gastrointestinal nematodes involved in bovine PGE has been reviewed by Armour and Ogbourne (1982), Armour (1989), Gibbs (1993), Miller (1993), Snider (1993) and Williams, Knox and Loyacano (1993).

The bovine abomasal parasite, *O. ostertagi* can be used to illustrate the epidemiology of PGE in cattle. Very often this species alone is responsible for severe parasitic gastritis in young calves in temperate or subtropical areas with a winter rainfall (Armour 1980). However, *Ostertagia* and species of the small intestinal genus *Cooperia*, are generally found in combination and it has been observed that a combination of *O. ostertagi* and *C. oncophora* produced adverse

effects in the host which exceeded those produced by either genus on its own (Entrocasso, Parkins, Armour, Bairden and McWilliam 1986a and Parkins, Taylor, Holmes, Bairden, Salman and Armour 1990). This contrasts with the observations of Kloosterman, Albers and Brink (1984) who demonstrated a negative interaction between these two genera in sequential infections which they attributed to different host immune responses to these nematodes. In addition, in studies with experimental concurrent infections with both species, under conditions designed to mimic natural conditions, no significant effect of *C. oncophora* on *O.ostertagi* infections was observed (Satrija and Nansen 1993).

Although *O. ostertagi* is predominantly a bovine parasite, its establishment experimentally in goats and, as a consequence of certain pasture management systems, in sheep has been documented (Bisset 1980; Le Jambre 1978 and Williams 1988). However, it is generally considered that the occurrence of *O. ostertagi* in small ruminants is of little epidemiological significance (Pandy 1971).

Distribution of Bovine Ostertagiosis

It is apparent that *O. ostertagi* has been found in almost all of the territory climatically suited to maturation and translation of its free living stages. Its occurrence has been documented in the United Kingdom (Armour 1980), The Netherlands (Kloosterman *et al.* 1984), Denmark (Steffan 1988), Belgium (Vercruysse, Dorney, Berghen and Geeraerts 1986), Norway (Helle and Tharaldsen 1976), France (Cabaret 1982), Switzerland (Eiseneger and Eckert 1975), Poland (Malczewski 1970), Sweden (Torniquist and Tolling 1983), Canada (Smith and Perrault 1972), New Zealand (Brunsdon 1971), Australia (Anderson 1988b), Brazil (Entrocasso 1988), Uruguay (Entrocasso 1988), Argentina (Eddi, Bianchin, Hones, Muniz, Caracostantogo and Nascimento 1993) and North America (Miller 1993 and Zimmerman, Worley and Rickard 1993).

Seasonal Pattern of Pasture Contamination

As described previously, the transmission of infection depends on the successful development of eggs into infective larvae and their successful translation from faeces to pasture. The primary factors affecting the development and survival of eggs and larvae are temperature and moisture and infective larvae and embryonated eggs are the least susceptible to adverse environmental conditions.

In temperate climates the development of eggs to infective larvae is dependent on an average temperature of over 10°C. Development is slow during spring, reaches a maximum by mid summer and then declines as the temperature drops during autumn (Smith and Archibald 1969 and Rose 1970). Eggs laid in the late autumn are unlikely to develop to infective larvae until the following spring.

Ostertagia infective larvae are fairly resistant to cold, therefore infective larvae arising from eggs deposited in the previous grazing season may survive over winter on pasture until the following spring (Gibbs 1980). Constant snow cover, by providing insulation, possibly aids in the overwintering of infective larvae on pasture (Zimmerman *et al.* 1993) as can migration into soil (Bairden, Parkins and Armour 1979 and Zimmerman *et al.* 1993). In this respect *Nematodirus helvetianus* has been shown to be capable of surviving longer on herbage than any other trichostrongyle as it is more resistant to cold (Steffan 1988). This agrees with field observations carried out in Canada by Smith and Archibald (1969) who observed that *N. helvetianus* survived over two winter periods , successfully overcoming unfavourable climatic conditions. In contrast, *Ostertagia* and *Cooperia* rarely survive longer than one year (Smith and Archibald 1969; Smith 1972 and Al Saqur, Bairden, Armour and Gettinby 1982a). In the Netherlands, *Cooperia* seems to be more successful in overwintering than *Ostertagia* (Steffan 1988). Recent observations have indicated that larvae may survive in the soil for an extended period and emerge onto the pasture from July onwards (Bairden *et al.* 1979 and Alsaqur *et al.* 1982a). In Australia, particularly in winter rainfall environments, the survival of infective larvae of *O. ostertagi* and

C. oncophora in desiccated dung pats, even after a period of 15 months drought, has been reported (Barger, Lewis and Brown 1984).

The estimation of herbage larval populations provides information essential for an understanding of parasite bionomics and epidemiology of helminth diseases. Assessments are generally carried out either by direct quantification of pasture larval levels or by using parasite naive tracer calves. Rose (1970) observed that by late April in the UK, herbage contamination was at a low level and that the role of the surviving infective larvae was to infect calves at a low level to ensure contamination of the pasture for the rest of the grazing season. In Europe overwintered larvae ingested by susceptible first season grazing calves in the spring become patent within about three weeks. However, infective larvae arising from this infection are not usually recovered from herbage until late July; this is a consequence of the relatively slow development of eggs to infective stages in the early spring. When the adult *Ostertagia* burden resulting from the ingestion of larvae during the grazing season exceeds a threshold, clinically apparent Type I disease can occur. This is usually seen in first season grazing calves during late summer (Anderson, Armour, Jennings, Ritchie and Urquhart 1965b; Entrocasso *et al.* 1986a and Vercruysse, Dorny, Berghen and Frankena 1987) with the youngest calves being most severely affected (Armour 1970). The occurrence of Type I disease in early July has also been documented in the UK (Taylor, Cawthorne and Kenny 1973; Bairden *et al.* 1979 and Jacobs *et al.* 1987d), Denmark (Nansen, Gronvold, Jorgensen, Henriksen, Foldager and Sejrsen 1989) and Belgium (Berghen, Dorny, Hilderson, Vercruysse and Hollanders 1990). These atypical outbreaks have been related to an alteration in epidemiological pattern perhaps associated with the emergence of overwintered *Ostertagia* L3 from the soil or the prevalence of climatic conditions such as a dry summer followed by a severe winter. Such conditions may retard the disintegration of dung pats and thus aid the successful overwintering of the L3.

Gibbs (1993) reviewed the available information on ostertagiosis in temperate regions of north America and concluded that in north-eastern USA

clinical disease in calves was usually of a sporadic nature and that subclinical infection was more usual.

Arrested Larval Development

As autumn progresses and the temperature falls, an increasing percentage of the ingested L3 do not mature but become inhibited at the early fourth stage (EL4) of development. At this stage large populations of *Ostertagia*, more than 100,000 parasites in many cases, have been recorded of which up to 70% (Armour, Jennings and Urquhart 1969), over 80% (Anderson *et al.* 1965b) or 94% (Gibbs 1988) have been reported to be arrested in development.

Inhibited larval development, or hypobiosis, is a mechanism of cessation of growth at an early stage of parasitic development. These non-pathogenic arrested larvae have a low metabolic rate enabling them to survive for a long period (Eysker 1993) and this may have evolved to avoid maturation and subsequent egg deposition into an unfavourable environment. This phenomenon has also been observed in other host-parasite relationships such as cattle/*Cooperia* spp. (Anderson *et al.* 1965b), cattle/*D. viviparus* (Gupta and Gibbs 1975), sheep/*H. contortus* (Blitz and Gibbs 1972) and ponies/*Cyathostomum* spp (Eysker, Boersma and Kooyman 1990).

Two distinct seasonal patterns of hypobiosis in *O. ostertagi* have been documented. Firstly, in the colder temperate regions of northern Europe (Armour, Duncan and Reid 1978 and Eysker 1993), Canada (Smith 1973), New Zealand (Brunsdon 1972 and Bissett 1983) and northern USA (Gibbs 1993; Rickard and Zimmerman 1992 and Miller 1993), *O. ostertagi* larvae show inhibited development during the autumn/winter with maturation in the following spring. In contrast in the warmer temperate and subtropical areas of southern USA (Snider 1993), North America (Entrocasso 1988) and Australia (Anderson 1988a), *O. ostertagi* inhibits during dry or hot summer conditions. Inhibited development is a complex phenomenon which is still not completely understood (Eysker 1993) although the stimuli that initiate hypobiosis have been studied extensively.

Armour (1970) observed a greater potential for inhibition in a field strain of *Ostertagia* compared with a laboratory strain. Similarly, in the Netherlands, two strains with different propensities for inhibition, have been observed (Borgsteede and Eysker 1987).

It may be that inhibited larval development is an adaptation by the parasite to its environment and is triggered by unfavourable climatic changes e.g. extreme cold (Armour 1970; Anderson *et al.* 1965b and Soulsby 1985). Blitz and Gibbs (1972) and McKenna (1973) confirmed the importance of cold as a trigger for hypobiosis. Their observations were based mainly on the occurrence of inhibited *Ostertagia* larvae in set-stocked as well as tracer calves in late autumn. Observations by Christensen, Nansen, Henriksen, Monrad and Satrija (1992) using a field strain of *O. ostertagi* also indicated that cold storage could increase the propensity for hypobiosis. In contrast, Bissett (1983) observed no significant levels of cold induced inhibition of *O. ostertagi* in grazing calves in New Zealand.

A third possible explanation for induction of hypobiosis is the involvement of acquired immunity (Michel 1963; Brunsdon 1972; Snider, William, Sheehan and Fuselier 1981 and Gronvold, Nansen, Gasbarre, Christensen, Larsen, Monrad and Midtigard 1992). The role of acquired immunity in initiating arrested development has been supported by a comparatively higher establishment of arrested larvae in calves previously exposed to infection than in parasite naive calves and also by consistently low levels of hypobiosis in tracer calves compared with permanently grazed calves. However, treatment of calves known to be harbouring substantial numbers of inhibited *Ostertagia* larvae with large doses of immunosuppressive corticosteroids did not result in the resumption of development of significant numbers of these hypobiotic larvae (Armour 1967) suggesting that host immunity does not play a vital role in inducing hypobiosis. However, as there is some evidence to suggest that both climatic conditions and host immunity play a role in hypobiosis the occurrence in one host of two separate inhibited populations, one seasonal and one immunological, has been suggested (Christensen *et al.* 1992 and Eysker 1993).

Whatever the initiating factors, it is known that arrested larvae can either recommence their development in large numbers synchronously, usually in spring (Armour 1970), giving rise to the Type II disease or gradually in small numbers over a period of time without any clinical consequences (Michel 1976).

EPIDEMIOLOGY OF BOVINE PARASITIC BRONCHITIS

The epidemiology of *D. viviparus* has been studied in Denmark (Jorgensen 1980 and Jorgensen, Ronne, Helsted and Iskander 1982), in the Netherlands (Eysker and de Leeuw 1987 and Eysker and van Miltenburg 1988) and in the UK (Michel and Parfitt 1956; Duncan, Armour, Bairden, Urquhart and Jorgensen 1979 and Oakley 1982) and the following facts have been established.

First, Dictyocaulosis is mainly a problem in dairy calves in August-September in temperate and moist regions of central and western Europe and is caused by second or third generation infective larvae.

Secondly, there are several differences in epidemiology between gastrointestinal nematodes and lungworm. The major difference is that unlike *Ostertagia*, for example, there is no clear and predictable seasonal pattern in the occurrence of *D. viviparus* infection. Another major difference is that clinical manifestation of disease is uncommon in yearlings and older cattle although these can act as carriers or reservoirs of infection.

It is known that outbreaks of parasitic bronchitis may arise from pasture contamination by carrier animals but they can also be a consequence of survival of overwintered infective larvae in pasture and soil.

Thirdly, a higher establishment of inhibited larvae has been observed both early and late in the grazing season and has been found to be negligible in mid season.

Finally, although *D. viviparus* larvae are very sluggish, rapid translation from the faeces has been observed which has been attributed largely to the role of the fungus *Pilobolus*.

PATHOGENESIS

PATHOGENESIS OF *O. ostertagi* INFECTION

Following ingestion, the infective larvae exsheath in the rumen and move to the abomasum where they enter the gastric glands; this takes four days. Here they moult to L4, grow and cause a mild tissue reaction. As the larvae develop the mucosal glands become distorted forming primary nodules on the abomasal surface. In this first phase, which persists for up to 17 days after infection, changes due to the parasite are confined mainly to the parasitised glands (Armour 1970).

Major cellular changes, as well as functional disturbances, are obvious in the second phase which takes place from 17 to 35 days post infection. These are associated with the mechanical damage due to the emergence of young adult parasites from the glands and the occurrence of pronounced inflammatory changes both in the parasitised and neighbouring glands. A marked loss of cellular integrity occurs, particularly in the parietal and zymogen cells; hyperplasia and loss of cellular differentiation are common and as a consequence the junctions between the cells are stretched. In severe infections, the lesions may coalesce to give a gross appearance some times described as 'crazy paving' or 'morocco leather' (Anderson *et al.* 1965a). Additionally, oedema, hyperaemia and enlarged regional lymph nodes are often present with some necrosis and sloughing of the abomasal mucosal surface.

The third phase, from day 35 onwards, is associated with a gradual loss of adult parasites after which normal morphology and function of the affected mucosa is restored. This occurs usually by 60 days post infection (Ritchie *et al.* 1966). Evidence of an immune response such as an intense lymphocyte accumulation has also been associated with this phase (Snider *et al.* 1981). It has been suggested that some of the pathological changes observed in ostertagiosis may be due to an IgE mediated Type I hypersensitivity response (Klesius 1988) as infection is associated with local increases in the numbers of mast cells and eosinophils (Wiggin and Gibbs 1987, 1990).

During the course of pre-Type II ostertagiosis there is little host reaction to the presence of arrested early fourth stage in the glands with no gross pathological lesions being obvious (Entrocasso, Parkins, Armour, Bairden and McWilliam 1986b). However, when maturation of large numbers of these hypobiotic larvae takes place synchronously clinical Type II ostertagiosis can occur. Lesions associated with this form of the disease are similar to those seen in Type I ostertagiosis.

PATHOGENESIS OF *D. viviparus* INFECTION

The pathogenesis of lungworm infection has been studied by Jarrett *et al.* (1957) using experimental infections and comprises the following phases,

(i) Penetration of the bowel and migration to mesenteric lymph nodes, considered to be the site of the primary immune stimulus, and subsequently to the lungs. This phase lasts approximately one week.

(ii) In the subsequent alveolar bronchial phase, lesions due to migrating larvae can be demonstrated in the lungs and pathological effects are caused by the pulmonary reaction to the parasites. The affected animals may show an occasional cough progressing to a decreased appetite and growth rate towards the end of this phase which normally lasts for three weeks.

(iii) In the patent phase, clinical signs are apparent and first stage larvae from eggs produced by mature worms can be demonstrated in the faeces. This phase extends from four to eight weeks.

(iv) In the late patent phase and post-patent recovery phase the numbers of adult worms in the bronchi gradually diminish although affected animals may continue to show some clinical signs.

PATHOPHYSIOLOGY

PATHOPHYSIOLOGY OF *O. ostertagi* INFECTION

The pathophysiology and clinical signs associated with bovine ostertagiosis are significant and have been well documented (Armour 1980 and

Holmes, Bairden, Ibarra Silva, Salman and McWilliam 1989) compared with *C. oncophora* infection in cattle which is considered to be less harmful (Armour, Bairden, Parkins, Ploeger, Salman and McWilliam 1987a). Detailed studies of the sequential development of the lesions and physiological changes associated with Type I ostertagiosis have been carried out (Al Saqur, Armour, Bairden, Dunn, Jennings and Murray 1982b; Entrocasso *et al.* 1986a,1986b; Snider, Williams, Knox, Marbury, Crowder and Willis 1988; Parkins *et al.* 1990 and Xiao, Gibbs and Yang 1991) and have been extensively reviewed (Armour and Ogbourne 1982; Holmes 1986; van Veen 1988 and Fox 1993). The most important physiological effects were associated with feed intake, gastrointestinal disturbances such as diarrhoea, protein, energy and mineral metabolism and body composition which, in general, had an adverse effect on the host.

Abomasal Changes

An increase in pH of the abomasal contents is a consistent feature of ostertagiosis (Xiao *et al.* 1991). This has been explained by the fact that a substantial number of acid secreting parietal cells are destroyed when the young worms emerge from the gastric glands (Murray *et al.* 1970). Defects in intercellular junctions also allow plasma proteins to pass freely into the abomasal lumen leading to hypoproteinaemia which may contribute to an increase in abomasal pH. The impairment of abomasal digestion (Entrocasso *et al.* 1986a) and the absorption of nutrients (Fox 1993) can be considerable. Failure to activate pepsinogen to pepsin and its effect on protein digestion together with an altered nitrogen metabolism may lead to increased losses of urinary and faecal nitrogen (Parkins, Bairden and Armour 1982; Entrocasso *et al.* 1986a). Diarrhoea, one of the main clinical manifestations of ostertagiosis, coincides with the increase in abomasal pH and it has been suggested that this rise in pH encourages bacterial growth (Jennings, Armour, Lawson and Roberts 1966). Initially it was assumed that the accumulation of nonactivated pepsinogen in the abomasum and its passage through defective intercellular junctions was the cause of elevated blood

pepsinogen concentrations (Jennings *et al.* 1966 and Murray *et al.* 1970). However this theory fails to explain why plasma pepsinogen concentrations remain elevated long after worm burdens have declined and abomasal damage healed (McKellar, Duncan and McWilliam 1986b). It has also been observed that plasma pepsinogen concentrations may remain high for considerable periods of time after resolution of any clinical signs of infection (Snider *et al.* 1981) or in older animals with few worms (Mylrea and Hotson 1969). Interestingly studies by Stringfellow and Maddsen (1979) suggested that the mucosal permeability of infected and non-infected calves is similar which is in contrast to the *Ostertagia* induced junctional complex leak theory. Also McKellar, Duncan, Armour and Lindsay (1986a) and McKellar *et al.* (1986b) detected elevated plasma pepsinogen concentrations within 24 hours of direct transfer of adult *Ostertagia* to parasite naive calves which implies a role for the adult worm in eliciting increased pepsinogen concentrations. It has also been demonstrated recently that excretory/secretory products of mature *Ostertagia* may increase mucosal permeability to macromolecules and that these secretory products may also stimulate zymogen cell activity resulting in an increase in pepsinogen production (McKellar, Mostofa and Eckersall 1990). Raised pepsinogen concentrations in blood may also be caused by an immune or hypersensitivity reaction in the abomasal wall following either rejection of recently ingested *Ostertagia* larvae or the continuous presence of adult worms (Chalmers 1983; Entrocasso *et al.* 1986a and Berghen *et al.* 1990).

More recently, changes in bovine plasma gastrin concentrations following infection with *O. ostertagi* have been reported (Entrocasso *et al.* 1986a and Fox, Girrelli, Pitt, Jacobs, Hart and Simmonds 1987). The marked rise in abomasal pH appears to be the main stimulus for the hypergastrinaemia (Fox *et al.* 1987), however, McKellar *et al.* (1986a) indicated that the presence of parasites or their secretions may also exert a direct stimulatory effect on gastrin secretion in the absence of any change in pH.

Changes in Voluntary Feed Intake

An important feature closely associated with bovine ostertagiosis is a marked change in voluntary feed intake (Entrocasso *et al.* 1986a and Fox, Gerrelli, Pitt, Jacobs, Gill and Simonds 1989a). Two comprehensive reviews (Holmes 1985 and Fox 1993) describe several factors which may be important in bringing about the reduced feed intake associated with ostertagiosis. One is related to hypergastrinaemia and a subsequent delay in abomasal emptying which, in turn, leads to stasis of ingesta and ultimately inappetance. Other possibilities are that the increase in abomasal pH and resultant changes in protein digestion, intestinal motility and associated pain may also lead to loss of appetite although none of these mechanisms is completely understood.

Changes in Bodyweight and Composition

Gastrointestinal nematode infection can have a significant effect on weight gain, especially in young grazing cattle. A live weight advantage of up to 80 Kg/calf/grazing season has been documented in calves prophylactically treated with ivermectin compared with untreated control animals (Suarez, Bedotti, Larrea and Garriz 1991). Similarly, Henriksen *et al.* (1976) observed gains on average of 40-50 Kg/animal/grazing season in calves treated with levamisole and moved to clean pasture compared with similar set-stocked, untreated calves. This lack of weight gain in untreated animals may have been partly due to exposure to a high larval challenge (Ploeger and Kloosterman 1993) and partly to lowered appetite (Fox, Gerrelli, Pitt and Jacobs 1989b).

Changes in body composition especially in fat, protein, minerals and water which may accompany *Ostertagia* infection have also been documented (Entrocasso *et al.* 1986b; Suarez *et al.* 1991 and Maclean, Bairden, Holmes, Mulligan and McWilliam 1992). Such changes directly affect carcass quality and result in poorer killing out percentages and related carcass measurements in control animals compared with those which had been prophylactically treated.

While in sheep there is evidence to suggest that gastrointestinal nematodes alter the mineral balance of the host, there is very little evidence for an impaired mineral metabolism associated with abomasal parasitism in cattle (Fox 1993).

Relatively few studies have been conducted into the pathophysiological significance of *C. oncophora* and *N. helvetianus* infections. Experimental infection of calves with single or trickle inoculations of *C. oncophora* L3, have been reported by Coop *et al.* (1979), Henriksen (1981), Kloosterman *et al.* (1984), Armour *et al.* (1987a); Parkins *et al.* (1990) and Satrija and Nansen (1992). In these studies the predilection site of *C. oncophora* was found to be the duodenum and the pathological changes comprised fusion and distortion of intestinal villi in the area surrounding the parasite (Parkins *et al.* 1990).

Experimental infections with *C. oncophora,* given at a level frequently encountered under natural conditions in the West of Scotland, have shown that such levels of infection could produce production losses in young parasite naive calves (Armour *et al.* 1987a). However, the fact that pathogenic infections with intestinal nematodes in cattle are rarely encountered in the field is probably due to the rapid acquisition of host immunity to these genera (Coop *et al.* 1979 and Armour *et al.* 1987a).

Although *N. helvetianus* has been isolated in outbreaks of bovine PGE, its pathogenic effect is debatable. *Nematodirus battus*, on the other hand can cause clinical disease in calves (Bairden and Armour 1987 and Armour, Bairden, Dalgleish, Ibarra-Silva and Salman 1988) and Bairden (1991) has reported clinical nematodirosis due to *N. battus* in set-stocked calves following a period of alternate grazing of calves and sheep.

PATHOPHYSIOLOGY OF *D. viviparus* INFECTION

The clinical consequences of *D. viviparus* infection have been well described and the pathophysiology of the disease is directly related to changes in the lungs and bronchi, which result in a parasitic pneumonia and bronchitis

(Jarrett *et al.* 1954 and 1957). Not surprisingly, poor weight gains and reduced feed intake have been observed.

IMMUNITY

Host-parasite relationships are biologically evolved systems in which both the host and the parasite are able to maintain a continuity of their relationship in a viable ecological state. Resistance or immunity to parasite infection can be evolved by the host to reduce a parasite burden and its impact upon health and productivity.

IMMUNITY TO BOVINE GASTROINTESTINAL NEMATODES

It is accepted that host immunity develops to all gastrointestinal nematodes of cattle although the rate of development and the level of immunity varies according to the species of parasite and the period of exposure to infection (Klesius 1988 and Armour 1989). The development of immunity to *Ostertagia* species is slow and very high worm burdens can be present at the end of a first grazing season (Xiao *et al.* 1991; Christensen *et al.* 1992 and Hilderson, Graff, Vercryusse and Berghen 1993). However, in Scotland, Entrocasso *et al.* (1986a) demonstrated that cattle at the end of their second grazing season had acquired immunity to infection with *O. ostertagi.* Similar results have been obtained in other studies (Armour, Bairden, Duncan, Jennings and Parkins 1979; Coop *et al.* 1979 and Vercruysse *et al.* 1986) and although adult cattle can occasionally harbour high abomasal worm burdens (Bairden and Armour 1981) it is generally accepted that older animals are immune.

The inability of young calves to show a rapid acquisition of immunity against *O. ostertagi* appears to be due in part to a modulation of the immune response of the host by the parasite (Klesius 1988) which presumably facilitates its survival (Klesius 1993). Considerable evidence now exists for an *Ostertagia* induced immune suppression in cattle (Kleus, Kloosterman and van Den Brink 1981; Cross, Kleus and Haynes 1986 and Wiggin and Gibbs 1990), yet the

underlying mechanism has still to be fully elucidated. There is also evidence to show that *Ostertagia* species possess excretory-secretory molecules that can communicate with host immune and accessory cells to down-regulate the response of the host (Klesius 1993). It has also been suggested that such an immunosuppressive mechanism does not operate in older animals so that their immune response can be fully expressed without interference (Kloosterman, Ploeger and Frankena 1991; Christensen *et al.* 1992 and Gronvold *et al.* 1992). It has been found that the establishment of *D. viviparus* was higher in calves infected with *O. ostertagi* and *C. oncophora* than in uninfected calves indicating that the presence of these gastrointestinal nematodes had caused a non-specific immunosuppression and a consequent increased establishment of lungworm (Kloosterman, Ploeger and Frankena 1990).

Although infection with *O. ostertagi* causes both humoral and cellular immune responses (Klesius 1988), the importance of antibodies in protective immune responses against *Ostertagia* remains uncertain (Christensen *et al.* 1992).

The situation with intestinal parasitism in cattle is different; *Cooperia* species and *Nematodirus* species have been found to provoke a significant immunity after relatively short periods of exposure (Coop *et al.* 1979; Steffan 1988; Armour 1989 and Kloosterman *et al.* 1991) and it is uncommon to find even low levels of infection with such intestinal nematodes in adult cattle (Gasbarre, Leighton and Davies 1993).

IMMUNITY TO *D. viviparus*

A strong immunity to *D. viviparus* infections is rapidly acquired under field conditions (Armour 1989 and Jarrett *et al.* 1957). Disease can develop due to a single large primary infection or due to repeated infections with second or third generation lungworm larvae. If, however, exposure to significant single or repeated lungworm larval infections does not cause the disease then it will most probably never occur because immunity will have developed. The development of immunity to *D. viviparus* is directly influenced by the level of infection but it has

been shown that this acquired immunity can decline if the host is not exposed to further natural challenge (Taylor *et al.* 1988).

DIAGNOSIS

DIAGNOSIS OF PGE

A presumptive diagnosis can usually be made based on clinical signs, herd history, geographical location and appropriate climatic and seasonal conditions. Traditionally one of the simplest confirmatory diagnostic techniques was the detection of parasite eggs in faeces although this does not always indicate the level of infection. However, if required, faecal egg counts and faecal culture for subsequent larval identification can be a useful aid to the diagnosis of Type I ostertagiosis where egg output may exceed 1,000 eggs per gram of faeces in severe cases. Faecal egg counts are of limited value , however, in the diagnosis of Type II disease as they are variable and seldom show any relationship to total parasite burdens. Faecal egg counts alone can be unreliable in indicating the level of parasitic infection or the presence of disease (Brunsdon 1971) since considerable variations occur depending, for example, on faecal consistency, host immune status and the species of parasite involved (Klesius 1988; Armour 1989 and Gronvold *et al.* 1992). Despite these limitations, Baker (1988) suggested that faecal egg counts applied on a herd basis rather than on individual animals, could produce useful information.

Biochemical analyses have also been used as an aid to the diagnosis of parasitism. For example, changes in pepsinogen concentrations have been widely used as an indicator of abomasal damage due to *O. ostertagi* infection (Snider *et al.* 1981; Entrocasso *et al.* 1986b; Berghen, Vercruysse, Dorny and Hilderson 1987 and Vercruysse *et al.* 1987). Van Veen (1988) and Berghen, Hilderson, Vercruysse and Dorny (1993) reviewed the value of serum or plasma pepsinogen estimation in the diagnosis of bovine ostertagiosis. Pepsinogen concentrations in parasite naive calves are generally below 1 iU tyrosine. With clinical ostertagiosis plasma pepsinogen concentrations may rise to above 3 iU tyrosine which, if it

occurs on a herd basis, is considered significant for a diagnosis of Type I disease in first season grazing calves. The value of pepsinogen assays as an indicator of parasitism in other classes of animals, however, has been debated (Chiejina 1978; Selman, Armour, Jennings and Reid 1977). It is less reliable in individuals (van Veen 1988) but together with clinical signs and faecal egg counts on a herd basis it can be useful (Baker 1988; van Veen 1988 and Hilderson, Berghen, Vercryusse, Dorny and Bream 1989).

Serological diagnosis can also be based on serum gastrin measurement as an indicator of size of the abomasal adult worm burden. The use of the gastrin assay to detect parasitism has been reviewed by van Veen (1988) and Berghen *et al.* (1993). Plasma gastrin concentrations in parasite naive calves vary between 100 to 106 pg per ml whereas during clinical outbreaks of Type I disease, group mean gastrin values frequently rise above 1,000 pg per ml (Entrocasso *et al.* 1986a and Yang, Gibbs, Xiao and Wallace 1993). It is of interest that Entrocasso *et al.* (1986a) also observed an increase in serum gastrin concentrations above 700 pg per ml in animals suffering from Type II ostertagiosis.

Finally, the use of necropsy procedures to detect characteristic pathological changes and establish worm burdens (Eysker and Kooyman 1993) is probably the most useful aid to diagnosing the different forms of ostertagiosis.

DIAGNOSIS OF PARASITIC BRONCHITIS

Diagnosis is generally based on clinical signs of coughing in groups of cattle at grass during summer and autumn.

A simple method of confirming lungworm infection is by detecting the L1 in faeces using the Baermann technique (Eysker, Boersema and Kooyman 1990a). It is important to remember that a low number of larvae or negative larval counts do not exclude the possibility of lungworms being present. The limitation of faecal examination for larvae is that it will not detect prepatent lungworm infections. Current serological methods such as the ELISA (Boss and Beekman 1985) and the complement fixation test (Jarrett *et al.* 1957) may be of use in large

sero-epidemiological surveys but are of limited diagnostic value unless they are negative in which case they would indicate negligible recent infections.

At post mortem examination the presence of worms in the bronchi or typical lesions of parasitic pneumonia will confirm the diagnosis of lungworm infection.

CONTROL

The control of parasitic infection in cattle has many beneficial effects related to productivity. These include improvements in weight gain, feed conversion, milk production, reproductive performance, carcass quality, immune status and reduced morbidity and mortality (Hawkins 1993).

CONTROL OF PGE

The options available to limit the prevalence of PGE have been extensively reviewed (Armour 1980; Brunsdon 1980; Anderson 1988b; Herd 1988; Prichard 1988; Williams and Knox 1988 and Nansen 1993). When gastrointestinal nematode control programmes are based on a sound knowledge of epidemiology, production benefits are maximum and the risk of developing anthelmintic resistance is minimal (Prichard and Ranjan 1993).

The aim of any control measure is to ensure that the parasite population does not exceed levels compatible with productivity of the host. It is unnecessary, and perhaps undesirable, to reduce infection to the lowest possible level as this may compromise the development of host immunity (Nansen 1993). This objective may be achieved by chemotherapy, grazing management or a combination of both.

Recently there has been increasing interest in biological control using nematode trapping fungi.

Chemotherapy and Prophylaxis

Currently, the prevention and control of clinical and sub clinical ostertagiosis in cattle relies mainly on the use of anthelmintics (Prichard and Ranjan 1993). The traditional and simple way to use anthelmintics was to cure existing infection or disease. This approach was not based on any epidemiological principles (Herd 1988).

Chemotherapy is still the only option when parasitic disease occurs in cattle but anthelmintics are now more commonly used in prophylactic regimes to reduce pasture contamination and prevent disease (Anderson 1988b).

The choice of drug for use in the control of bovine PGE is wide. The most recently introduced classes of anthelmintics are avermectins such as ivermectin, doramectin and abamectin and milbemycins such as moxidectin; modern benzimidazoles such as albendazole, fenbendazole and oxfendazole are also widely used in cattle. All of these compounds are active against both developing and arrested stages of *O. ostertagi* (Prichard 1988 and Prichard and Ranjan 1993) whereas levamisole, morantel, some older benzimidazoles and probenzimidazoles such as febental are only effective against adult and developing stages.

The first of the avermectins to be developed was ivermectin and its efficacy has been studied extensively in cattle (Armour, Bairden, Pirie and Ryan 1987b and Jacobs, Fox and Ryan 1987c). Despite a broad spectrum of activity, its efficacy against small intestinal nematodes such as *Cooperia* and *Nematodirus* is not absolute and their elimination often requires the use of higher dose rates (Bogan and McKellar 1988 and Steffan 1988). This failure to totally remove some of the sheep intestinal nematodes at standard dose rates has also been documented with regard to the milbemycin, moxidectin (Bairden, Duncan and Mudd 1993). This may be attributed to a combination of species susceptibility and drug concentration at the site of action (Lanusse and Prichard 1993). However, for the control of bovine ostertagiosis, three doses of ivermectin at 3, 8 and 13 weeks post turnout to set-stocked calves has been recommended (Armour *et al.* 1987b and Jacobs *et al.* 1987c). This schedule results in negligible pasture contamination

with parasite eggs thereby lowering pasture infectivity for the remainder of the grazing season. This regime is based on the persistent activity of ivermectin, each treatment preventing reinfection of calves with *Ostertagia* for up to 14 days (Barth 1983). Several other compounds show similar properties of high efficacy and persistency e.g. moxidectin (Ranjan, Trudeau, Prichard, Kulzleben and Carrier 1992; Taylor, Kenny and Edgar 1993 and Wang and Smith 1993), abamectin (Cobenas, Eddi, Caracostantogolo, Nolzco, Gross, Guerrero and Muscotena 1993) and doramectin (Weatherley, Hong, Harris, Smith and Hammet 1993).

Recent advances in continuous and controlled release technology have influenced the presentation of anthelmintics which effectively minimises labour input. There are now pulse or sustained release devices for cattle which incorporate oxfendazole, ivermectin, albendazole, fenbendazole or morantel. The efficacy and pharmacokinetics of these devices or boluses have been studied extensively and it has been found that they are efficacious in controlling gastrointestinal and, in some cases, lung worms (Armour, Bairden, Duncan and Jones 1981; Bairden, Armour and Reid 1983; Egerton, Suhayda and Eary 1986 and Jacobs, Pilkington, Foster, Fox and Oakly 1987).

Increasingly there is concern that the use of highly effective anthelmintic prophylactic regimes will affect the acquisition of host immunity to both gastrointestinal nematodes and lungworm in cattle and that this may leave second grazing season animals or even adult cattle more vulnerable to infection (Armour 1989). Reduced host immunity to gastrointestinal nematodes and lungworm has been evident after the use of either the morantel sustained release bolus (Bairden 1991) and the oxfendazole pulse release bolus (Jacobs *et al.* 1987c).

Nansen (1993) suggested that such intensive control systems may translocate the parasite problem from young to older animals with the latter being economically more important. Other potential disadvantages of controlled release technology include unacceptable tissue residues (Anderson 1985), the development of anthelmintic resistance (Herd 1984; Anderson 1985 and Donald 1985) and ecotoxicity (Waller 1993a). It is accepted that the more effective the

anthelmintic treatment, the greater the potential for development of resistance (Prichard, Hall, Kelly, Martin and Donald 1980) but to date anthelmintic resistance in cattle has been shown to occur infrequently (Eagleson and Bowie 1986 and Anderson and Lord 1979).

Prophylactic treatment regimes are basically designed to minimise the effect of overwintered larvae which are ingested during the early part of the grazing season and initiate the infection/contamination cycle. The beneficial effect of such early season regimes on growth performance has been well documented (Jones 1981; Herd 1988; Prichard 1988 and Ploeger and Kloosterman 1993).

Control by Grazing Management

Grazing management systems for the control of bovine PGE have been widely advocated. This may be achieved simply by grazing calves on new grass leys or on pastures where there is little immediate risk of gastrointestinal nematode infection; for example hay or silage aftermaths. This method of control however may not allow exposure of calves to parasite challenge and acquisition of immunity resulting in a pool of susceptible animals (Whitelaw and Fawcett 1982). In the Netherlands, some success has been obtained by delaying the turnout of calves until mid summer when overwintered pasture larval numbers have fallen to extremely low levels (Henriksen *et al.* 1976).

Another management system, alternate grazing, is the term used to describe the practice of sequentially stocking a pasture with different host types or species. The advantages of this system can be exploited by one of several combinations; for example by the use of different age groups of cattle or by alternating different host species such as cattle and sheep.

The leader/follower or dilution system is a rotational grazing method where calves are only allowed to graze the leafy upper parts of herbage before being moved to the next paddock, thus avoiding the infective larvae which tend to be concentrated lower down the herbage and which will then be ingested by the following older immune cattle (Leaver 1970; Nagle, Brophy, Cafrey and

O'Nuallain 1980). One consequence of such a system with different age classes of cattle may be the exposure of older animals to a higher level of infective larvae than is usual and this may lead to an accumulation of arrested larvae resulting in Type II ostertagiosis (Morley and Donald 1980).

Alternating cattle with another host species, such as sheep, followed by an annual crop ideally utilises grazing land over a three year period and has proven beneficial for both cattle and the alternate host (Morley and Donald 1980). This method is based on the assumption that the life span of most *Ostertagia* species infective larvae is under one year and that cross transmission between different host species is of little consequence (Morley and Donald 1980). Bairden (1991) however, demonstrated that the effect of annual alternation of cattle and sheep over a four year period did not prevent the acquisition of substantial burdens of bovine gastrointestinal nematodes by the cattle grazed on the alternated pasture in the third and fourth years. Also cross transmission of *N. battus* from sheep to cattle (Morley and Donald 1980 and Bairden 1991) make this method less successful especially when *N. battus* establishes in calves and develops to a patent infection (Bairden and Armour 1987; Armour *et al.* 1988 and Coop, Jackson and Jackson 1991). Cross transmission and establishment of *O. leptospicularis*, which is normally found in sheep and deer, has also been reported as a potential problem (Al Saqur, Armour, Bairden, Dunn and Jennings 1980 and Bissett 1980).

Another animal management system, mixed grazing, where calves are grazed together with nonsusceptible animals such as older immune cattle or sheep is also based on the assumption that cross transmission of infection is minimal between the two host groups. When two age categories of cattle are grazed together, a low herbage infectivity can be expected since immune adults produce few eggs in their faeces. Nansen, Steffan, Manrad, Gronvold and Henriksen (1990) showed that first season grazing calves benefit from mixed grazing with older immune animals by acquiring fewer parasites. Furthermore, in the same study, it was observed that no adverse consequences occurred in the older age group of animals. Unlike the situation in cattle, adult sheep can significantly

contaminate a pasture so the same philosophy cannot be applied in sheep nematode control. Control by mixed grazing of sheep and cattle all year round on permanent pasture, has been evaluated in Australia and although effective, it was not popular among farmers (Edwards, Wroth, de Chaneet, Besier, Karlsson, Morcombe, Dalton-Morgan and Roberts 1986).

Bransby (1993) in a comparison of two systems of grazing management, found no advantage of rotational grazing over the set-stocked situation. It has been considered that the high stocking densities, associated with rotational grazing, encouraged animals to graze closer to the ground and nearer to dung pats and also to spread dung more with their hooves than under a set-stocked system and thus any advantage of the rotational system was lost. Further, it has been suggested that the survival of free living stages of parasites and the rate of reinfection could be enhanced using a rotational grazing system compared with set-stocked conditions. This was explained by the enhanced growth of pasture during the resting period in rotational grazing which provided favourable conditions for parasite development.

Control by a Combination of Chemotherapy and Grazing Management

The Dose and Move system devised by Michel (1966), is still widely used in the northern hemisphere. This involves calves being moved to clean-pasture e.g. hay or silage aftermath, in mid summer, combined with an anthelmintic treatment at the time of moving to prevent contamination of the new pasture. The method is based on the knowledge that the annual increase of infective larvae on set-stocked areas occurs after mid July and that the numbers of overwintered larvae are rarely high enough to cause early season disease, although unexpected deviations from the normal herbage infectivity pattern may occur (Bairden *et al.* 1979 and Bairden 1980). Some disadvantages of this method include sub optimal growth during the first part of the grazing season and the dependency on availability of clean grazing. Also, in New Zealand, the Dose and Move system combined with a single anthelmintic treatment proved unsuccessful in eliminating the effects of ostertagiosis (Brunsdon 1980). In the long term, however, a Dose

and Move system may reduce the rate of selection for drug resistance as the use of anthelmintic is minimal (Morley and Donald 1980).

Biological Control

Compared with other control options biological control of nematodes, focusing on the free living stages, has received little attention (Waller 1993a). One such control method involves the use of nematophagous fungi. These either produce nematode trapping organs (predators) or they kill nematodes by infecting them with their spores (endoparasitic). The interaction between nematode destroying fungi and the infective larvae of various species of parasitic nematodes of animals has been reported (Nansen, Gronvold, Henriksen and Wolstrup 1988; Larsen and Nansen 1991; Gronvold, Wolstrup, Nansen and Henriksen 1993; Gronvold, Wolstrup, Nansen, Henriksen, Larsen and Bresiani 1993a and Waller 1993a). In one laboratory experiment it was observed that *O. ostertagi* and *C. oncophora* had a greater capacity to induce the formation of fungal traps than some other gastrointestinal nematodes (Gronvold *et al.* 1993). It has also been shown that the trap forming potential of *D. viviparus* infective larvae is poor (Nansen *et al.* 1988).

Other fungi such as the oyster mushroom, *Pleurotus pulmonarius*, have shown a powerful immobilising effect on the free living stages of *O.ostertagi* and *C. oncophora* (Larsen and Nansen 1991). If fungi are to be used as a practical method of control, they must be capable of growing and trapping preparasitic stages of nematodes in the faeces following passage through the gastrointestinal tract of animals. *Duddingtonia flagrans* has been used successfully in the control of *O.ostertagi* and *C. oncophora* infective larvae in a field experiment (Gronvold *et al.* 1993a) however, further studies are necessary to determine details such as the minimal amount of fungal material required to maximise trapping capability in faeces.

CONTROL OF PARASITIC BRONCHITIS

Vaccination

Immunisation of calves using an irradiated larval vaccine has become a common method of control of disease caused by the bovine lungworm *D. viviparus* (Jarrett *et al.* 1957). The vaccination schedule comprises two doses of approximately 1000 irradiated larvae administrated four weeks apart and calves are not turned out until at least two weeks after the last dose of vaccine.

Chemoprophylaxis

The use of early season chemoprophylaxis such as ivermectin treatments at 3, 8 and 13 weeks post turnout (Taylor, Mallon and Kenny 1985 and Armour *et al.* 1987a) or an oxfendazole pulse release bolus at turnout (Jacobs, Pitt, Foster and Fox 1987d and Vercryusse *et al.* 1987) has been reported to be successful in the control of lungworm infection. Although these treatments were successful in controlling lungworm infection in calves during their first grazing season they may, as in the case of gastrointestinal nematodes, interfere with the development of immunity to lungworm in cattle (Jacobs *et al.* 1987d).

Pasture Management

As a rapid contamination of pasture by lungworm larvae is possible due to dispersal of larvae by *Pilobolus* sporangia, many pasture management systems designed for the control of gastrointestinal parasites will not necessarily prevent patent lungworm infections. Rotational grazing is one possibility but any pasture management system developed for lungworm control in cattle should be compatible with measures designed to control gastrointestinal nematodes. Eysker, Boersema, Cornelissen, Kooyman and de Leeuw (1992) studied the possibility of reducing lungworm infections in first season animals by rotational grazing weekly on six paddocks and found that the animals in the rotated group were less immune to lungworm than animals in the control group. Another disadvantage of this

pasture management system was the occurrence of serious parasitic gastro-enteritis although clinical lungworm infection was controlled.

CHAPTER 1

GENERAL INTRODUCTION

Anthelmintics are commonly used for the control of nematodes in cattle (Waller 1993a) and approximately $ 1.7 billion is spent annually throughout the world for the control of helminth diseases in ruminants (Lanusse and Prichard 1993).

The history of anthelmintics and their role in the treatment and control of parasitic diseases of animals has been reviewed by Gibson (1965). Early anthelmintics included plant materials followed by chemical compounds such as copper sulphate and other copper and arsenic salts which were still in use until 1940. The discovery of drugs such as phenothiazine (Knipling 1938), piperazine (Swanson, Stone and Wade 1957) and thiabendazole (Baily, Diamond and Walker 1961) improved the status of anthelmintics in terms of much improved efficacy and lack of toxic side effects. Subsequently, other efficient compounds such as levamisole and various benzimidazoles were discovered.

In the last 20-30 years a number of non-toxic modern benzimidazole drugs with improved efficacy have been developed and in 1980 a highly efficient anthelmintic, ivermectin, which has a broad spectrum of activity against many external as well as internal parasites, became available. Recently, further groups of similar drugs, the avermectins and milbemycins, have been developed.

Originally most anthelmintics were administered orally or by injection but in the last 10-15 years novel delivery systems such as sustained or pulse release rumen boluses and formulations for topical application have been introduced.

Recently Baggot and McKellar (1994) reviewed the classes of anthelmintics and their representatives which are effective against bovine gastrointestinal parasites and the bovine lungworm, *Dictyocaulus viviparus*.

These can be broadly classified into four groups, i.e. the Benzimidazoles, Imidazothiozoles plus tetrahydropyrimidines, Avermectins and Milbemycins.

The benzimidazole class of anthelmintics comprises some of the most commonly used anthelmintics to control gastrointestinal nematodes and lungworm in cattle; for example thiabendazole, fenbendazole, oxfendazole and albendazole. The earliest compound of this group marketed was thiabendazole which has

limited efficacy against the bovine lungworm *D. viviparus* and is not efficacious against fourth stage *O. ostertagi* arrested larvae. The benzimidazoles subsequently introduced have a broader spectrum of activity and are generally highly effective against all stages of worm development including inhibited larval stages of *O. ostertagi* (Prichard and Ranjan 1993).

Most of the benzimidazoles are insoluble and in ruminants they are often formulated for oral administration as suspensions; their relatively slow passage through the gut allows absorption of most of the drug (Baggot and McKellar 1994). Several benzimidazoles are now available as controlled or continuous release boluses and their efficacy has been well documented. For example the oxfendazole pulse release bolus (Bairden *et al.* 1983 and Jacobs *et al.* 1987) and the fenbendazole slow release bolus (Bauer 1993). These boluses release active anthelmintic as single doses at pre-determined periods, or in a sustained manner over a longer period, and a single bolus administration at turnout is recommended to protect first season calves from parasitic gastroenteritis (PGE) infection for a whole grazing season. Such devices work by preventing the establishment of patent infections and thus prevent pasture contamination. They have an added advantage in that they decrease the labour required for repeated dosing.

The imidazothiozole and tetrahydropyrimidine classes of anthelmintics, which are commonly used against cattle nematodes, are represented by levamisole and morantel. Although levamisole and morantel are effective against adult and developing parasite stages their efficacy against inhibited or arrested larval forms is questionable (Prichard and Ranjan 1993). For cattle levamisole is available in oral, injectable and topical formulations while morantel is available only as a sustained release bolus for oral administration. The morantel slow release bolus (MSRB, Paratect, Pfizer Ltd.) was the first such device to appear commercially and it has been shown to be very effective in controlling bovine gastrointestinal nematodes (Armour *et al.* 1981 and Jones 1981). One major disadvantage of this original bolus was the presence of a non-biodegradable metal casing which interfered with gut processing at the abattoir (McKellar 1994). This

was overcome by the development of a morantel slow release trilaminate biodegradable bolus (Paratect-Flex, Pfizer Ltd.).

The avermectins belong to a group of macrolide antibiotics and are derived from the fermentation products of *Streptomyces avermectilis*. Members of this class of anthelmintics are available as oral, injectable or topical formulations for the control of parasitic gastroenteritis and bronchitis in cattle. The first commercially available member of the avermectin class of anthelmintics, ivermectin, is a semisynthetic product which appeared in 1981. Ivermectin is known as an endectocide and is now considered by many as the drug of choice for the control of nematode and arthropod parasites in livestock and companion animals (Campbell 1989). Three doses of ivermectin at 3, 8 and 13 weeks post turn out have been shown to effectively control PGE in young set-stocked calves. This regime is based on a two week persistent anthelmintic activity of ivermectin and a three week pre-patent period of the common abomasal and small intestinal nematodes of cattle (Armour *et al.* 1987b). This treatment can also prevent parasitic bronchitis effectively in first season calves as shown by Taylor, Mallon, Green, McLoughlin and Bryson (1988). Ivermectin is also available as a sustained release bolus and its efficacy in helminth control has been documented (Egerton *et al.* 1986).

Abamectin is a natural fermentation product of *Streptomyces avermectilis* and also belongs to the avermectin class of anthelmintics. It was introduced as an agricultural pesticide and antiparasitic drug in 1985. The efficacy and persistency of abamectin are similar to ivermectin (Eagleson, Scott and Gross 1992). Treatment with abamectin at turn out and 6 weeks later has been shown to be effective in controlling infection with gastrointestinal nematodes in calves (Jacobs, Fisher, Hutchinson, Bartram and Vercruysse 1995).

One other member of the avermectin class of anthelmintics which has appeared recently is doramectin and its efficacy and persistence of activity are similar to the other avermectins (Vercruysse, Dorny, Hong, Harris, Hammet,

Smith and Weatherley 1993 and Yazwinsky, Featherston and Tucker 1994a). Currently, doramectin is only available for use in cattle by subcutaneous injection.

Moxidectin belongs to the class Milbemycin and is synthesised from *Streptomyces cyanogriseus* subspecies *noncyangenus*. Milbemycins are macrocyclic lactones from the same family as the avermectins. Moxidectin, although experimentally tested in several formulations, is as yet only available as an injectable for use in cattle. Moxidectin is a highly efficient anthelmintic and has a persistent activity of up to 35 days against *Dictyocaulus* and *Ostertagia* in calves (Uriarte, Gracia and Almeria 1994 and Hubert, Kerboeuf, Cardinaud and Bloud 1995).

Traditionally, anthelmintics were used as a curative measure and that trend continued until Michel (1966) introduced the 'Dose and Move' system for the control of PGE. This was based on a knowledge of the pattern of herbage larval counts and consisted of a mid-summer move to clean pasture combined with a single anthelmintic treatment. With only one anthelmintic treatment during the grazing season selection for drug resistance was unlikely to become a problem using this system; The Dose and Move method of control also allowed animals to be exposed to a significant parasite challenge and thus acquire an immunity to substantial reinfection and disease in subsequent years. However, there are limitations to this method of nematode control; for example it depends on the availability of clean pasture in July and, in some years, unexpectedly high overwintered herbage larval levels may be encountered and result in outbreaks of disease before the scheduled mid-summer anthelmintic treatment.

In addition, early season (Armour *et al.* 1987b), mid season (Taylor, Mallon, Kenny and Edgar 1995) and late season (Steffan and Nansen 1990a and 1990b) prophylactic regimes were tested for the control of bovine PGE. For example Taylor *et al.* (1995) compared the effects of ivermectin (3, 8 and 13 or10, 15 and 20 weeks post turnout) or the morantel slow release trilaminate (at turnout or 10 weeks post turnout) in prophylaxis and recorded better weight gains at the end of two years in the animals which received the early season treatments. Late

season anthelmintic prophylaxis, whilst preventing clinical consequences which may be encountered in the second half of the grazing season is not usually sufficient to counteract production losses. Therefore, early season chemoprophylaxis remains the method of choice to prevent production losses resulting from gastrointestinal nematodiosis.

For the control of parasitic bronchitis in calves, two doses of live attenuated vaccine at 6 and 2 weeks before being exposed to potential larval challenge from pasture have been recommended (Jarrett *et al.* 1957). In an early field study to evaluate the effect of vaccination against lungworm in calves exposed to either mild or severe *Dictyocaulus* pasture challenge, Downey (1965) observed that vaccinated calves were better protected than similarly exposed non-vaccinated calves. In addition, Duncan *et al.* (1979) and Pouplard and Pecheur (1981) observed that strategic anthelmintic prophylactic strategies designed to control gastrointestinal nematodiosis were not always sufficient to prevent lungworm infection and supported vaccination as the best means of controlling parasitic bronchitis.

One concern, especially with the advent of anthelmintic boluses, is the possibility of detrimental effects on the environment of some of the new highly efficient compounds. For example, the bulk of administered ivermectin is excreted unchanged through faeces (Prichard 1987) and since it has an effect against both nematodes and arthropods possible environmental hazards were anticipitated. In this respect Madsen, Gronvold and Nansen (1988) observed that a longer time was taken for the degradation of dung pats from ivermectin treated animals compared with controls and they related this to the larvicidal effect of ivermectin on the higher diptera which use dung pats as breeding sites. However, there are contrasting results which suggest that the effect of ivermectin on dung degradation is minimal (Jacobs, Pilkington and Fisher 1988; McKeand, Bairden and Ibarra-Silva 1988; Barth, Heinze-Mutz, Roncalli, Schluter and Gross 1993 and Forbes 1993). This variation has been related to differences in both methodology and climate (Barth 1993 and Strong 1993). Forbes (1993) reviewed the literature on

the possible effect of ivermectin and abamectin on the environment and concluded that the use of these anthelmintics, since their commercial availability in 1981 and 1985 respectively, has had little adverse effect on the environment.

Another concern regarding anthelmintics with high persistency profiles or controlled release anthelmintic technology is that unacceptable drug residue levels may occur in tissues (Anderson 1985). Anthelmintics such as ivermectin (Armour, Bairden, Batty, Davison and Ross 1985), abamectin (Eagleson *et al.* 1992), doramectin (Yazwinsky *et al.* 1994a) and moxidectin (Hubert *et al.* 1995) which are used in the control of cattle gastrointestinal nematodes all show an extended persistence and may therefore contribute to tissue residues for a considerable period. Also, although anthelmintics such as the benzimidazoles have comparatively shorter persistency profiles, when they are formulated as controlled or sustained release devices they may also prolong the period in which tissue residues can be detected.

The major potential disadvantage inherent in the use of anthelmintics with high efficiency either repeatedly or in controlled release technology is the development of anthelmintic resistance. There are several reports of bovine *Ostertagia* being resistant to levamisole (Anderson 1977; Anderson and Lord 1979; Borgsteede, Geerts, de Deken, Kumar and Brandt 1985a and Geerts, Brandt, Kumar and Biesemans 1987) and benzimidazole (BZ) resistance in *Trichostrongylus axei* (Eagleson and Bowie 1986). However, anthelmintic resistance in cattle is not as widespread as in small ruminants and this has been related to the less frequent use of anthelmintics in cattle rather than to a lack of genetic diversity (Waller 1993b).

Another negative influence of such effective anthelmintics and formulations could be on the development of host immunity to parasitism. It is known that the acqusition of immunity depends on different lengths of exposure to different parasite genera (Armour 1989). The administration of anthelmintics to control nematodes alters the length of host exposure and hence the subsequent acquisition of immunity to these parasites. One early report on the influence of

anthelmintic treatment on the development of immunity to gastrointestinal nematodes was that of Gibson, Parfitt and Everett (1970) who observed that treatment of lambs with thiabendazole weekly for a six month period left the lambs as susceptible as helminth naive controls to gastrointestinal nematodes. Recently, evidence that long term parasite suppression by anthelmintics can reduce the level of acquired immunity to nematodes has been documented (Jacobs *et al.* 1987d; Bairden 1991; Nansen 1993 and Taylor *et al.* 1995).

It is likely that the introduction of new, highly effective and persistent anthelmintic compounds together with the advances in drug release technology will prove beneficial both in terms of animal welfare and the agricultural economy. However, there can be limitations to the use of effective chemprophylactic regimes and these should always be considered by those who design and/or recommend their use in farming systems.

The studies reported in this thesis were carried out to (1) review the literature on PGE and parasitic bronchitis in cattle with special emphasis on epidemiology, immunity and control (2) to evaluate the influence of chemoprophylaxis with two highly efficient anthelmintics, ivermectin and moxidectin, on the acquisition of immunity in one to two year old cattle (3) to obtain background data on the parasite burdens and pepsinogen concentrations of adult dairy cattle in relation to *Ostertagia* and (4) to compare two techniques commonly used for the recovery of fourth larval stages from bovine abomasa and for the determination of serum pepsinogen concentrations and assess their merits in relation to efficiency and convenience.

CHAPTER 2

MATERIALS AND METHODS

ANIMALS

Three types of animals were used.

CALVES: Three to six month old Friesian calves were used for the production of helminth cultures and as seeders to contaminate pasture where appropriate.

YEARLINGS: Eighteen to twenty month old male Friesian or Friesian cross animals were used in the second grazing season. As first season calves these animals had been used in a strategic dosing field experiment. At turnout their average weight was 268 Kg . Animal weights were recorded using a standard cattle weigh scale and crush.

ADULTS: These provided the material used in the abbattoir study and were Friesian cattle aged between two to four years .

MANAGEMENT

HOUSING: was within the grounds of Glasgow University Veterinary School. The animals were kept in concrete floored and walled pens and offered concentrates at up to 2 Kg/animal/day; hay and water were available *ad libitum.*

GRAZING: the grazing plots used in this study were situated about forty five miles from the Veterinary School. The total pasture area of 18 acres was divided into three equal paddocks and the animals were rotated regularly throughout each paddock. No supplementary feeeding was given during the grazing period.

INFECTION

The experimental animals were infected either naturally and/or artificially. To ensure an adequate natural challenge in the early part of the grazing season four seeder animals, each infected with 10000 *O. ostertagi,* 10000 *C. oncophora* and 500 *D. viviparus* L3, grazed the experimental plots for several weeks prior to the principal cattle being introduced. For artificial infections infective larvae of field strains of *O. ostertagi* and *C. oncophora,* isolated and maintained at the

Department of Parasitology of Glasgow University Veterinary School, were used. *D. viviparus* L3 were supplied by Intervet U K. Ltd..

ANTHELMINTICS

Moxidectin injectable and moxidectin pour-on were supplied by Cyanamid UK limited and the other anthelmintics used were the commercially available ivermectin (Ivomec, MSD Agvet) and fenbendazole. (Panacur, Hoechst).

CLINICAL EXAMINATION

The animals were observed daily and their condition was assessed during regular handling.

PARASITOLOGICAL TECHNIQUES

FAECAL ANALYSES

Faeces were taken directly from the rectum and trichostrongyle worm egg counts were determined using a modified McMaster salt flotation technique which was based on the original McMaster method as described by Gordon and Whitlock (1939). In this method 3 grams of faeces were homogenized with 42 ml of water and the suspension passed through a coarse mesh sieve of aperture size 250 μ to remove larger particles of debris. A fifteen ml sub-sample of the filtrate was transferred to a test tube and spun at 2000 rpm for two minutes. The supernatant was then discarded, the faecal deposit mixed thoroughly using a whirlmixer (Whirlmixer, Scientific and Industrial Ltd) and the tube filled to its former level with saturated sodium chloride solution. After mixing gently by inversion, enough of the suspension to fill both chambers of a McMaster slide (Gelman Hawksley Ltd.) was transferred by pipette and the preparation examined under the ×25 objective of a stereomicroscope. The number of eggs found under the etched squares of both chambers was multiplied by 50 and expressed as the numbers of trichostrongyle eggs per gram of faeces based on the following calculation.

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

Volume under one square equals 0.15 ml.

No. of eggs seen in one square × 100 equals No. of eggs/gram.

No of eggs seen in two squares × 50 equals No. of eggs/gram.

For the recovery of lungworm larvae the Modified Baermann method described by Henriksen (1965) was used. Here ten grams of faeces placed in a gauze square were suspended in lukewarm water contained in a 250 ml conical measure and allowed to stand for at least six hours at room temperature. The supernatant was then siphoned off and 10 ml of the sediment retained. Using an Eel counting chamber (Gelman Hawksley Ltd.) the larvae present in 1 ml of the sediment were counted. When no larvae were found in the first aliquot the remainder of the sediment was examined.

HERBAGE ANALYSES

Pasture samples were collected randomly by hand following a W-shaped route across each plot. Fifty evenly spaced stops were made along the route and at each stop four plucks of grass were taken to get a total of 200 plucks per paddock. The samples were processed by a method adapted from that described by Parfitt (1955). The basis of the technique relies on the liberation of larvae from herbage by washing and concentration by sieving or sedimentation prior to subsequent identification. In this technique the grass samples were weighed and then soaked in six litres of lukewarm water to which a few drops of detergent were added. After six hours soaking, the washings were passed through a 38μ seive (Endecotts Ltd.) and the material retained by the sieve collected and stored at 4°C. The grass was then soaked again in fresh lukewarm water for a further six hours, the washings sieved as before and the material retained added to the first sample.

The grass was then spread on a tray, dried in an incubator at 70° C and the dry weight of the sample determined. The dry weight was used for final calculation and expression of larval numbers per Kg dried herbage.

The combined washings were Baermannised. In this method the washings containing the larvae in suspension were drawn through a coarse filter paper (Whatmans grade 113, 18.5 cm) using a Buchner funnel and vacuum pump. Using a milk filter (Maxa milk filters, A. McCaskie ltd., Stirling) as a support, the preparation was placed on a Baermann apparatus so that the larvae retained on the filter paper could pass through to the lukewarm water in the funnel. After a minimum of six hours, 10 ml of fluid was withdrawn from the bottom of the funnel. One drop of 45% iodine was added to the sample and the larvae in 1ml counted using an Eel counting chamber and differentiated using the morphological criteria outlined by Keith (1953). These were body length, prolongation of the sheath beyond the tail of the third stage larva and the presence of refractile structures. The numbers of infective larvae of each genus per Kg dry herbage were calculated by dividing the number of larvae counted x1000 by the herbage dry weight.

WORM RECOVERY AT POST MORTEM

Prior to necropsy all animals were housed for at least two weeks. This allowed discrimination of arrested fourth stage larvae from developing stages. At necropsy the lungs, abomasum and small intestine were removed and processed. The animals were euthanased using a captive bolt pistol and immediately exsanguinated. To prevent mixing of abomasal and intestinal contents the pyloric/duodenal junction was ligatured as soon as possible after death. The

abomasum and small intestine with their contents were then separated and processed individually for the estimation of worm burdens. The lungs plus heart were removed intact from the thoracic cavity.

For gastrointestinal worm burdens the abomasum and small intestine were processed according to Ritchie *et al.* (1966). The organs were freed of excess fat and mesenteric attachments and treated seperately. The abomasal contents were collected by opening the abomasum along its greater curvature, washing the mucosal surface under a slow running jet of water making the total volume of washings up to 4 litres. When a large amount of material was present, the volume of washings was increased to 6 or 8 litres. After thorough mixing, duplicate aliquots of 200 ml were transferred to bottles and preserved with 1 ml 45% iodine solution. In addition to preservation the iodine stained the sample and facilitated subsequent observation of parasites.

The abomasum was then divided into two longitudinally along the lesser curvature and the mucosa of one half was scraped off. The mucosal scrapings were then digested with a pepsin/HCL acid mixture at 42° C according to a method based on that described by Herlich (1956) for the recovery of arrested larvae. After 6 hours, the digest was transferred to a bucket and made up to a final volume of 4 litres. Again, duplicate 200 ml samples were taken and preserved as before for subsequent examination.

The other half of the abomaum was soaked in 0.85% sodium chloride solution in a tray at 37° C. After six hours, the half abomasum was rinsed thoroughly to release larvae lying in the folds of the mucosa then discarded and the washings collected and made up to 4 litres. As before, duplicate samples of 200 ml were taken, stained and preserved for subsequent examination.

The small intestine was opened along its length and its contents collected by washing under a jet of water while rubbing the mucosal surface to maximise parasite recovery. The tissue was then discarded and the washings made up to 4 litres and duplicate 200 ml samples taken as before.

Aliquots of abomasal contents, abomasal mucosal digests and washings plus intestinal contents were examined for worms and their developing stages at × 25 magnification under a stereomicroscope. Three to four ml of 5% sodium hypochlorite solution was added to the aliquots prior to microscopy to destain the background. Ten 4 ml aliquots from each 200 ml sub-sample were examined for nematodes and results expressed were based on the final volume of the washings.

For lungworm recovery a perfusion method similar to that described by Inderbitzen (1976) was employed. In this method the pulmonary artery was exposed, an incision made and a rubber tube inserted pointing towards the tracheal bifurcation. The tube was ligatured *in-situ*, the remaining great vessels 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 and returned *via* the bronchial tract and the trachea. Twenty litres of washings were collected from each set of lungs and passed through a milk filter/nylon sieve using the apparatus described by Jorgensen (1975; shown in **Plate 1**). From the material retained by the filter a total count of worms was made and these were differentiated into adults and developing stages.

BIOCHEMICAL ANALYSES

Blood samples were drawn in to sterile silicone coated vacutainers and allowed to clot overnight. The clots were then discarded and the serum

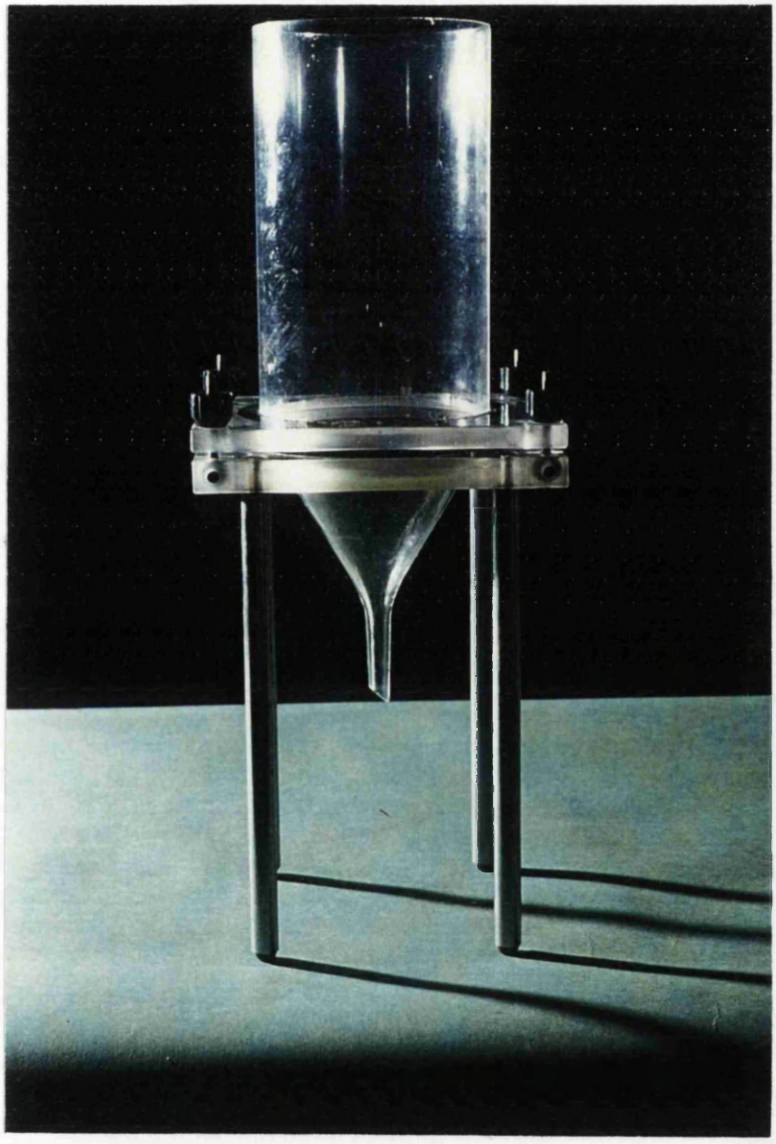


Plate 1 System for the recovery of parasites after lung perfusion (adapted from Jorgensen 1975)

centrifuged at 2500 rpm for 20 min. Aliquots of 1.5 ml serum were stored at minus 20°C for analysis.

Two different assays were used for the estimation of serum pepsinogen. These assays determine serum pepsinogen concentrations by quantifying the products of its proteolytic activity. The basis is the acid medium which converts serum pepsinogen to pepsin and the subsequent digestion of a protein substrate. The resultant small peptic fragments can be measured spectrophotometrically.

The standard method employed was based on that described by Edwards, Jepson and Wood (1960). In this method serum was incubated at 37°C with a substrate, bovine serum albumin (BSA), at pH 1.5 for 24 hours and the tyrosine like phenolic amino acids liberated were determined using Folin-Ciocalteu reagent. Corrections were made for the normal or non-incubated content of tyrosine-like substances and the release of these substances due to any acid hydrolysis of BSA during the incubation period. Serum pepsinogen concentrations were expressed as iU where one unit equals one μmol tyrosine released/min/litre of serum.

The other assay used for the estimation of serum pepsinogen has been described by Paynter (1992). This method involves a shorter incubation period and the use of a glycine-buffered BSA. In this method, serum samples were incubated with BSA fraction V substrate (3.2% BSA, 1% Glycine, pH 1.6) for 30 minutes at 37°C. Undigested substrate was precipitated by the addition of 10% perchloric acid and separated by centrifugation at 14000 rpm for 10 minutes. The concentration of acid soluble peptide fragments was determined using a Bicinchonic acid/CuSo4 mixture (50:1 v/v) and reading on a Dynatec MR 5000

ELISA reader at an absorbance of 560 nm. Comparison was made to standards containing Tyrosine and the results were expressed as iU.

Procedures of both methods for the estimation of serum pepsinogen have been detailed in **Appendix 1**.

CHAPTER 3

THE INFLUENCE OF CHEMOPROPHYLAXIS ON THE ACQUISITION OF IMMUNITY TO GASTROINTESTINAL NEMATODES AND LUNGWORM

INTRODUCTION

Bovine parasitic gastroenteritis and bronchitis are widespread and economically important diseases. In temperate climates, *Ostertagia* and *Cooperia* are the commonest causes of parasitic gastroenteritis in cattle (Armour 1989) while parasitic bronchitis is caused solely by *Dictyocaulus viviparus.* Calves in their first grazing season are most susceptible to these infections and can show signs of inappetence, disturbance of gastrointestinal function and body metabolism (Fox 1993) and various respiratory signs (Jarrett *et al.* 1954).

Acquired immunity to *O. ostertagi* and *D. viviparus* is an important factor in the epidemiology of disease caused by these two parasites and the rate of development of such immunity varies with the parasite and the period of exposure (Armour 1989). Acquired immunity to *Ostertagia* only develops following a long exposure to infection with the parasite while in the case of *C. oncophora* and *Dictyocaulus* a strong degree of immunity is induced following a shorter period of exposure. Experimental evidence suggests that calves exposed to *Ostertagia* during the first grazing season are still susceptible in the following year (Armour 1967 and Gronvold *et al.* 1992) and that a solid immunity, characterized by low egg counts and abomasal worm burdens, is not acquired until the end of the second grazing season (Entrocasso *et al.* 1986b and Gronvold *et al.* 1992). The immunity to *Ostertagia* observed in adult cattle is predominantly due to previous antigenic exposure (Armour and Ogbourne 1982 and Kleus 1988). This view has been confirmed by the low worm egg counts detected at the end of a second year of observation by Gronvold *et al.* (1992) and higher weight gains in second year animals which had been exposed to an adequate challenge during their first grazing season (Ploeger, Kloosterman, Borgsteede and Eysker 1990). Although recent studies have indicated the presence of some degree of age immunity to *Ostertagia* (Gronvold *et al.* 1992) this plays a minor role (Michel, Lancaster and Hong 1979).

Although acquired immunity to *Dictyocaulus* is strong, differences in functional immunity to this genus, shown between different age categories of

animals have been observed (Taylor *et al.* 1988) but this is, again, considered to be of little practical significance. Immunity to *Cooperia* differs from that to both *Ostertagia* and *Dictyocaulus* in that an age related immunity to this genus is substantial (Kloosterman *et al.* 1991).

Current control of nematode infections is based on an understanding of parasite bionomics and the epidemiology of disease and relies mainly on the use of anthelmintics and grazing management practices (Brunsdon 1980). The commonest approach of all preventive measures against infection with gastrointestinal nematodes is chemoprophylaxis but, for the control of parasitic bronchitis in calves, two doses of a live attenuated vaccine at 6 and 2 weeks before being exposed to pasture challenge have been shown to be highly effective (Jarrett *et al.* 1957).

The successful use of prophylactic regimes such as ivermectin treatment at 3, 8 and 13 weeks post turn out (Taylor *et al.* 1985 and Armour *et al.* 1987b), moxidectin injectable and moxidectin pour-on at turnout and 5 weeks post turnout (Ranjan *et al.* 1992; Taylor *et al.* 1993 and Whang, Bauer, Kollmann and Burger 1994) for the control of PGE and parasitic bronchitis in first season calves have been documented. The use of such modern anthelmintics in prophylactic strategies in grazing calves invariably results in lower worm burdens, reduced pasture contamination and subsequent lower re-infection potential. However, highly effective anthelmintics, when used prophylactically, may prevent sufficient antigenic stimulation of the host during the first grazing season and therefore interfere with the development of immunity in subsequent years (Armour 1989 and Nansen 1993). This type of suppression of immunity with a consequent high incidence of parasitic gastroenteritis and bronchitis in yearlings has been reported, for example, after prophylaxis with an oxfendazole pulse release bolus (Jacobs *et al.* 1987d, Herbert and Probert 1987 and Vercruysse *et al.* 1987), or the morantel sustained release trilaminate (Bairden 1991 and Vercruysse, Dorney, Hilderson and Berghen 1992) or the ivermectin 3, 8 and 13 weeks regime (Armour, Bairden and Ryan 1988b and Vercruysse, Hilderson and Claerbout 1995). In contrast,

some authors have demonstrated no significant adverse effect on the development of immunity following such prophylactic treatments; for example with the oxfendazole or morantel boluses (Downey 1988 and Eysker, Boersema and Kooyman 1990 and Entrocasso *et al.* 1986b) or with the ivermectin 3, 8, and 13 weeks regime (Taylor *et al.* 1988). These contrasting results may be related to the use of an anthelmintic treatment at the beginning of the second grazing season, different degrees of pasture challenge or the efficacy of the anthelmintic used.

AIMS

The study reported here was initiated to assess and compare the effects of first season chemoprophylaxis of grazing calves with moxidectin injectable or pour-on at turnout and 5 weeks later or ivermectin injectable at 3, 8 and 13 weeks post turnout on the immune status of these animals to *Ostertagia, Cooperia* and *Dictyocaulus* during their second grazing season.

EXPERIMENTAL DESIGN

YEAR 1

Forty eight first season calves were divided into four groups, each of 12 animals, which were set stocked on separate permanent cattle grazing paddocks. Anthelmintic treatments were administered as shown in **Table 1**.

Table 1 First season anthelmintic treatment schedule

Group	Anthelmintic	Administration	Schedule	Dose rate
1	Moxidectin 1%	Injection	0 and 5 *	200 µg/Kg
2	Moxidectin 0.5%	Pour-on	0 and 5 *	500 µg/Kg
3	Ivermectin 1%	Injection	3, 8 and 13 *	200 µg/Kg
4	Untreated	-	-	-

* Number of weeks after turnout

Fortnightly faecal, blood and herbage samples were collected and the animals were also weighed at this time. At the end of the first grazing season a selection of animals from each group was housed overwinter. At housing they were treated with 10% fenbendazole at the recommended dose rate of 7.5mg/Kg.

At the end of the first housing period, and prior to turnout for a second season, 5,4,4 and 2 animals from former treatment Groups 1,2,3 and 4 respectively were selected and challenged, experimentally, with 50,000 L3 each of *O. ostertagi* and *C.oncophora* and 1,000 *D.viviparus* L3. They were then necropsied 23-25 days post challenge and their worm burdens established.

YEAR 2

The remaining 28 yearlings (7 animals from each former group) were set-stocked together on permanent cattle grazing. To ensure an adequate pasture larval challenge four seeder calves previously infected with *O. ostertagi, C.oncophora* and *D.viviparus* were grazed ahead of the principal animals and removed after four weeks grazing. As the pasture was sub-divided into three paddocks, all of the animals were rotated regularly through each paddock.

At the end of the grazing season the remaining animals were housed and treated with fenbendazole. Two weeks post housing they were challenged, as previously, with 50,000 *O. ostertagi* and 50,000 *C.oncophora* L3 and 1,000 *D.viviparus* L3 and necropsied 23-25 days post challenge. With each experimental challenge a group of parasite naive calves were included as an infectivity control.

Observations

The animals were observed daily with faecal and herbage samples being collected fortnightly. In addition, monthly blood samples were taken when the animals were also weighed.

Statistical Methods

Data were subjected to two-way factorial analysis of variance with repeated measures using the least square method using a computer statistical package (Animal Designs, Data International Service, Glasgow). The results from the analysis of variance were then used in the Newman-Keuls multiple range test to identify treatment differences. The worm count comparisons were analysed by one-way analysis of variance followed by a between group comparison using the Newman-Keuls multiple range test. Before being analysed, faecal egg, larval and worm counts were log transformed in order to stabilize variance. Comparisons were considered significantly different at $P<0.05$.

RESULTS

YEAR 1

During the first grazing season the calves were exposed to a substantial gastrointestinal parasite larval challenge as confirmed by faecal egg counts and pasture larval recoveries. The level of exposure was sufficient to precipitate an outbreak of PGE in the calves of control Group 4 and, although no treatment had been scheduled for these animals, they were dosed with fenbendazole in July of their first grazing season. Exposure to lungworm infection was considered minimal since no evidence of a patent *Dictyocaulus* infection was detected in any animal throughout the grazing season.

YEAR 2

Clinical Observations

In the second grazing season clinical signs of parasitic bronchitis, including coughing and dyspnoea, were observed by the 30th of June which was six weeks post turnout. As the condition of the animals gradually deteriorated, therapeutic treatment, as shown in **Table 2**, was instigated on the 19th of July (Week 9). At this time two animals were selected from each group and necropsied for worm burden estimation.

This treatment resulted in a rapid improvement in condition although moderate coughing persisted in most of the animals for several weeks or longer. Five weeks after turnout, on the 23rd of June, mild signs of intermittent diarrhoea and inappetence, suggestive of PGE were also noted.

Table 2 Second season anthelmintic treatment schedule

Group	Anthelmintic	Dose rate
1	Moxidectin 1%	200 µg/Kg
2	Moxidectin 0.5%	500 µg/Kg
3	Ivermectin 1%	200 µg/Kg
4	Fenbendazole 10%	7.5 mg/Kg

Bodyweights

Group mean bodyweights are shown in **Figure 1** with individual data being recorded in **Appendix 2**. At the beginning of the second grazing season the mean live weights of Groups 1, 2, 3 and 4 were 279, 271, 246 and 279 Kg respectively. Low weight gains were observed in the animals of all four groups until therapeutic treatment (Week 9) after which a marked increase in group bodyweight was recorded. At the end of the grazing season, mean bodyweights of 422, 402, 373 and 415 Kg were observed for the animals of groups 1, 2, 3 and 4 respectively. There were no significant live weight differences observed between groups and in the second grazing season the animals of Groups 1, 2, 3 and 4 gained an average of 143, 131, 127 and 136 Kg respectively.

Biochemical Analyses

Serum pepsinogen concentrations are illustrated in **Figure 2** and detailed in **Appendix 3**. At the beginning of the season the ex-control animals had a significantly higher group mean serum pepsinogen concentrations than those of

Figure 1 Group mean bodyweights

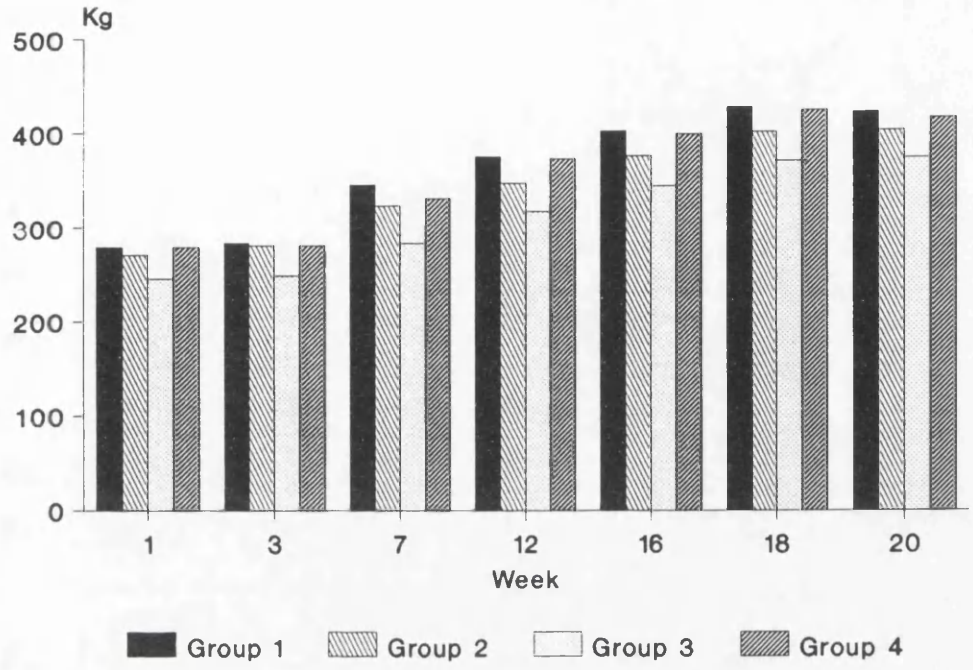
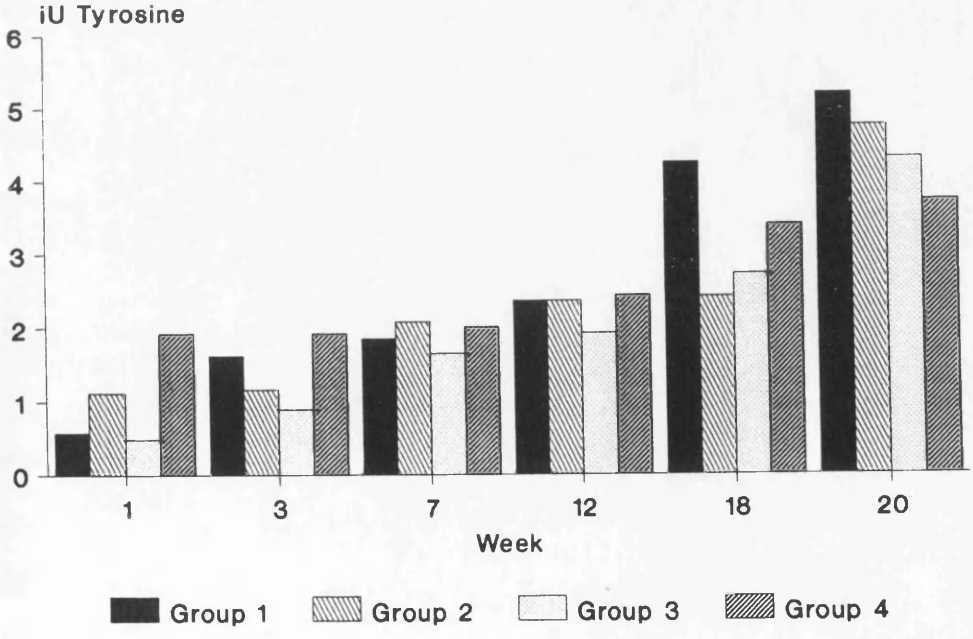


Figure 2 Group mean serum pepsinogen concentrations



the prophylactically treated groups. Mean pepsinogen concentrations of 0.57, 1.11, 0.48 and 1.92 iU were recorded from Groups 1, 2, 3 and 4 respectively. These levels increased gradually over the season with the highest levels being observed at the end of the grazing period when group mean serum pepsinogen concentrations of 5.18, 4.74, 4.28 and 3.71 were recorded from Groups 1, 2, 3, and 4 respectively. For the second grazing season, there was a significant group by time interaction indicating that at certain times there were significant differences in mean serum pepsinogen concentrations between different groups.

Parasitological Analyses

Faecal Egg Counts

Mean faecal egg counts (epg) for each group of yearlings are illustrated in **Figure 3** with individual data being recorded in **Appendix 4.** At the beginning of the second grazing season mean egg counts of 7, 0, 0 and 14 epg were observed in the animals of Groups 1, 2, 3 and 4 respectively. Mean maximum egg counts of 142 (Week 7), 307, 275 and 79 epg (Week 9) were recorded from the animals of Groups 1, 2, 3 and 4 respectively. This mid season rise of egg counts coincided with mild signs of PGE. Following anthelmintic therapy for parasitic bronchitis on the 19th of July (Week 9) lower egg counts were then observed for the remainder of the grazing season with mean maximum values of 80, 30, 120 and 50 epg being recorded for the animals of Groups 1, 2, 3 and 4 respectively.

***D. viviparus* Faecal Larval Counts**

Group mean *D. viviparus* faecal larval counts are illustrated in **Figure 4** and individual data detailed in **Appendix 5**. Animals of all four groups started the grazing season with negative larval counts which remained negative until the 19th of July (Week 9). At this time, coincident with the outbreak of parasitic bronchitis involving most animals in all four groups, group mean maximum larval counts of 41, 144, 114 and 57 lpg were recovered from Groups 1, 2, 3, and 4 respectively.

Figure 3 Group mean faecal egg counts

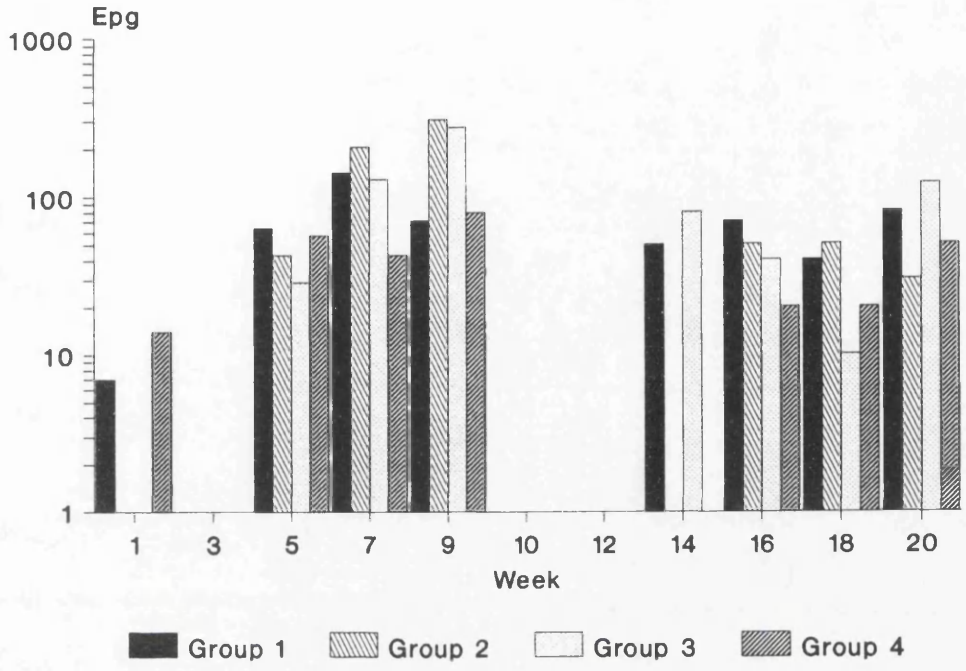
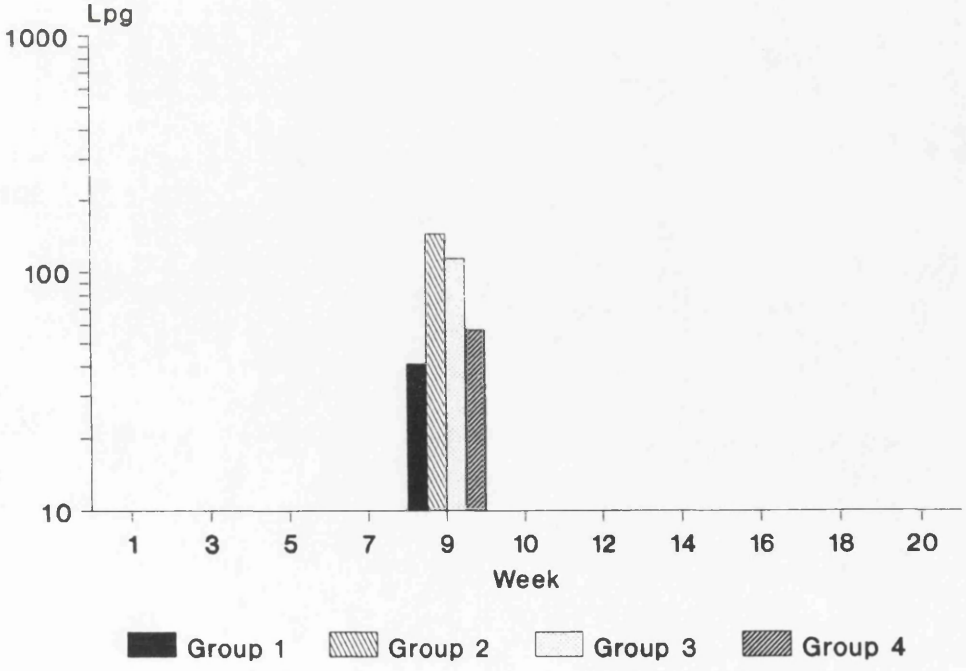


Figure 4 Group mean faecal larval counts



After therapeutic treatment faecal larval counts fell again to zero and remained negative for the rest of the grazing season.

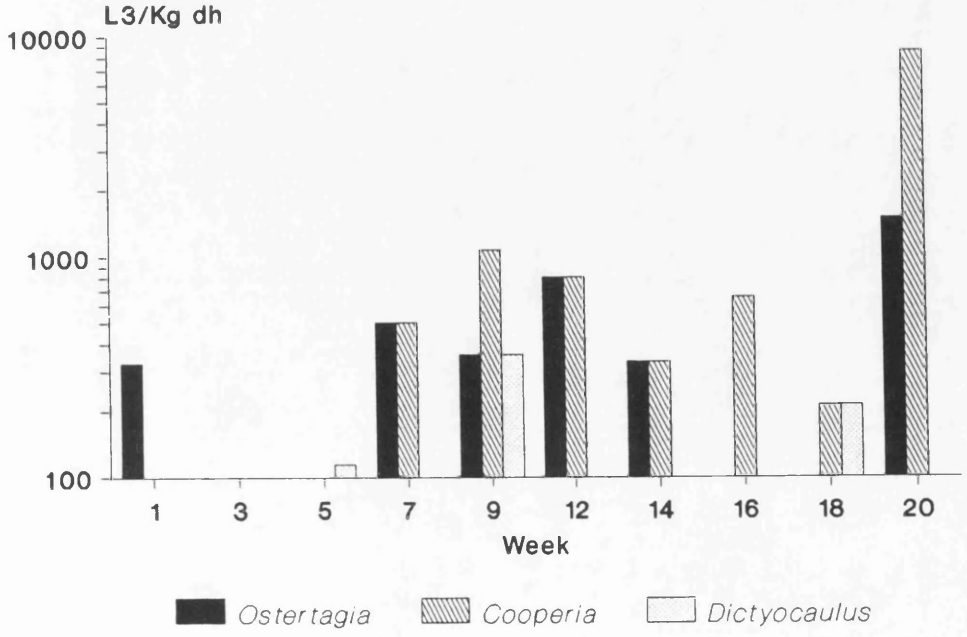
Pasture Larval Recoveries

Average herbage larval recoveries from the three pastures are presented in **Figures 5a, b** and **c** and individual paddock and average counts detailed in **Appendix 6.**

Paddock 1

During the first 4 weeks of grazing low pasture larval counts were observed. A moderate increase in larval numbers was observed during midseason with maximum recoveries of 1470 L3/Kg dh (*Ostertagia*) and 8461 L3/Kg dh (*Cooperia*) recorded at the time of housing. An increase in the numbers of *Dictyocaulus* larvae on herbage was observed during midseason with maximum recoveries of 357 L3/Kg dh coinciding with the outbreak of parasitic bronchitis on the 19th of July (Week 9).

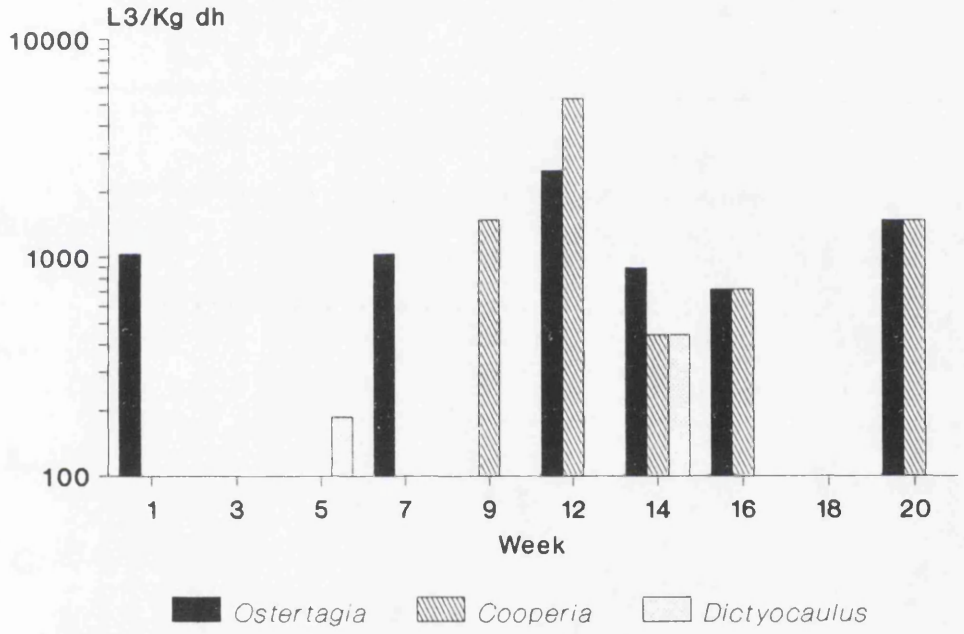
Figure 5a Herbage larval recoveries from Paddock 1



Paddock 2

Levels of *Ostertagia* and *Cooperia* larvae slightly higher than those from Paddocks 1 and 3 were detected early in the season and thereafter counts fluctuated with maximum recoveries of 2500 and 5312 L3/Kg dh respectively for these two genera at twelve weeks post turnout. During the grazing season, *Dictyocaulus* larvae were recovered from this area on only two ocassions with a maximum recovery of 444 L3/Kg dh being recorded fourteen weeks post turnout.

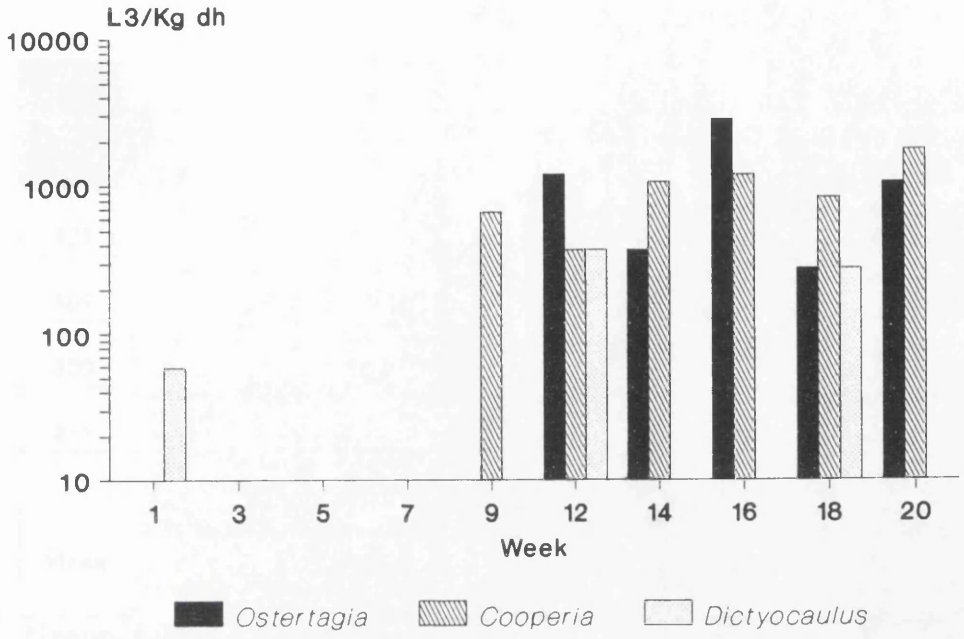
Figure 5b Herbage larval recoveries from Paddock 2



Paddock 3

Initially low pasture larval recoveries were observed but these increased later in the grazing season. The maximum *Cooperia* recovery of 1754 L3/Kg dh was recorded at housing while the highest *Ostertagia* and *Dictyocaulus* recoveries of 2807 L3/Kg dh and 370 L3/Kg dh were recorded at sixteen and twelve weeks post turnout respectively.

Figure 5c Herbage larval recoveries from Paddock 3



Worm Burdens

Individual and group mean worm burdens from the animals challenged and necropsied at the end of the first housing period are given in **Table 3.**

Table 3 Individual and group mean worm burdens at the end of the first housing Period

Group 1-Moxidectin Injectable

	Ostertagia			*Cooperia*			*Dictyocaulus*
No.	Adult	L4	Total	Adult	L4	Total	Total
832	5700	0	5700	900	0	900	52
835	6900	200	7100	1300	0	1300	81
844	11800	200	12000	17200	0	17200	77
865	7800	0	7800	900	0	900	0
866	10200	400	10600	16000	0	16000	0
Mean	**8480**	**160**	**8640**	**7260**	**0**	**7260**	**42**

Table 3 (continued)

Group 2-Moxidectin Pour-on

	Ostertagia			*Cooperia*			*Dictyocaulus*
No.	Adult	L4	Total	Adult	L4	Total	Total
828	8300	0	8300	1900	0	1900	113
869	8100	0	8100	1600	0	1600	146
880	8200	0	8200	3200	0	3200	129
885	9800	400	10200	400	0	400	0
Mean	**8600**	**100**	**8700**	**1775**	**0**	**1775**	**97**

Group 3-Ivermectin Injectable

	Ostertagia			*Cooperia*			*Dictyocaulus*
No.	Adult	L4	Total	Adult	L4	Total	Total
827	15500	4400	19900	2500	0	2500	441
855	10600	0	10600	2500	0	2500	36
857	7600	0	7600	8000	0	8000	171
874	8600	0	8600	1800	0	1800	24
Mean	**10575**	**1100**	**11675**	**3700**	**0**	**3700**	**168**

Group 4-Untreated Control

	Ostertagia			*Cooperia*			*Dictyocaulus*
No.	Adult	L4	Total	Adult	L4	Total	Total
152	13600	800	14400	100	0	100	156
858	11000	1000	12000	7500	0	7500	282
Mean	**12300**	**900**	**13200**	**3800**	**0**	**3800**	**219**

Table 3 (continued)

Group 5-Challenge Control

No.	*Ostertagia*			*Cooperia*			*Dictyocaulus*
	Adult	L4	Total	Adult	L4	Total	Total
P14	12000	0	12000	10600	0	10600	209
P15	13900	0	13900	11400	0	11400	304
P16	12200	0	12200	10800	0	10800	303
P17	12000	0	12000	10000	0	10000	283
Mean	**12525**	**0**	**12525**	**10700**	**0**	**10700**	**275**

Mean *Ostertagia* burdens in the animals of Groups 1, 2, 3 and 4 were 8640, 8700 11675 and 13200 compared with a recovery of 12525 from the challenge control calves. *Cooperia* burdens of 7260, 1775, 3700 and 3800 and lungworm recoveries of 42, 97, 168 and 219 were recovered from Groups 1, 2, 3 and 4 compared with 10700 and 275 respectively from the challenge control calves. Analysis of variance showed no significant differences in worm recoveries between groups.

Ostertagia, Cooperia and *Dictyocaulus* worm recoveries from the animals necropsied midway through the second grazing season are presented in **Table 4**. Mean *Ostertagia* burdens of 14900, 40900, 22900 and 16150, *Cooperia* burdens of 0, 4700, 3600 and 300 and *Dictyocaulus* burdens of 133 1268, 2185 and 208 were recovered from the Groups 1, 2, 3 and 4 respectively. The low number of animals in each group prevented any meaningful statistical analysis of results.

Table 4 Individual and group mean midseason worm burdens

Group 1-Moxidectin Injectable

	Ostertagia			*Cooperia*			*Dictyocaulus*
No.	Adult	L4	Total	Adult	L4	Total	Total
842	6800	4200	11000	0	0	0	9
875	18800	0	188800	0	0	0	267
Mean	**12800**	**2100**	**14900**	**0**	**0**	**0**	**133**

Group 2-Moxidectin Pour-on

	Ostertagia			*Cooperia*			*Dictyocaulus*
No.	Adult	L4	Total	Adult	L4	Total	Total
852	45200	200	45400	4800	0	4800	537
861	36400	0	36400	4600	0	4600	2000
Mean	**40800**	**100**	**40900**	**4700**	**0**	**4700**	**1268**

Group 3-Ivermectin Injectable

	Ostertagia			*Cooperia*			*Dictyocaulus*
No.	Adult	L4	Total	Adult	L4	Total	Total
837	22000	0	22000	4200	200	4400	4058
868	23800	0	23800	2800	0	2800	312
Mean	**22900**	**0**	**22900**	**3500**	**100**	**3600**	**2185**

Table 4 (continued)

Group 4-Untreated Control

		Ostertagia			*Cooperia*		*Dictyocaulus*
No.	Adult	L4	Total	Adult	L4	Total	Total
838	11500	200	11700	400	0	400	253
845	20600	0	20600	200	0	200	163
Mean	**16050**	**100**	**16150**	**300**	**0**	**300**	**208**

Ostertagia, Cooperia and *Dictyocaulus* worm burdens after artificial challenge at the end of the second grazing season are shown in **Table 5.** *Ostertagia* L4 burdens in the former prophylactically treated and control groups were significantly higher than those of the challenge control group with mean counts of 13360, 2280,1560, 3160 and 0 being recovered from groups 1, 2, 3, 4 and the challenge control group respectively.

Table 5 Individual and group mean worm burdens at the end of the second. grazing season

Group 1-Moxidectin Injectable

		Ostertagia			*Cooperia*		*Dictyocaulus*
No.	Adult	L4	Total	Adult	L4	Total	Total
851	0	2000	2000	100	0	100	0
854	8200	55800	64000	0	0	0	0
871	4400	600	5000	0	0	0	0
876	7600	7200	14800	200	0	200	0
877	3600	1200	4800	400	0	400	0
Mean	**4760**	**13360**	**18120**	**140**	**0**	**140**	**0**

Table 5 (continued)

Group 2-Moxidectin Pour-on

No.	*Ostertagia* Adult	*Ostertagia* L4	*Ostertagia* Total	*Cooperia* Adult	*Cooperia* L4	*Cooperia* Total	*Dictyocaulus* Total
833	2000	1000	3000	0	0	0	0
841	12400	3000	15400	1400	100	1500	0
847	8000	600	8600	1500	200	1700	0
860	5400	6600	12000	0	0	0	0
872	3700	200	3900	0	0	0	0
Mean	**6300**	**2280**	**8580**	**580**	**60**	**640**	**0**

Group 3-Ivermectin Injectable

No.	*Ostertagia* Adult	*Ostertagia* L4	*Ostertagia* Total	*Cooperia* Adult	*Cooperia* L4	*Cooperia* Total	*Dictyocaulus* Total
849	1600	0	1600	0	0	0	0
853	6200	3000	9200	0	0	0	0
863	3000	1200	4200	0	0	0	0
864	1000	1600	2600	0	0	0	0
884	8600	2000	10600	0	0	0	0
Mean	**4080**	**1560**	**5640**	**0**	**0**	**0**	**0**

Table 5 (continued)

Group 4-Untreated Control

No.	*Ostertagia* Adult	*Ostertagia* L4	*Ostertagia* Total	*Cooperia* Adult	*Cooperia* L4	*Cooperia* Total	*Dictyocaulus* Total
153	0	0	0	0	0	0	0
816	0	0	0	0	0	0	0
836	6200	13800	20000	0	0	0	0
846	4200	1000	5200	0	0	0	0
878	4200	1000	5200	0	0	0	0
Mean	**2920**	**3160**	**6080**	**0**	**0**	**0**	**0**

Group 5-Challenge Control

No.	*Ostertagia* Adult	*Ostertagia* L4	*Ostertagia* Total	*Cooperia* Adult	*Cooperia* L4	*Cooperia* Total	*Dictyocaulus* Total
P34	21000	0	21000	14500	0	14500	118
P35	18500	0	18500	16800	0	16800	96
P36	19100	0	19100	18200	0	18200	220
P37	16000	0	16000	21000	0	21000	106
P38	23400	0	23400	14500	0	14500	72
Mean	**19600**	**0**	**19600**	**17000**	**0**	**17000**	**122**

Ostertagia adult counts of 4760, 6300, 4080 and 2920 were recovered from Groups 1, 2, 3 and 4 respectively compared with 19600 from the challenge control group. Mean total *Cooperia* worm burdens in Groups 1 , 2 , 3 and 4 were 140, 640, 0 and 0 which was significantly lower than the mean burden of 17000 recovered from the challenge control calves. No lungworms were found in any of

the treatment groups compared with the mean of 122 recovered from the challenge control animals.

CONCLUSION

Based on both parasitological and biochemical analyses it appeared that none of the first season grazing calves were immune to infection with gastrointestinal nematodes or lungworm during the second grazing season. Comparatively lower serum pepsinogen concentrations and faecal egg counts in the animals of the former control group throughout the second grazing season might imply that these cattle were slightly more immune to gastrointestinal nematodes. However at necropsy of the challenged animals, at the end of the housing period, and those removed midway through the second grazing season, the gastrointestinal and lungworm burdens between all former grazing groups were very similar; this does not provide any evidence for a greater immune response by the control group.

The lack of significant immunity to challenge with gastrointestinal nematodes seen at the end of the first housing period may reflect a waning of any immunity acquired after exposure to infection during the first grazing season. Surprisingly, the establishment of *Ostertagia* in the two animals of the ex-control group was similar to that seen in the parasite naive control group. That the previously grazed animals showed no immunity to lungworm might however be related to lack of exposure due to the negligible pasture larval challenge during the first grazing season.

At the end of the second grazing season, analysis of worm burdens following experimental challenge indicated that animals in all groups had variable numbers of arrested fourth stage *Ostertagia* larvae compared with a complete absence of such larvae in the parasite naive controls. The higher establishment of *Ostertagia* EL4 in some of the yearlings in all of the treatment groups is most likely the result of an immune response but the consequences of any such arrested populations in adult cattle is not known. As expected, a sound immunity to

Cooperia and *Dictyocaulus* appeared to have developed by the end of the second grazing season.

CHAPTER 4

AN ABATTOIR SURVEY OF *Ostertagia* WORM BURDENS AND SERUM PEPSINOGEN CONCENTRATIONS IN ADULT DAIRY CATTLE

INTRODUCTION

The importance of ostertagiosis in young dairy calves is well recognised (Armour 1974 and Berghen *et al.* 1990) but clinical ostertagiosis in adult cattle has also been reported (Wedderburn 1970 and Smith and Perault 1972) although it is less common. However, there is a danger that a change of epidemiological pattern of *Ostertagia* infection may lead to a greater prevalence of ostertagiosis in older animals with increased economic loss (Nansen 1993). It has been suggested that this problem could arise under intensive nematode control programmes or new cattle management systems e.g. aggressive chemoprophylaxis with highly efficient anthelmintics or intensive grazing systems.

As there is little or no age immunity to *Ostertagia* infection (Martin 1977 and Kloosterman *et al.* 1991) ostertagiosis may occur in older animals if they are not adequately exposed to infection during their first or second grazing seasons and recent studies suggest that immunity to *Ostertagia* can be affected by the use of highly efficient anthelmintics or long term anthelmintic suppression (Herbert and Probert 1987 and Jacobs *et al.* 1987d).

An early report on the occurrence of Type II ostertagiosis in a group of older cattle by Wedderburn (1970) related this outbreak of disease to a lack of nutrition and the consequent relaxation of immunity to *Ostertagia*. Unexpectedly high larval counts from pasture grazed by a group of second year cattle have also been reported (Armour 1989): At the end of their second grazing season these animals, which had experienced a shortage of grass and inadequate nutrition, also had high *Ostertagia* worm burdens.

It has also been suggested that some factors associated with stress e.g. parturition and lactation also interfere with the immunity to *Ostertagia* acquired by cows resulting in higher worm egg counts around parturition (Borgsteede 1978; Michel *et al.*, 1979 and Armour 1989). This periparturient increase in faecal egg output could be due, as in sheep, to the resumption of development of inhibited EL4, the establishment of new infections or an increase in the egg laying capacity of existing female parasites.

The effect of sub-clinical ostertagiosis on milk yield in cows has been studied extensively (Bliss and Todd 1977; Bisset, Marshall and Morrison 1987 and Ploeger, Schoenmaker, Kloosterman and Borgsteede 1989). However worm egg counts from lactating cows are generally low (Borgsteede 1982) and while worm burdens are low to moderate, some individual high counts are occasionally recorded (Gutierres, Todd and Crowley 1979; Burrows, Davison and Best 1980; Bairden and Armour 1981; Borgsteede and Burg 1982; Vercruysse *et al.* 1986 and Marnu, Wintersteller and Prosl 1987).

Current information suggests that older animals may be playing a more important epidemiological role in outbreaks of ostertagiosis in dairy cattle but as yet there is no clear understanding of this role. There have been few recent studies of *Ostertagia* burdens in adult dairy cattle since the report by Bairden and Armour (1981) from Scotland and information on serum pepsinogen concentrations in older cattle is also limited.

AIMS

This study was undertaken in adult dairy cattle to determine the level of abomasal worm burdens at necropsy, their seasonal variation and any relationship with serum pepsinogen values.

EXPERIMENTAL DESIGN

From April 1994 to March 1995 the abomasa of adult Friesian cattle were collected from an abattoir in Larkhall, West of Scotland. As far as possible the abomasa and blood samples from five animals were collected each week. The total number of abomasa and blood samples collected was 201 and these were processed in the laboratory for worm counts and serum pepsinogen concentrations respectively. Because of the blood collection method, i.e from the heart at necropsy, some of the samples were found to be unsuitable for pepsinogen determination.

To determine the seasonal fluctuations in worm burdens and serum pepsinogen concentrations, the data were analysed according to winter (December to February), spring (March to May), summer (June to August) and autumn (September to November) and also according to calendar months.

RESULTS

Ostertagia BURDENS

Frequency distribution of *Ostertagia* burdens and their corresponding serum pepsinogen levels are shown in **Table 6** while individual worm counts are detailed in **Appendix 7.** Thirty nine of the abomasa had no worms present. One hundred and twenty five animals, the largest group, showed low to moderate worm burdens (< 10000) with thirty seven of the animals having moderate to high worm burdens (>10000); this included five animals with worm counts >100000.

Table 6 Frequency distribution of *Ostertagia* worm counts and their mean pepsinogen concentrations (iU) of 201 cattle

Worm burden	No. of animals	Percentage	iU tyrosine
0	39	19.4%	1.59
< 100	1	0.4%	*
101-1000	41	20.3%	1.81
1001-10000	83	41.2%	1.60
10001-100000	32	15.9%	1.69
>100000	5	2.4%	1.82

* no serum sample

The highest individual worm count recorded was 393500 which included almost 100% L4.

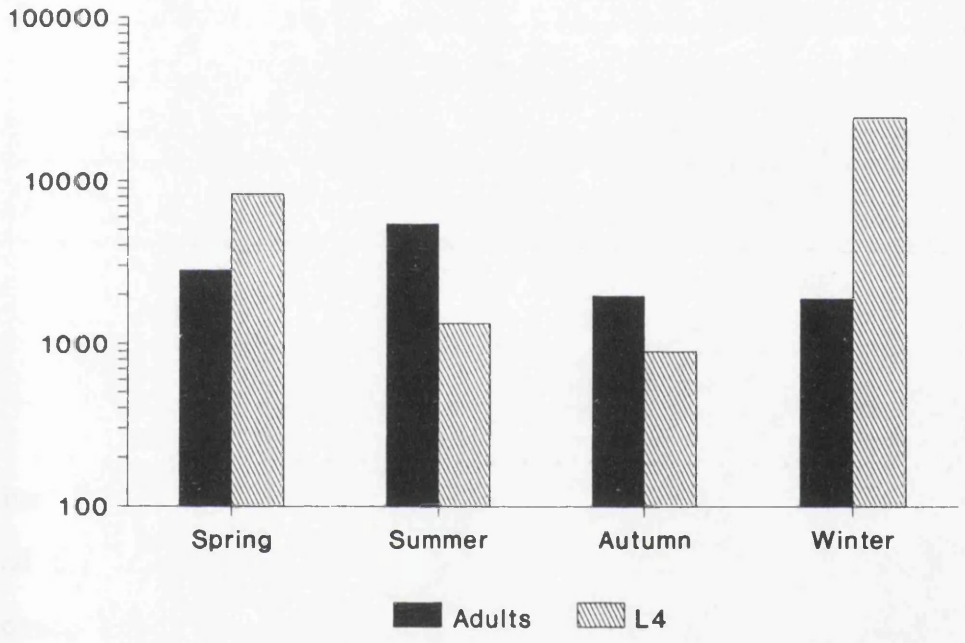
Mean seasonal worm burdens, together with the percentage of L4 are shown in **Table 7** and illustrated in **Figure 6**.

Table 7. Mean seasonal *Ostertagia* worm burdens

	O. o L4	O. o Total
Spring	8240 (74.4%)	11064
Summer	1334 (20.4%)	6534
Autumn	895 (31.0%)	2851
Winter	24377 (93.8%)	26271

Ostertagia L4 counts were significantly lower during summer and autumn than those recorded during winter and spring. No significant differences were found between seasons in mean *Ostertagia* adult worm burdens.

Figure 6 Seasonal variation of mean *Ostertagia* adult and L4 worm burdens

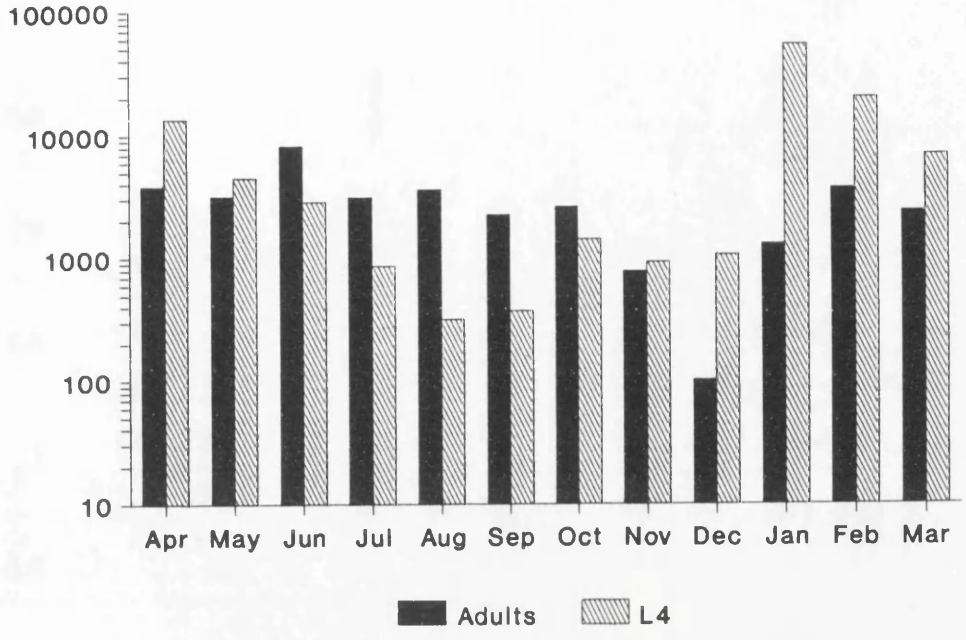


For both *Ostertagia* adults and L4, monthly fluctuations were observed (**Figure 7**) with the highest incidence of L4 being seen in January. *Ostertagia* adult counts were slightly higher in the spring and summer months than in most other months of the year.

SERUM PEPSINOGEN CONCENTRATIONS

Serum pepsinogen concentrations are illustrated in **Figures 8** and **9** with individual data being recorded in **Appendix 7**. Again a seasonal pattern was

Figure 7 Mean monthly *Ostertagia* adult and L4 worm burdens



observed with the highest mean pepsinogen concentrations being seen in summer and the lowest in spring. Statistical analysis showed that serum pepsinogen concentrations in summer were significantly higher than those in autumn, winter and spring serum pepsinogen concentrations were significantly lower than those recorded in summer and autumn. Monthly analysis of data revealed that, with the exception of November, significantly higher serum pepsinogen concentrations occurred in June, July, August and September than in any other month. There was no significant correlation between serum pepsinogen concentrations and L4 , adult or total *Ostertagia* worm counts.

CONCLUSION

The overall prevalence of *Ostertagia* infection in adult dairy cattle was 80.6% this observation contrasting to that previously described by Bairden and Armour (1981) for the West of Scotland. The magnitude of the *Ostertagia* burdens had also increased when compared to Bairden and Armour's study. The

Figure 8 Seasonal variation of mean serum pepsinogen concentrations

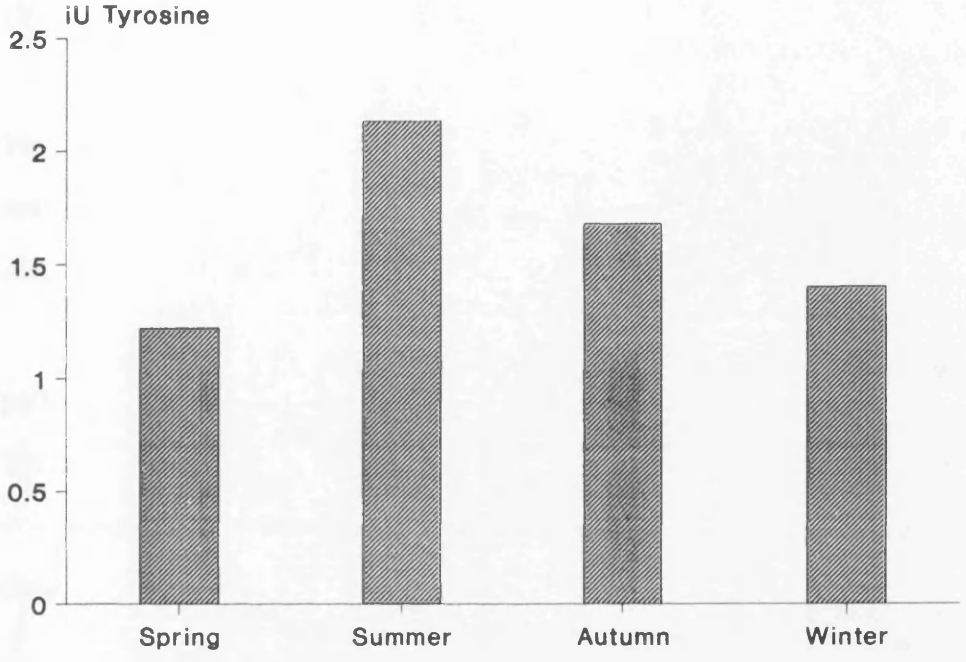
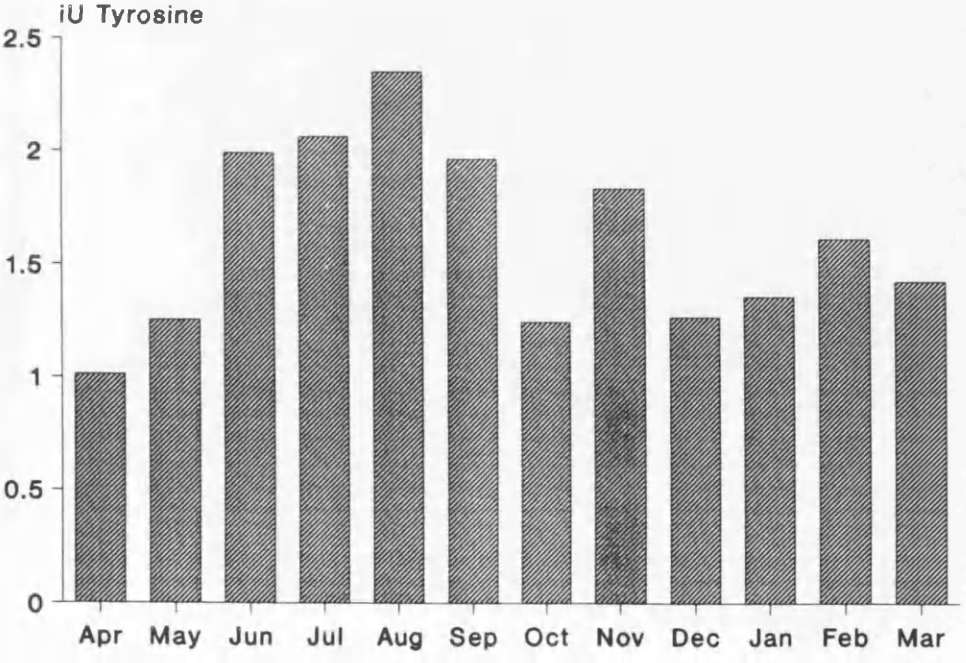


Figure 9 Mean monthly serum pepsinogen concentrations



intensive nematode control methods and other management systems introduced in the later part of the eighties might have contributed to these unexpected results. However these results are in agreement with those recorded in other countries (Vercruysse *et al.* 1986).

The observed seasonal and monthly fluctuations in the *Ostertagia* worm burdens resemble the pattern described for young cattle. Also these results confirm the findings of similar studies carried out for older cattle (Bairden and Armour 1981 and Marnu *et al.* 1987).

A summer increase of serum pepsinogen concentrations was observed particularly during July and August and this observation confirms that of Vercruysse *et al.* (1986).

There was no correlation between serum pepsinogen concentrations and *Ostertagia* adult, L4 and total worm counts.

CHAPTER 5

EVALUATION OF TWO TECHNIQUES FOR THE RECOVERY OF *Ostertagia* FOURTH STAGE LARVAE FROM BOVINE ABOMASA AND TWO METHODS COMMONLY USED FOR THE ESTIMATION OF BOVINE SERUM PEPSINOGEN CONCENTRATIONS

INTRODUCTION

Several laboratory techniques are used in the diagnosis of nematode infections of ruminants and these techniques demonstrate the presence of parasites either directly, for example postmortem worm counts and faecal egg counts or indirectly, for example serum pepsinogen concentrations.

Although useful in detecting the presence of infection or confirming a diagnosis, different techniques used to assess a particular parameter may give different results in different laboratories thus complicating any comparison or interpretation of results. These variations are attributed mainly to differences in methodology employed.

AIMS

The aims of the studies reported in this chapter were to evaluate several of the laboratory methods used in the experiments described earlier in this thesis. The techniques assessed were those used to estimate serum pepsinogen concentrations (Edwards *et al.* 1960) and *Ostertagia* L4 recoveries (Herlich 1956); these were compared for efficacy and convenience, with other techniques currently in use for pepsinogen estimations (Paynter 1992) and *Ostertagia* L4 recoveries (Williams *et al.* 1977) in other laboratories.

A COMPARISON OF TWO METHODS FOR THE RECOVERY OF *Ostertagia* L4 FROM THE BOVINE ABOMASUM

INTRODUCTION

The retrieval of *Ostertagia* L4 from the abomasa of ruminants forms an essential part of diagnostic parasitology and research work. One established method for the recovery of L4 from the abomasal mucosa of ruminants is by digestion in a pepsin/HCL mixture for 6 hours (Herlich 1956 and Ritchie *et al.* 1966). Although results from this technique are satisfactory it is time consuming, some of the reagents required, for example pepsin, are expensive and larvae may

be damaged in the process of recovery (Downey 1981). An alternative method, based on soaking of the abomasum in lukewarm physiological saline or tap water for 4-6 hours at 37°C, has also been described (Williams *et al.* 1977 and Snider, Williams, Knox, Roberts and Rommairre 1985).

These two methods have been evaluated and compared in sheep by Jackson, Jackson and Smith (1984) and in cattle by Downey (1981) who concluded that the incubation method was as efficient as digestion. However, results obtained from parasitological studies carried out over a number of years in the Parasitology Department of Glasgow University Veterinary School suggested that *Ostertagia* L4 recoveries obtained by the digestion method were higher than those obtained by Baermannisation.

Larval inhibition occurs during late autumn and winter in northern temperate climates and *Ostertagia* EL4 persist for long periods in the abomasal mucosal glands. During these periods it is probable that larval motility is minimal and, since Baermannisation is based on the active movement of L4 from the mucosal glands, larval recovery by this method would seem to be less efficient than digestion although the results of Downey's comparison of these two techniques (Downey 1981) do not support this view.

This study was designed to compare the efficacy and convenience of digestion and Baermannisation methods in recovering *Ostertagia* L4 and to determine whether seasonal differences in "depth" of *Ostertagia* larval arrest contributed to the efficacy of such larval recoveries.

EXPERIMENTAL DESIGN

The study was carried out from April 1994 to March 1995. The abomasa of 201 Friesian and Friesian cross bred adult cattle obtained during an abattoir survey were examined for *Ostertagia* L4 recoveries. As far as possible 5 abomasa were processed each week.

Within 3 hours of necropsy abomasa were transported to the laboratory. After washing the abomasa were bisected and one half digested with pepsin/HCL

for six hours (Ritchie *et al.* 1966) and the other half soaked in normal saline for six to eight hours (Williams *et al.* 1977 and Snider *et al.* 1985). Counting of *Ostertagia* L4 and identification techniques were as described in the Materials and Methods.

Efficacy of recovery was determined by comparing the numbers of *Ostertagia* L4 recovered by each method. Seasonal and monthly L4 recoveries by each method were compared.

RESULTS

Tables 8 and **9** show the mean monthly and seasonal *Ostertagia* L4 recoveries from each half of the mucosa and their significance levels with individual data being detailed in **Appendix 8**. The seasonal and monthly variation of *Ostertagia* L4 recoveries are shown in **Figures 10** and **11**.

Table 8 Monthly variation of mean *Ostertagia* L4 recoveries by Digestion and Baermannisation

Month of necropsy	Mean L4 recoveries/ Digestion	Mean L4 recoveries/ Baermannisation	Significance P value
April	13631	6168	0.93
May	1539	4156	0.09
June	2653	2053	0.97
July	817	1083	0.00
August	300	520	0.56
September	350	350	0.93
October	1400	360	0.20
November	757	643	0.97
December	1040	2280	0.21
January	22835	19505	0.53
February	10567	17772	0.13
March	7357	7057	0.25

Table 9 Seasonal differences in *Ostertagia* L4 recoveries by Digestion and Baermannisation

Season	L4 recoveries/ digestion	L4 recoveries/ Baermannisation	Significance P value
Spring mean	7509	5793	0.12
range	(0-192600)	(0-66400)	
Summer mean	1257	1219	0.39
range	(0-13000)	(0-9600)	
Autumn mean	835	451	0.41
range	(0-9600)	(0-7200)	
Winter mean	11480	13185	0.45
range	(0-171200)	(0-232000)	

Figure 10 Seasonal variation of mean *Ostertagia* L4 recoveries by Digestion and Baermannisation

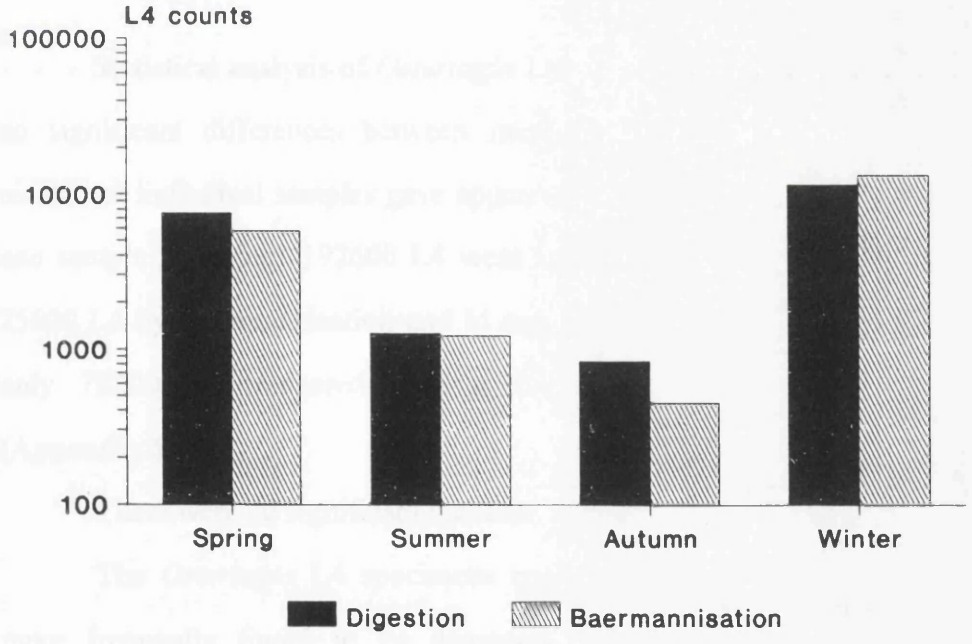
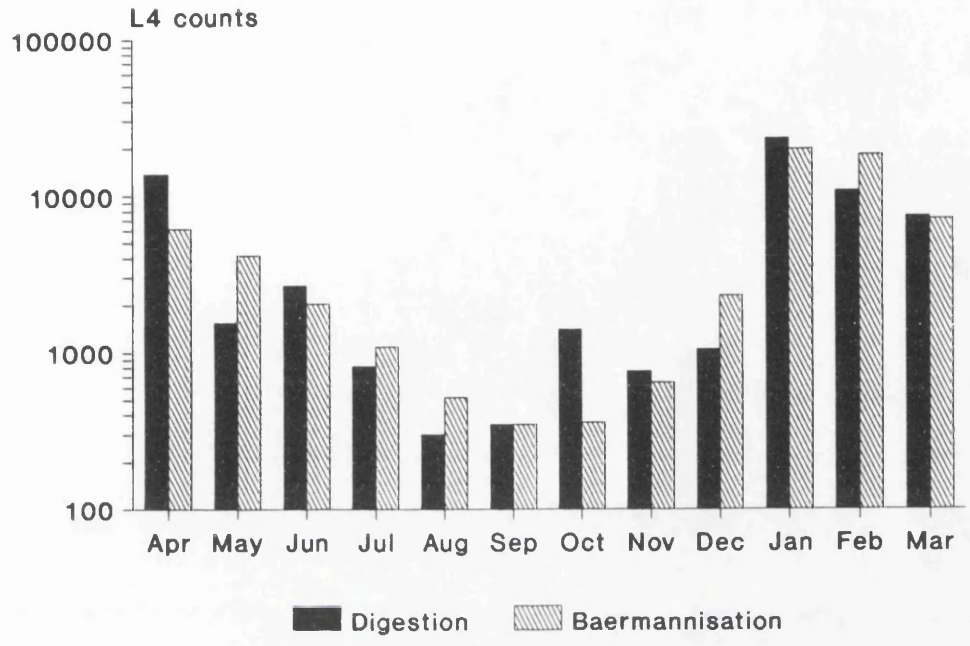


Figure 11 Mean monthly *Ostertagia* L4 recoveries by Digestion and Baermannisation



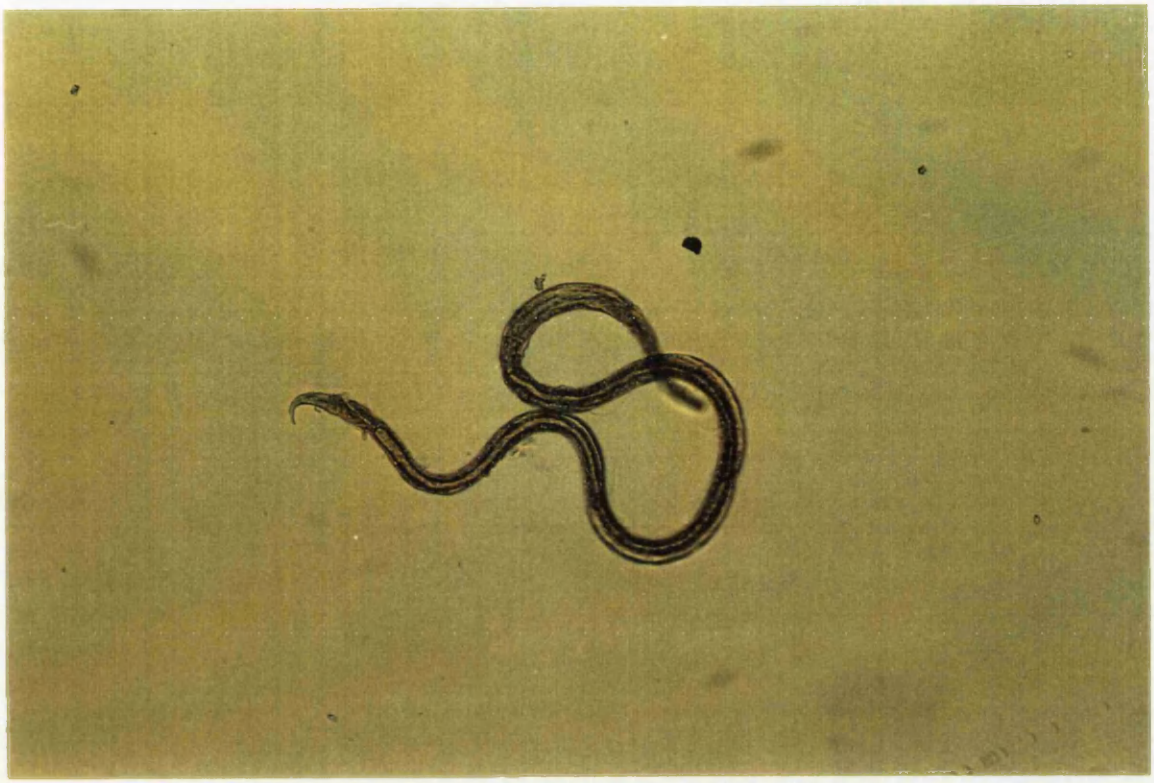
Statistical analysis of *Ostertagia* L4 recoveries from 201 abomasa yielded no significant differences between methods. However very occasionally the results of individual samples gave apparently conflicting results. For example in one sample in spring 192600 L4 were recovered by digestion compared with 25600 L4 by Baermannisation and in one sample in winter digestion recovered only 78200 L4 compared with a Baermannisation recovery of 232000 (**Appendix 8**).

There were no significant seasonal differences between methods.

The *Ostertagia* L4 specimens recovered by the digestion method were more frequently found to be damaged compared with those recovered by Baermannisation (**Plates 2** and **3**).

Plate 2 *Ostertagia* 4th stage larvae recovered by mucosal Baermannisation

Plate 3 *Ostertagia* 4th stage larvae recovered by mucosal Digestion



CONCLUSION

The method known as saline incubation (Williams *et al.* 1977 and Snider *et al.* 1985) is described here as saline Baermannisation as the recovery of *Ostertagia* L4 was based on larval motility.

The results of this work confirm that both methods were, in the majority of cases, equally efficient in recovering *Ostertagia* L4 from cattle abomasa. However, Baermannisation has some advantages over digestion and could be recommended for routine laboratory use. The advantages of Baermannisation include the fact that the technique requires no expensive material, the specimens of L4 larvae recovered are in good condition and less time is taken to process the abomasa and subsequently examine samples. The Baermannisation method may be preferable in anthelmintic evaluation studies which involve post mortem worm counting as early as 7-9 days after treatment since it has been suggested that fourth stage larvae, despite being killed by treatment may persist for up to 9 days post treatment (Snider *et al.* 1985); if the digestion technique were used in such cases it would be very difficult to differentiate between L4 which were alive or dead at the time of necropsy.

A COMPARISON OF TWO COLOURIMETRIC ASSAYS FOR THE ESTIMATION OF SERUM PEPSINOGEN CONCENTRATIONS

INTRODUCTION

Since pepsin was discovered and named by Schwann in 1836 (Hirschowitz 1984) considerable knowledge has accumulated on its chemical, biological, physiological and pathophysiological features in a wide range of animals. Pepsinogen is secreted from the chief cells of the stomach of man and animals and

it requires an acid medium to become the active proteolytic enzyme, pepsin. The activation of pepsinogen to pepsin is extremely rapid at pH 2 or less. The pH optimum for the digestion of substrate varies for different substrates e.g for haemoglobin and albumin it is 1.8 and 2.3 respectively.

In humans, serum pepsinogen concentrations have been used as an aid in the diagnosis of duodenal ulcers and functional dyspepsia (Edwards *et al.* 1960) and in the 1960's estimation of serum pepsinogen concentrations was a common procedure in clinical monitoring of gastric ulcers and other related conditions.

Among the domesticated species, elevated serum pepsinogen concentrations have been identified as one of the major pathophysiological consequences of ostertagiosis in cattle (Anderson *et al.* 1965a) and sheep (Scott, Stear and McKellar 1995) and haemonchosis in sheep (Meana, Miro and Rojo-Vazquez 1991) and goats (Fox, Jacobs and Sani 1991) and it is widely accepted as a reliable indicator of abomasal damage in ostertagiosis and haemonchosis (Anderson *et al.* 1965a; Armour 1970; Berghen, Dorny and Vercruysse 1987; Michel, Lancaster, Hong and Berrett 1978; Vercruysse *et al.* 1987; Williams *et al.* 1987, Fox *et al.* 1991 and Berghen *et al.* 1993). Serum concentrations of pepsinogen have been shown to increase by more than 3 iU of tyrosine in response to *O. ostertagi* infections in cattle (Selman *et al.* 1977) and pepsinogen concentrations have been shown to correlate with different levels of experimental infection with *O. ostertagi* (Jennings *et al.* 1966).

Most of the techniques described for the estimation of serum pepsinogen concentrations are based on the measurement of total proteolytic activity of pepsin. Thus results are affected by incubation time, pH, temperature, substrate and enzyme concentration (Hirschowitz 1984 and Berghen *et al.* 1987). For veterinary use most laboratories use different versions of the original method developed for the diagnosis of duodenal ulcers in humans (Edwards *et al.* 1960) and since a number of factors can influence the rate of proteolysis, the results obtained by different laboratories are not necessarily comparable. Although other methods have been developed for the measurement of serum pepsinogen e.g the

enzyme linked immunosorbent assay (ELISA, Turner and Shanks 1982) and radial diffusion (Jensen 1977) these are not commonly used because they require expensive materials (ELISA) or are semiquantitative (radial diffusion).

In performing serum pepsinogen assays Berghen *et al.* (1987) observed that a pH difference of 0.5 could make considerable differences in tyrosine release. As this indicated that the stability of pH within a pepsinogen assay is important, they suggested that the use of glycine-buffered BSA as a substrate would reduce variation by enhancing the stability of pH within the assay. In the Department of Veterinary Parasitology, Glasgow University Veterinary School the pepsinogen assay which has been routinely used in studies of ostertagiosis is a modification of the original method devised by Edwards *et al.* (1960). The main difference between this method and that described by Berghen *et al.* (1987) is that it uses an unbuffered-BSA substrate. Recently, Paynter (1992) introduced a method which, in addition to using a glycine-buffered BSA, has the advantage of a shorter incubation period.

Two of the most important requirements of any assay are precision and reproducibility. It is important to know that any observed differences in values are the result of pathological changes in the abomasum following infection and not due to the inability of the assay employed to give repeatable results. This can be measured by inter-assay variation. When intra-assay variation is high the ability of an assay to identify differences between animals is markedly reduced.

This study was carried out to evaluate and compare the repeatability and convenience of the methods described by Edwards *et al.* (1960, original method) and Paynter (1992, micro method) for the quantification of pepsinogen concentrations in bovine serum.

EXPERIMENTAL DESIGN

Detailed procedures of both methods are given in **Appendix 1.**

Serum samples were obtained from the animals used in the chemoprophylaxis study described in Chapter 4. On the basis of their pepsinogen

values (previously determined using the method of Edwards *et al.* 1960) sera with high or low pepsinogen activities were combined into batches to obtain a sufficient volume of serum for comparative testing.

Within Day Variation

Five replicates of ten serum samples, five with a high level of activity and five with a low level of activity, were assayed on a single day (50 tests/assay). The mean, SD and coefficient of variation (CV) were calculated separately for low and high pepsinogen activity samples.

Day to Day Variation

Ten serum samples, five with a high level of activity and five with a low level of activity, were assayed on five consecutive days (50 tests/assay) including the first assay from the within day variation. The mean, SD and CV were calculated separately for low and high pepsinogen activity samples.

RESULTS

Comparison of Tests

The micromethod gave values approximately 400% higher than the original method and the results are therefore not directly comparable. The results are shown in **Table 10**.

Within Day Variation

For the high pepsinogen samples tested with the original method, the CV was 17.0% compared with 17.3% for the low pepsinogen samples. For comparison, the CV of the same samples tested with the micromethod were 21.2% for the high pepsinogen samples and 48.6% for the low pepsinogen samples.

Table 10 Individual and mean±SD serum pepsinogen concentrations (iU)

LOW PEPSINOGEN ACTIVITY SAMPLES

Original Method

Same Day
Replicates

Serum Batch	1	2	3	4	5	Mean	SD
A	0.70	0.62	0.99	0.88	0.79	**0.80**	**0.15**
B	0.78	0.83	0.88	0.92	0.76	**0.83**	**0.07**
C	0.55	1.14	0.73	1.06	0.62	**0.82**	**0.27**
D	0.85	1.14	0.94	0.89	1.09	**0.98**	**0.13**
E	1.02	1.03	1.17	1.03	0.92	**1.03**	**0.09**

Micro Method

Same Day
Replicates

Serum Batch	1	2	3	4	5	Mean	SD
A	2.97	3.14	3.99	2.00	1.61	**2.74**	**0.95**
B	1.06	3.51	1.28	5.02	1.56	**2.49**	**1.72**
C	1.43	3.07	1.74	0.94	4.00	**2.24**	**1.26**
D	2.31	3.32	2.76	2.46	2.95	**2.76**	**0.40**
E	0.34	0.81	1.56	0.60	1.32	**0.93**	**0.51**

Original Method

Consecutive Days
Days

Serum Batch	1	2	3	4	5	Mean	SD
A	0.70	0.87	0.96	0.85	1.19	**0.91**	**0.18**
B	0.78	0.74	0.77	0.71	0.73	**0.75**	**0.03**
C	0.55	0.55	0.37	0.48	0.65	**0.52**	**0.10**
D	0.85	1.13	0.95	0.95	0.95	**0.97**	**0.10**
E	1.02	0.46	0.46	0.49	1.01	**0.69**	**0.30**

Micro Method

Consecutive Days
Days

Serum Batch	1	2	3	4	5	Mean	SD
A	2.97	4.53	3.94	3.51	2.21	**3.43**	**0.89**
B	1.06	4.23	4.24	2.78	3.33	**3.13**	**1.31**
C	1.43	4.12	2.23	1.14	2.28	**2.24**	**1.16**
D	2.31	1.52	3.93	4.17	1.65	**2.72**	**1.26**
E	0.34	4.53	2.17	2.64	1.53	**2.24**	**1.54**

Table 10 (continued)

HIGH PEPSINOGEN ACTIVITY SAMPLES

Original Method

Same Day
Replicates

Serum Batch	1	2	3	4	5	Mean	SD
F	2.59	3.17	2.37	2.68	2.85	**2.73**	**0.30**
G	3.06	3.12	2.86	2.96	3.28	**3.00**	**0.16**
H	2.41	2.87	3.71	2.81	2.40	**2.84**	**0.53**
I	1.82	2.42	3.52	2.36	2.92	**2.61**	**0.64**
J	2.89	2.36	2.22	3.07	3.66	**2.84**	**0.58**

Micro Method

Same Day
Replicates

Serum Batch	1	2	3	4	5	Mean	SD
F	16.92	15.67	13.13	11.97	11.24	**13.79**	**2.43**
G	15.73	14.49	11.94	16.72	12.63	**14.30**	**2.02**
H	14.58	9.10	4.69	10.09	8.71	**9.43**	**3.54**
I	16.79	15.99	10.68	13.66	11.19	**13.60**	**2.75**
J	9.95	6.67	7.85	8.75	7.33	**8.11**	**1.28**

Original Method

Consecutive Days
Days

Serum Batch	1	2	3	4	5	Mean	SD
F	2.59	4.80	3.17	3.63	4.23	**3.68**	**0.87**
G	3.06	5.48	2.21	6.27	3.93	**4.19**	**1.68**
H	2.41	3.60	2.62	2.38	3.18	**2.84**	**0.53**
I	1.82	3.59	2.38	3.08	3.36	**2.85**	**0.73**
J	2.89	4.26	3.06	2.64	3.83	**3.34**	**0.68**

Micro Method

Consecutive Days
Days

Serum Batch	1	2	3	4	5	Mean	SD
F	16.92	18.89	18.02	15.18	14.83	**16.77**	**1.76**
G	15.73	11.33	14.70	18.67	9.47	**13.98**	**3.64**
H	14.58	14.01	13.97	9.91	8.70	**12.23**	**2.72**
I	16.79	7.28	8.89	8.55	8.48	**10.00**	**3.85**
J	9.95	13.89	12.67	14.32	13.20	**12.81**	**1.72**

Variation Among Days

For the high pepsinogen samples tested with the original method over 5 days the CV was 21.4% compared with 21.1% for the low pepsinogen samples. With the micromethod the CV was 21.4% for the high pepsinogen samples and 36.9% for the low pepsinogen samples.

CONCLUSION

Similar, and only minor, variation was observed with both tests on samples with a high pepsinogen activity. With low pepsinogen activity samples the micromethod showed greater variation but the results recorded were acceptable in the low range for this method.

CHAPTER 6

GENERAL DISCUSSION

Control of infection with bovine gastrointestinal nematodes relies mainly on the strategic administration of anthelmintics based on a knowledge of parasite epidemiology. Almost ten years ago the successful use of early season chemoprophylaxis in first season grazing calves was reported (Armour *et al.* 1987b and Jacobs *et al.* 1987). Such treatments limit faecal egg output considerably during the first half of the grazing season thus reducing the accumulation of infective larvae on pasture in late summer. However it has been suggested that the use of highly efficient anthelmintics to minimise pasture larval burdens may interfere with host immune responses to parasitic nematodes (Herbert and Probert 1987 and Jacobs *et al.* 1987d and Vercruysse, Hilderson and Claerebout 1995). As there is little or no age immunity to *Ostertagia* infection (Martin 1977 and Kloosterman *et al.* 1991) disease may occur in older animals if they are not adequately exposed to larval challenge in previous grazing seasons. Nansen (1993) has also suggested that aggressive anthelmintic control may lead to the translocation of parasite problems from young calves to adult cattle resulting in a greater economic loss.

In relation to gastrointestinal parasites, the epidemiological data obtained from this study was similar to that described for the West of Scotland (Armour *et al.* 1979; Armour *et al.* 1988b and Bairden 1991) with low levels of overwintered larvae being present at turnout. That these larvae were cycled by the yearlings was shown by eggs appearing in the faeces 5 weeks post turnout. This contamination together with that from the seeder calves resulted in a moderate increase in larval challenge during the summer. This coincided with an outbreak of parasitic bronchitis and sub clinical PGE in most of the yearlings irrespective of previous treatment. This indicated that the yearlings, including the previous control animals, were not immune to *Ostertagia* or *Dictyocaulus* during the first half of the second grazing season. The susceptibility of the yearlings to *D. viviparus* was not surprising since there was no evidence of significant exposure to lungworm during their first grazing season. Berghen *et al.* (1990) have also observed an outbreak of parasitic bronchitis in a group of yearlings which they

related to an inadequate acquisition of immunity during their first grazing season. However, the cattle in this study had been exposed to both *Ostertagia* and *Cooperia* and it could have been expected that a moderate immunity, at least to *Cooperia* would have developed. In a similar study by Armour *et al.* (1979) yearlings infected with *Ostertagia* and *Cooperia* in their first grazing season, were immune to natural challenge with these parasites in the second season. This was also found by Berghen *et al.* (1990) and Gronvold *et al.* (1992) who have observed that yearlings pass negligible numbers of nematode eggs during their second grazing season provided that they have been adequately exposed during their first grazing season.

Despite the low egg counts in the study reported here, gastrointestinal nematode larval levels on pasture fluctuated throughout the grazing season with a moderate autumn increase which could result in a significant overwintered population on the herbage for the following spring.

With regard to weight gain, there was little or no difference in bodyweights between the groups early in the grazing season. A marked increase in the group mean bodyweights of all four groups was observed after anthlelmintic therapy in July and this trend continued for the rest of the season. The observed increase in group mean bodyweights after treatment could be due to several factors. Obviously it was partly due to a reduction in adult worm burdens (Fox 1993) which was demonstrated by the fall in faecal egg and larval counts. Secondly, by the removal of eight animals from the paddock, the stocking rate was decreased thus increasing pasture availability per animal and thirdly, the lighter, clinically ill animals from each group were selected for necropsy which artificially increased the group average bodyweights. Analysis of bodyweights, after removal of data pertaining to the animals necropsied mid-season, suggsted that the apparent increase in group bodyweight was caused by the absence of these lighter animals. There was no significant difference between the prophylactically treated groups and the controls in mean live weight gain over two grazing seasons and during the first grazing season, the animals receiving

anthelmintic prophylaxis had shown no difference in bodyweights compared to the control group (Dr. K. Bairden unpublished data). Since growth performance of yearlings may be affected by trichostrongyle worm burdens (Conder and Jones, 1983; Borgsteede, Kloosterman, Oostendoop and Van Tarriji, 1985a), it is possible that the therapeutic treatment of the controls with fenbendazole (FBZ) on Day 109 of the first grazing season, their anthelmintic treatment with FBZ at housing and the further therapeutic treatment with FBZ on Day 63 of the second grazing season would account for their reasonable performance in terms of weight gain. Bairden (1991) in an anthelmintic prophylaxis study comparing ivermectin at 3,8 and 13 weeks post turnout and an MSRT given at turnout, observed no difference in bodyweights between the prophylactically treated and control animals during a second grazing season and related this to a therapeutic treatment instigated during the first grazing season to control an outbreak of PGE in the animals of the control group.

From turnout in this study until the time of therapeutic anthelmintic treatment, faecal egg counts increased at a moderate rate in all three prophylactically treated groups and at a slightly lower rate in the control animals. Although not statistically significant, this could indicate that the control animals had acquired some immunity to gastrointestinal nematodes (Armour 1989). The mid-season therapeutic treatment eliminated faecal egg output for at least 4 weeks after which positive egg counts were observed. Evidence of patent infections was not anticipated so quickly since both moxidectin and ivermectin have current persistent activity claims of fourteen to forty two days against the common gastrointestinal nematode parasites of cattle. The mild signs of PGE which included intermittent diarrhoea and reduced appetite together with the increased faecal egg counts observed in most of the animals during the first half of the season suggested that they were harbouring moderate gastrointestinal worm burdens. This, together with the necropsy results at the end of the first housing period suggests that the animals of all four groups were only partially immune to gastrointestinal parasites and non immune to lungworm challenge during the first

half of their second grazing season. It may be that in the absence of therapeutic involvement the normal course of the gastrointestinal infection would have progressed resulting in clinical PGE. This hypothesis is supported by two factors. Firstly, the animals of Groups 1 and 3 had positive egg counts a few weeks after anthelmintic therapy indicating that they were susceptible to reinfection. Secondly, the necropsy results at the end of the second grazing season showed that all of the former prophylactically treated animals and controls, after an artificial challenge, harboured moderate to substantial levels of *Ostertagia* EL4. It is interesting to note that one animal (No. 854) harboured an *Ostertagia* burden in excess of the larval challenge given. Although experimental error could not be dismissed it may be that the anthelmintic treatment at housing did not remove all of the *Ostertagia* burden acquired after mid season therapy.

Faecal larval counts reflected the *Dictyocaulus* larval recoveries from pasture. During the first 8 weeks of grazing no larvae were found in faeces and during this period pasture *Dictyocaulus* larval recoveries were negative or very low. The highest lungworm larval recoveries from faeces coincided with the outbreak of parasitic bronchitis. Patent lungworm infections were present by Day 58 which was followed by treatment of all groups on Day 63. The course of the lungworm infection after treatment, as judged by the negative faecal larval counts for the rest of the grazing period implies that although the animals were susceptible during the first half of the grazing season they were immune thereafter. This was confirmed by the high establishment of lungworm challenge infection in the animals necropsied at the end of the first housing period, the high lungworm counts in the animals removed in the middle of the grazing season and the zero establishment of *Dictyocaulus* after challenge infection at the end of the second grazing season. In the current study therefore the yearlings were susceptible at least until Day 63 and after treatment they acquired a solid immunity to *Dictyocaulus* confirming earlier observations by Armour (1989) and Jarrett *et al.* (1957).

At the beginning of the grazing season serum pepsinogn concentrations in the control group were significantly higher than those of the prophylactically treated groups. This is in agreement with the findings of Bairden (1991) and Entrocasso *et al.* (1986b) in similar studies conducted over two years. Although serum pepsinogen concentrations increased gradually in all four groups, by the end of the second grazing season the pepsinogen concentrations of the control group were the lowest of all. As all animals had been given, at the end of their first grazing season, a housing treatment of FBZ, an anthelmintic with activity against inhibited *Ostertagia* (Duncan *et al.* 1978, Anderson and Lord 1979 and Nagle *et al.* 1980), the possibility of abomasal damage due to the development of arrested larvae being responsible for the higher pre-turnout pepsinogen concentrations is unlikely. However, Bairden (1991), in a study to compare the use of ivermectin at 3, 8 and 13 weeks post turnout or an MSRT bolus at turnout on acquired immunity, also observed similar changes in serum pepsinogen concentrations in the control group which he related to the severe abomasal damage suffered by these animals during the first grazing season. Entrocasso *et al.* (1986b) also observed higher concentrations of pepsinogen in control cattle at the beginning of their second grazing season compared with animals which had received an MSRB at turnout as calves. However, in his study, the control concentrations of serum pepsinogen did not fall below the levels observed in the animals of the prophylactically treated groups towards the end of the grazing season. This however, may have been due to the fact that a second bolus treatment was given to the previously bolused yearlings at the beginning of their second grazing season. It is reasonable to assume that this second bolus application prevented higher infection levels and consequently less abomasal damage and lower serum pepsinogen concentrations in the treated animals compared with the controls.

Until the time of therapeutic intervention, the serum pepsinogen concentrations of most of the animals in this study increased slowly but after treatment increased levels (> 2 iU Tyrosine) were observed in all four groups. Pepsinogen concentrations remained elevated for the rest of the grazing season.

Unlike the situation with the bodyweights, where removing the lightest animals had the effect of artificially increasing the group mean values, the group mean pepsinogen concentrations still increased despite the animals with the highest pepsinogen concentrations being removed.

Since pasture *Ostertagia* larval counts increased in the latter half of the grazing season indicating that the yearlings were under larval challenge over this period these elevated pepsinogen concentrations could be related to an immune reaction of the abomasal wall to ingested larvae resulting in an increased permeability of the mucosa to pepsinogen as suggested by Armour *et al.* (1979) and Entrocasso *et al.* (1986b).

Although the differences between groups were not significant, the comparatively lower serum pepsinogen concentrations in the control group, as with their egg counts, throughout the second grazing season imply that the animals of the control group may have had a slightly greater level of immunity to gastrointestinal nematode infection during this period. However, these observations were not supported by the necropsy results which suggested no difference in immunity between groups.

The worm burdens of the animals necropsied at the end of the first housing period showed that all of the animals, irrespective of whether they were in treatment groups or controls, were not immune to single challenge infections with *Ostertagia, Cooperia* and *Dictyocaulus*. This could be, as suggested by Xio *et al.* (1991) and Hilderson *et al.* (1993), due to a poor development of immunity to these nematodes during the first grazing season perhaps reflecting the chemoprophylactic measures and other treatments employed during the first grazing season, or, as Martin (1977) and Armour (1989) have suggested, a waning of any immunity acquired in the first grazing season during the housing period. As mentioned previously, the susceptibility of the yearlings to lungworm infection was not surprising as the animals had apparently encountered very little *Dictyocaulus* larval challenge in their first grazing season. As almost two grazing seasons are necessary to acquire protective immunity to *Ostertagia* (Entrocasso *et*

al. 1986b and Armour 1989), the observed lack of immunity to this genus at the end of first housing period could probably be related more to a slow acquisition of immunity rather than to the effects of any anthelmintic treatments or the waning of immunity. However, it is very difficult to determine exactly the reasons for this lack of immunity as their immune status, as determined by experimental challenge, was not evaluated at the end of the first grazing season

In the yearlings necropsied at the time of anthelmintic therapy high *Ostertagia* worm burdens were observed in the animals of all four groups with the highest *Dictyocaulus* burdens being seen in the moxidectin pour-on and ivermectin treated groups. However, the numbers of animals necropsied at this time were insufficient to provide any meaningful analysis.

The necropsy results following experimental challenge at the end of the second grazing season were interesting. The fact that the establishment of *Cooperia* and *Dictyocaulus* were extremely low in the animals of all four groups compared with the challenge controls indicated clearly that all of the animals had acquired a good immunity to these parasites by the end of the second grazing season. This is in agreement with the findings of Entrocasso *et al.* (1986b) in their two year study carried out to evaluate the efficacy of a morantel slow release bolus or early season FBZ treatments over two consecutive grazing seasons.

When compared with the challenge controls the establishment of inhibited *Ostertagia* larval populations in all four groups were significantly higher. Presumably such larval populations could resume development in a trickle manner or resume development *en masse* and give rise to the Type II disease (Michel 1976) or as shown by Taylor *et al.* (1995) eventually disappear without causing any marked clinical effect.

One of the problems with the abattoir survey, carried out to establish the parasitological status of adult cattle with respect to their *Ostertagia* worm burdens and serum pepsinogen levels, was that the previous grazing and treatment histories of the animals were not known. Also, since there was no indication as to whether these animals had been housed for any length of time prior to slaughter, it

was difficult to assess the L4 status with regard to inhibition. However, useful data which could be considered in relation to similar surveys carried out earlier in both the West of Scotland and elsewhere, were obtained.

The observed seasonal and monthly fluctuations in the *Ostertagia* worm burdens in this survey resemble those described earlier for young cattle in the West of Scotland by Anderson *et al.* (1965a) and also confirm the results of similar studies carried out in other countries for older cattle, for example in Austria (Marnu *et al.* 1987); Belgium (Vercruysse *et al.* 1986); The Netherlands (Borgsteede and Burg 1981); UK (Burrows, Denison and Best 1980; Bairden and Armour 1981) and the USA (Gutierres *et al.* 1979).

The overall prevalence of *Ostertagia* infection in this study was around 81%, which is comparable with the 100% prevalence of *Ostertagia* detected in studies carried out in Germany over 10 years ago (Barth, Bernhard and Lamina 1980) and in Italy (Vigilani 1981). However, at the same time Bairden and Armour (1981) in a study of *Ostertagia* worm burdens of cull cows in the West of Scotland, reported a much lower prevalence of 38%. This marked increase in the apparent prevalence of *Ostertagia* infection in adult cattle in the West of Scotland during the last 14 years may be due to a change in *Ostertagia* epidemiology. This could be the result of the introduction of intensive nematode control methods or new management systems in the latter part of the 1980's which may have interfered with the development of a high level of immunity in older cattle.

There are of course, other factors which can contribute to an increase in worm numbers in adult cattle. For example it is known that during the stress of pregnancy and lactation the immune response to gastrointestinal nematodes may be relaxed (Michel *et al.* 1979 and Armour 1989) and it is possible that the physiological status of some of the animals sampled in this study may have contributed to the high worm burdens and prevalence rate recorded.

The average total number of *Ostertagia* worms recorded in this study was almost 11600 which was approximately three times higher than that observed by Bairden and Armour (1981). In the USA Gutierres *et al.* (1979) found an average

Ostertagia burden of around 1000 worms in Wisconsin dairy cows but because of differences in management practices and climate it is difficult to compare the worm burden data recorded in studies from other countries. Bairden and Armour (1981) related the relatively low *Ostertagia* burdens in their survey to a high level of immunity acquired under the intensive grazing conditions encountered in the West of Scotland which again raises the question as to whether the immunity to *Ostertagia* in adult cattle is declining with the introduction of new management systems and/or intensive nematode control methods.

Significant differences in adult *Ostertagia* worm counts between seasons was not demonstrated in this study but when data was analysed on a monthly basis, significantly higher adult *Ostertagia* worm counts were recorded in the months of June, July, August, January, March and April compared with November. The pasture challenge encountered during summer grazing and the accumulation of EL4 in the latter part of winter and spring presumably contributed to the higher adult *Ostertagia* worm populations observed in these months. A similar adult *Ostertagia* worm distribution has been observed for young calves (Armour 1974).

There was a seasonal pattern in *Ostertagia* L4 counts which were significantly higher in winter than in summer and autumn. This supports the earlier observations of Bairden and Armour (1981) but the numbers of *Ostertagia* L4 recovered in their study were generally lower which may have been related to the source of the abomasa most of which came from a knackers yard and were in various stages of decomposition at the time of processing.

When analysed monthly a clear pattern of L4 burdens was also apparent with significantly higher L4 numbers in January, April and May than in July and in April than in August. The increased *Ostertagia* L4 numbers in January, April and May were likely to reflect inhibition of larvae ingested over the latter part of the grazing season. Over the same period the adult numbers declined. The reverse was observed when these larvae resumed development resulting in an increase in the adult burdens in July and August.

A clear seasonal variation in serum pepsinogen concentrations was observed with the pattern resembling that described by Vercruysse *et al.* (1986) who recorded an increase of pepsinogen concentrations during summer when pasture availability of larvae is generally at its highest. Similarly, Armour *et al.* (1979) maintained that serum pepsinogen concentrations in young calves reflect the availability of larvae from pasture. In this study, serum pepsinogen concentrations in the summer were significantly higher than in spring and autumn again suggesting that they followed the pattern of larval availability.

There was a clear monthly variation of serum pepsinogen concentrations with significantly higher serum pepsinogen concentrations in July than in January, March, April and May and in August than in April and May. Vercruysse *et al.* (1986) also observed an increase in serum pepsinogen concentrations between May and August.

Little correlation was found between the serum pepsinogen concentrations and either *Ostertagia* adult, L4 or total worm counts. This confirms the lack of correlation found by Vercruysse *et al.* (1986) and Barth *et al.* (1980) between these parameters. Although increases in serum pepsinogen concentrations are not specific for *Ostertagia* infection (Vercruysse *et al.* 1986) the estimation of serum pepsinogen remains a useful aid in the diagnosis of ostertagiosis in adult cattle where faecal egg output is generally low (Anderson *et al.* 1965a).

Traditional parasitological and biochemical techniques for the recovery of mucosal fourth stage *Ostertagia* larvae (Herlich 1956) and serum pepsinogen estimation (Edwards *et al.* 1960) were used in these studies. Another larval recovery method based on saline incubation (Williams *et al.* 1977 and Snider *et al.* 1985) and described here as saline Baermannisation since it is largely dependent on larval motility, was compared with the digestion technique of Herlich (1956) for the recovery of *Ostertagia* L4 from the bovine abomasal mucosa.

The efficacy of the two techniques was determined by examining the data for *Ostertagia* L4 recoveries and both were found to give similar results. This

supports the findings of Downey (1981) in cattle and Jackson *et al.* (1984) in sheep. Downey (1981) compared these two techniques using abomasa from naturally infected cattle and concluded that both methods were equally efficient in recovering *Ostertagia* EL4 while Jackson *et al.* (1984) found that saline Baermannisation was as efficient as digestion in recovering EL4 from sheep infected experimentally with *Ostertagia circumcincta.*

An important point worth noting is that saline Baermannisation was as efficient as the digestion method in recovering *Ostertagia* L4 all through the year indicating that L4 motility was not dependent on stage of development or inhibition.

It has been suggested that the digestion method is less suitable for anthelmintic evaluation studies as it has been observed that, even after 7-9 days post treatment, dead and degenerating *Ostertagia* larvae could still be recovered from abomasa (Snider and Williams 1980 and Snider *et al.* 1985). As the digestion of the abomasal mucosa kills all larvae, those killed by anthelmintic treatment, but still recoverable, cannot be distinguished from larvae still viable at the time of necropsy resulting in difficulties in interpreting the significance of L4 numbers found.

Another disadvantage of the digestion method is that it is relatively time consuming from scraping the mucosa, which can require considerable effort, to examination of aliquots of digested material for L4. The samples obtained by Baermannisation had less debris such as undigested fat and were easier to examine.

However, there are limitations of Baermannisation which have been reported. Firstly, the method may be suitable only for freshly killed animals as larvae will migrate from the mucosal glands shortly after necropsy (Downey 1981) and secondly, lesions in the abomasum may interfere with larval movement out of the mucosa which may make this method less suitable for the recovery of L4 from animals with chronic abomasal nodules (Gasbarre 1987).

The only biochemical technique used in these studies was the serum pepsinogen assay. The original assay introduced by Edwards *et al.* (1960) was compared with a micro method described by Paynter (1992). This study did not involve any controlled assessment of the accuracy of the assay , but both methods measured serum pepsinogen concentrations based on proteolytic activity. The within day variation of the two assays was determined on assessment of CV's obtained from the same day tests and the day to day variation of the assays was obtained on assessment of CV's from tests carried out over five consecutive days.

As expected the values obtained by the micro method were higher than those obtained by the original method, for the same pooled serum sample (Scott, Stear and McKellar 1995). These differences in serum pepsinogen concentrations obtained by different assays confirm the need to establish baseline concentrations of pepsinogen in serum for each method. For example for the original method serum pepsinogen concentrations >2 iU tyrosine (Selman *et al.* 1977) and for the micro method levels >8 iU tyrosine (Paynter 1992) are considered to be clinically significant.

With low pepsinogen activity samples higher day to day and within day variations were a consistent feature with the micro method and this probably could be related to a greater margin of operator error and other variations.

With high pepsinogen activity samples both methods showed similar day to day and within day variations.

Overall the original method was better in terms of low variation recorded both within day and among days. This may reflect a relatively low operator error factor due to the use of a higher quantity of materials in the original method.

Some advantages of the micromethod included a shorter incubation period and the fact that it requires very small quantities of serum and other reagents. The original method, however, could be improved by the use of glycine buffered BSA (Berghen *et al.* 1987) or if batches of samples could be analysed at the same time.

In summary, the micro method was less reliable than the original method for estimating low pepsinogen concentrations. This may be a reflection of the very

small quantity of materials used and associated operator error. For sera with a high concentrations of pepsinogen both methods gave results with an acceptable range. However, while the micro method has the convenience of a shorter incubation period and uses smaller quantities of reagents, the original method could be improved with the use of buffered substrate or, when it is possible, to analyse samples collected over a period, in one day.

In conclusion, studies carried out in yearlings revealed that previous chemoprophylaxis with moxidectin or ivermectin as first season calves interfered slightly with the development of immunity to gastrointestinal nematodes, as judged by the comparatively lower faecal egg counts and serum pepsinogen concentrations in the control animals, during the second grazing season. However, this observation was not confirmed by the worm burdens at necropsy at the end of the study when there was little difference in the apparent level of immunity between groups. A similarly high establishment of *Ostertagia* EL4 in all four groups was also observed at the end of the second grazing season compared with parasite naive challenge controls although it should be remembered that the latter animals were much younger than the principals.

The abattoir survey indicated an increase in the prevalence and magnitude of *Ostertagia* worm burdens in adult cattle in the West of Scotland. The effect of these on production is uncertain and needs further investigation. The observed clear monthly and seasonal patterns of *Ostertagia* worm burdens and serum pepsinogen concentrations in adult cattle confirm the results obtained in other studies.

Evaluation of two techniques used in these studies indicated that baermannisation of abomasal mucosae for the recovery of *Ostertagia* L4 had some advantages over the digestion method and an original method described by Edwards *et al.* (1960) for the estimation of serum pepsinogen concentrations was better in terms of low within day and among day variations. From the data generated in this study the micro method appears to give poor reproducibility perhaps making it less suitable for either diagnostic or experimental work.

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Appendix 1. Serum pepsinogen estimation

Original Method (Edwards *et al.* 1960)

Procedure

1. BSA blanks (in duplicate):two test tubes for each BSA blank (incubated and nonincubated) were prepared, 2.5 ml acidified BSA and 0.5 ml distilled water added to each tube and the tubes incubated for 24 hours at 37°C. The 'nonincubated BSA blanks' were precipitated by the addition of 4% trichloroacetic acid immediately after the addition of distilled water.

2. Serum tests: two test tubes for each sample ('Control' and 'Test') were prepared, 2.5 ml acidified BSA (pH 1.5) and 0.5 ml serum added to each tube and the 'Test' tubes incubated for 24 hours at 37°C. The 'Control' was precipitated by the addition of 4% trichloroacetic acid immediately after the addition of serum.

3. The precipitated 'Controls' and 'nonincubated' samples were allowed to stand for 10 minutes after mixing, to ensure efficient flocculation of the precipitate, and then centrifuged.

4. After the 24 hours incubation period 5 ml of 4% trichloroacetic acid was added to each tube and the undigested BSA precipitated as before.

5. A summary of this part of the technique is shown below:

	BSA blanks 1	BSA blanks 2	Serum (Control)	Serum (Test)
Acid/BSA	2.5 ml	2.5 ml	2.5 ml	2.5 ml
Distilled water	0.5 ml	0.5 ml	-	-
Serum	-	-	0.5 ml	0.5 ml
	Precipitated with 5 ml 4% TCA	Incubated at 37°C for 24 hours	Precipitated with 5 ml 4% TCA	Incubated at 37°C for 24 hours
		Precipitated with 5 ml 4% TCA		Precipitated with 5 ml 4% TCA

Appendix 1 (continued)

Treatment of Supernatant

1. 2 ml of each supernatant was transferred to suitably labelled test tubes containing 5 ml of 0.25N NaOH.

2. Duplicate tubes were prepared containing 0.5 ml of each working tyrosine standard (i.e. 0.2 μ mols and 0.4 μ mols).

3. A reagent blank was prepared containing 0.5 ml distilled water with 5 ml 0.25N NaOH.

4. 3 ml diluted Folin-Ciocalteu's reagent was added to all the test tubes.

5. After 30 minutes, the blue colour density was determined by a spectrophotometric analysis at 720 nm.

6. A summary of the treatment of the supernatant is shown below:

	0.25 N NaOH	Water	0.2 μ mols tyrosine	0.4 μ mols tyrosine	Supernatent
Folin Reagent (Blank)	5 ml	0.5 ml	-	-	-
Duplicate BSA	5 ml	-	-	-	0.5 ml
Blanks	5 ml	-	-	-	0.5 ml
Duplicate Standard 0.2 μ	5 ml	-	0.5 ml	-	-
mols tyrosine	5 ml	-	0.5 ml	-	-
Duplicate Standard 0.4 μ	5 ml	-	-	0.5 ml	-
mols tyrosine	5 ml	-	-	0.5 ml	-
Sample 1 'Test'	5 ml	-	-	-	0.5 ml
'Control'	5 ml	-	-	-	0.5 ml
Sample 2 'Test'	5 ml	-	-	-	0.5 ml
'Control'	5 ml	-	-	-	0.5 ml

3 ml diluted Folin-Ciocalteu's Reagent added to each tube
After 30 minutes read at 720 nm in spectrophotometer

Appendix 1 (continued)

Results were calculated as follows

1.'Folin reagent blanks' were subtracted from all spectrophotometer readings.

2.A factor for the conversion of all spectrophotometer readings to μ mols tyrosine was calculated and all readings were converted into eqivalent 'μ mols tyrosine' accordingly.

3.Example of calculation

If incubated BSA and serum ('Test')	=	A
and non-Incubated BSA and serum (Controls')	=	B
Then total release of tyrosine on incubation	=	A-B
If incubated BSA-Blank and	=	C
non-Incubated BSA-Blank	=	D
Then tyrosine released from BSA substrate due to incubation alone		
(no pepsinogen)	=	C-D
Tyrosine in μ mols released on incubation due to action of		
activated pepsinogen in 0.125 ml serum in 24 hrs	=	(A-B)-(C-D)

4. Calculated 'tyrosine in μ mols' released by 1000 ml serum per minute

=

(A-B)-(C-D) × factor 5.56)

= iU

Appendix 1 (continued)

Micro Method (Paynter 1992)

1. 1 ml Eppendorf tubes are prepared as follows:

Reagent	Non-Incubated	Incubated
Tyrosine standard 0%	50 µl	50 µl
5%	50 µl	50 µl
10%	50 µl	50 µl
20%	50 µl	50 µl
30%	50 µl	50 µl
50%	50 µl	50 µl
Sample 1 (serum)	50 µl	50 µl
Sample 2 (serum)	50 µl	50 µl

1. To each tube 75 µl glycine buffered BSA was added.

2. Each tube was vortexed, flash-spun for 5 seconds at 14000 rpm and incubated for 30 minutes at 37°C.

3. The 'Non-Incubated' (NI) tubes were then removed and 250 µl of 10% perchloric acid added following which the tubes were vortexed and cetrifuged at 14000 rpm for 10 minutes.

4. The incubated (I) tubes were incubated for a further 3 hours following which 250 µl of 10% perchloric acid added was added and the tubes were vortexed. All the tubes were then centrifuged at 14000 rpm for 10 minutes.

Appendix 1 (continued)

5. A 96-well microtitre plate was treated as follows:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 ST	5 ST	10 ST	20 ST	30 ST	50 ST	1 NI	1 I	2 NI	2 I	3 NI	3 I
B												
C												
D												
E	4 NI	4 I	5 NI	5 I	6 NI	6 I	7 NI	7 I	8 NI	8 I	9 NI	9 I
F												
G												
H												

6. 200 µl of Piers BCA protein reagent was added to each well.

7. 10 µl of perchloric acid supernatant was added in quadruplicate to 200 µl of Pierce BCA protein reagent and this was mixed by pipetting up and down.

8. The mixture was shaken and read at absorbance 560 nm on a 'Dynatech MR 5000' microplate reader.

Appendix 2 Individual and group mean bodyweights

Group 1-Moxidectin Injectable

No.	week 1	3	7	12	16	18	20
842	260	265	340	-	-	-	-
851	280	275	330	375	385	410	400
854	270	270	335	360	400	410	410
871	290	310	365	375	405	435	425
875	240	250	350	-	-	-	-
876	305	320	360	385	420	455	435
877	310	300	340	380	400	425	440
Mean	**279**	**284**	**346**	**375**	**402**	**427**	**422**

Group 2-Moxidectin Pour-on

No.	week 1	3	7	12	16	18	20
833	255	230	275	305	325	350	365
841	215	220	270	275	300	320	325
847	260	290	310	330	360	395	395
852	240	250	290	-	-	-	-
860	360	385	430	475	505	525	523
861	270	295	350	-	-	-	-
872	300	300	330	350	390	415	400
Mean	**271**	**281**	**323**	**347**	**376**	**401**	**402**

Appendix 2 (continued)

Group 3-Ivermectin Injectable

No.	week 1	3	7	12	16	18	20
837	230	220	240	-	-	-	-
849	225	265	285	315	355	380	385
853	270	310	355	390	420	455	440
863	290	270	300	325	335	365	365
864	195	195	215	260	285	305	320
868	295	275	315	-	-	-	-
884	215	210	260	295	325	345	355
Mean	**246**	**249**	**283**	**317**	**344**	**370**	**373**

Group 4-Untreated Control

No.	week 1	3	7	12	16	18	20
153	310	320	350	385	400	430	425
816	340	335	375	390	420	445	445
836	285	255	300	335	370	400	365
838	235	240	320	-	-	-	-
845	225	225	300	-	-	-	-
846	280	310	360	390	415	435	450
878	280	285	310	365	385	410	390
Mean	**279**	**281**	**331**	**373**	**398**	**424**	**415**

Appendix 3 Individual and group mean serum pepsinogen concentrations

Group 1-Moxidectin Injectable

No.	week 1	3	7	12	18	20
842	0.41	2.14	2.07	-	-	-
851	0.32	1.22	1.22	1.94	2.33	2.65
854	0.53	0.85	1.65	3.02	5.42	6.32
871	0.40	2.03	2.40	2.69	5.15	6.55
875	1.22	1.77	2.27	-	-	-
876	0.24	1.11	2.03	2.32	3.10	2.99
877	0.86	2.27	1.30	1.79	5.20	7.40
Mean	**0.57**	**1.62**	**1.84**	**2.35**	**4.24**	**5.18**

Group 2-Moxidectin Pour-on

No.	week 1	3	7	12	18	20
833	0.83	0.75	1.74	1.74	0.66	2.06
841	1.22	0.39	2.18	2.18	2.80	6.65
847	1.32	0.96	1.70	2.84	4.58	6.90
852	1.03	1.56	2.84	-	-	-
860	1.66	1.80	2.68	2.68	3.33	5.98
861	0.62	0.87	2.30	-	-	-
872	1.09	1.72	1.02	2.30	0.64	2.23
Mean	**1.11**	**1.15**	**2.07**	**2.35**	**2.40**	**4.74**

Appendix 3 (continued)

Group 3-Moxidectin Injectable

No.	Week 1	3	7	12	18	20
837	0.39	0.71	1.10	-	-	-
849	0.32	1.11	1.80	1.84	2.80	4.23
853	0.71	0.90	1.81	2.19	3.00	2.84
863	0.86	1.10	1.59	1.71	4.30	8.38
864	0.10	0.72	1.48	1.96	1.69	3.05
868	0.40	0.51	1.95	-	-	-
884	0.58	1.15	1.79	1.84	1.78	2.89
Mean	**0.48**	**0.88**	**1.64**	**1.91**	**2.71**	**4.28**

Group 4-Untreated Control

No.	Week 1	3	7	12	18	20
153	1.42	1.66	1.74	3.24	4.40	3.91
816	1.44	2.77	2.34	1.82	3.60	4.90
836	1.15	1.59	1.74	2.23	1.86	2.66
838	1.23	1.36	1.24	-	-	-
845	2.23	2.09	2.20	-	-	-
846	3.32	2.10	2.41	2.62	2.89	2.45
878	2.69	1.82	2.39	2.20	4.17	4.65
Mean	**1.92**	**1.91**	**2.00**	**2.42**	**2.38**	**3.71**

Appendix 4 Individual and group mean faecal egg counts.

Group 1-Moxidectin Injectable

	Week										
No.	1	3	5	7	9	10	12	14	16	18	20
842	0	0	0	0	0	-	-	-	-	-	-
851	0	0	0	0	0	0	0	50	100	50	150
854	0	0	50	0	0	0	0	50	0	0	50
871	0	0	150	350	100	0	0	100	100	0	100
875	0	0	50	200	0	-	-	-	-	-	-
876	0	0	200	100	300	0	0	50	50	150	0
877	50	0	0	200	100	0	0	0	100	0	100
Mean	**7**	**0**	**64**	**142**	**71**	**0**	**0**	**50**	**70**	**40**	**80**

Group 2-Moxidectin Pour-on

	Week										
No.	1	3	5	7	9	10	12	14	16	18	20
833	0	0	0	100	0	0	0	0	0	0	0
841	0	0	100	300	200	0	0	0	0	0	0
847	0	0	100	450	200	0	0	0	200	200	100
852	0	0	50	150	700	-	-	-	-	-	-
860	0	0	0	50	50	0	0	0	50	0	0
861	0	0	0	300	250	-	-	-	-	-	-
872	0	0	50	100	750	0	0	0	0	50	50
Mean	**0**	**0**	**43**	**207**	**307**	**0**	**0**	**0**	**50**	**50**	**30**

Appendix 4 (continued)

Group 3-Ivermectin Injectable

	Week										
No.	1	3	5	7	9	10	12	14	16	18	20
837	0	0	50	350	-	-	-	-	-	-	-
849	0	0	50	150	600	0	0	300	100	0	100
853	0	0	0	50	0	0	0	50	100	0	400
863	0	0	0	0	850	0	0	0	0	0	50
864	0	0	0	100	0	0	0	0	0	0	0
868	0	0	0	150	50	-	-	-	-	-	-
884	0	0	100	100	150	0	0	50	0	50	50
Mean	**0**	**0**	**29**	**129**	**275**	**0**	**0**	**80**	**40**	**10**	**120**

Group 4-Untreated Control

	Week										
No.	1	3	5	7	9	10	12	14	16	18	20
153	50	0	0	0	0	0	0	0	50	50	100
816	50	0	0	0	100	0	0	0	0	0	0
836	0	0	100	0	50	0	0	0	0	0	50
838	0	0	50	100	150	-	-	-	-	-	-
845	0	0	0	0	50	-	-	-	-	-	-
846	0	0	0	0	100	0	0	0	0	0	0
878	0	0	250	200	100	0	0	0	50	50	100
Mean	**14**	**0**	**57**	**43**	**79**	**0**	**0**	**0**	**20**	**20**	**50**

Appendix 5 Individual and group mean faecal larval counts

Group 1-Moxidectin Injectable

	Week										
No.	1	3	5	7	9	10	12	14	16	18	20
842	0	0	0	0	10	-	-	-	-	-	-
851	0	0	0	0	12	0	0	0	0	0	0
854	0	0	0	0	8	0	0	0	1	2	1
871	0	0	0	0	1	0	0	0	0	0	0
875	0	0	0	0	200	-	-	-	-	-	-
876	0	0	0	0	45	0	0	0	0	0	0
877	0	0	0	0	11	0	0	0	0	0	0
Mean	**0**	**0**	**0**	**0**	**41**	**0**	**0**	**0**	**0**	**0**	**0**

Group 2-Moxidectin Pour-on

	Week										
No.	1	3	5	7	9	10	12	14	16	18	20
833	0	0	0	0	0	0	0	0	0	0	0
841	0	0	0	0	50	0	0	0	0	0	0
847	0	0	0	0	150	0	0	0	0	0	0
852	0	0	0	0	100	-	-	-	-	-	-
860	0	0	0	0	57	0	0	0	0	0	0
861	0	0	0	0	200	-	-	-	-	-	-
872	0	0	0	0	450	3	0	0	0	0	0
Mean	**0**	**0**	**0**	**0**	**144**	**0**	**0**	**0**	**0**	**0**	**0**

Appendix 5 (continued)

Group 3-Ivermectin Injectable

	Week										
No.	1	3	5	7	9	10	12	14	16	18	20
837	0	0	0	0	0	-	-	-	-	-	-
849	0	0	0	0	250	1	0	0	0	0	0
853	0	0	0	0	8	0	0	0	0	0	0
863	0	0	0	0	0	0	0	0	0	0	0
864	0	0	0	0	121	0	0	0	0	0	0
868	0	0	0	0	300	-	-	-	-	-	-
884	0	0	0	0	2	0	0	0	0	0	0
Mean	**0**	**0**	**0**	**0**	**114**	**0**	**0**	**0**	**0**	**0**	**0**

Group 4-Untreated Control

	Week										
No.	1	3	5	7	9	10	12	14	16	18	20
153	0	0	0	0	8	0	0	0	0	0	0
816	0	0	0	0	4	0	0	0	1	0	0
836	0	0	0	0	76	0	0	0	0	0	0
838	0	0	0	0	200	-	-	-	-	-	-
845	0	0	0	0	8	-	-	-	-	-	-
846	0	0	0	0	0	0	0	0	0	0	0
878	0	0	0	0	100	0	0	0	1	0	0
Mean	**0**	**0**	**0**	**0**	**57**	**0**	**0**	**0**	**0**	**0**	**0**

Appendix 6 Pasture larval recoveries (L3/Kg dh)

Paddock 1

Genus	Week 1	3	5	7	9	12	14	16	18	20
Ostertagia	328	0	0	500	357	800	333	0	0	1470
Cooperia	0	0	0	500	1071	800	333	652	212	8461
Dictyocaulus	0	0	114	0	357	0	0	0	212	0

Paddock 2

Genus	Week 1	3	5	7	9	12	14	16	18	20
Ostertagia	1034	0	0	1034	0	2500	889	714	0	1470
Cooperia	0	0	0	0	1481	5312	444	714	0	1470
Dictyocaulus	0	0	187	0	0	0	444	0	0	0

Paddock 3

Genus	Week 1	3	5	7	9	12	14	16	18	20
Ostertagia	0	0	0	0	0	1190	370	2807	278	1053
Cooperia	0	0	0	0	667	370	1053	1186	833	1754
Dictyocaulus	59	0	0	0	0	370	0	0	278	0

Mean Pasture Larval Recoveries from Paddocks 1, 2 and 3

Genus	Week 1	3	5	7	9	12	14	16	18	20
Ostertagia	454	0	0	511	119	1497	531	1174	93	1331
Cooperia	0	0	0	167	1073	2161	610	851	348	3895
Dictyocaulus	20	0	100	0	119	123	148	0	163	0

Appendix 7 Individual and mean monthly *Ostertagia* worm counts and serum pepsinogen concentrations. (- samples unsuitable for pepsinogen assay)

April

No	L4	L5	Adults	Total	iU tyrosine
1	0	0	15200	15200	0.42
2	0	0	16400	16400	0.87
3	5800	0	1400	7200	0.87
4	8600	400	0	9000	0.86
5	5200	0	0	5200	0.52
6	8400	0	5100	13500	1.26
7	3600	600	1800	6000	0.46
8	1200	0	1400	2600	2.39
9	600	0	3200	3800	0.97
10	192600	400	1200	194200	0.83
11	5800	1200	15600	16800	1.31
12	19200	800	7500	8000	1.58
13	1800	0	1200	1200	-
14	0	0	0	0	0.59
15	4500	600	1800	2400	1.10
16	0	0	1200	1200	1.20
17	200	0	200	400	0.97
18	0	0	400	400	0.91
19	0	0	0	0	1.15
Mean	**13552**	**210**	**3812**	**15889**	**1.01**

May

No.	L4	L5	Adults	Total	iU tyrosine
20	9200	0	0	9200	1.38
21	3200	0	0	3200	1.87
22	1000	0	4000	5000	0.93
23	600	0	1000	1600	0.52
24	5500	600	7400	13500	1.16
25	400	400	6500	7300	0.75
26	500	400	6200	7100	0.64
27	2600	400	7800	10800	-
28	3800	800	7800	12400	0.93
29	0	0	0	0	0.23
30	1500	600	4600	6700	1.23
31	0	0	0	0	1.00
32	5300	3600	5700	14600	1.79
33	0	0	0	0	1.44
34	0	200	300	500	1.50
35	1000	100	1700	2800	1.76
36	0	0	0	0	1.92
37	66800	1000	18000	85800	2.20
38	0	0	0	0	0.35
39	0	0	200	200	0.66
40	1400	400	1800	3600	1.89
41	0	0	0	0	2.19
42	0	0	0	0	1.33
Mean	**4469**	**369**	**3174**	**8013**	**1.25**

Appendix 7 (continued)

June

No	L4	L5	Adults	Total	iU tyrosine
43	800	800	4000	5600	1.19
44	1000	0	1800	2800	1.49
45	0	0	0	0	0.67
46	13000	1100	3100	17200	3.68
47	4400	2200	24800	31400	3.51
48	10800	9400	26000	46200	4.18
49	0	0	800	800	1.13
50	0	0	0	0	1.56
51	2200	800	9200	12200	2.50
52	3000	1000	16800	20800	2.65
53	1200	300	200	1700	0.89
54	800	400	1200	2400	1.51
55	1000	100	3000	4100	2.34
56	300	0	4300	4600	1.31
57	4000	1600	26800	32400	1.36
Mean	**2833**	**1180**	**8133**	**12147**	**1.99**

July

No	L4	L5	Adults	Total	iU tyrosine
58	0	400	200	600	1.66
59	400	0	400	800	0.44
60	4200	0	2800	7000	0.71
61	800	0	0	800	1.47
62	0	0	400	400	1.23
63	0	0	200	200	2.68
64	0	0	4000	4000	1.22
65	0	0	800	800	1.36
66	100	200	0	300	4.09
67	400	0	1600	2000	3.85
68	0	200	1600	1800	2.21
69	0	0	0	0	1.73
70	0	0	1400	1400	-
71	0	0	0	0	4.14
72	600	0	2300	2900	-
73	400	0	3200	3600	-
74	0	300	200	500	-
75	8800	600	7100	16500	-
76	2200	400	800	3400	-
77	900	100	2800	3800	-
78	1600	400	23200	25200	-
79	0	400	4600	500	-
80	200	200	4600	500	-
81	0	0	11600	11600	-
Mean	**855**	**133**	**3075**	**3433**	**2.06**

Appendix 7 (continued)

August

No.	L4	L5	Adults	Total	iU tyrosine
82	200	0	0	200	1.75
83	200	0	600	800	3.21
84	1600	700	5300	7600	2.17
85	400	0	0	400	-
86	0	0	0	0	1.60
87	400	300	2400	3100	2.41
88	200	600	4000	4800	3.54
89	500	1300	3300	5100	3.12
90	600	0	0	600	1.32
91	0	0	500	500	1.90
92	0	0	0	0	0.93
93	0	0	0	0	2.99
94	0	0	0	0	2.77
95	0	0	0	0	-
96	200	0	400	600	-
97	0	0	8400	8400	-
98	0	0	35000	35000	1.59
99	200	0	4600	4800	1.60
100	200	200	2800	3200	3.53
101	1600	200	2400	2600	3.22
Mean	**315**	**165**	**3542**	**4022**	**2.35**

September

No.	L4	L5	Adults	Total	iU tyrosine
102	0	0	400	400	-
103	0	0	200	200	-
104	2800	1000	11000	14800	-
105	200	0	200	400	-
106	0	0	0	0	-
107	0	200	5400	5600	3.01
108	0	400	5800	6200	2.33
109	0	0	2100	2100	0.82
110	400	0	2800	3200	1.90
111	0	0	1500	1500	3.78
112	400	200	6100	6700	-
113	1600	0	400	2000	2.35
114	1200	200	1600	3000	1.24
115	0	0	0	0	2.74
116	0	0	0	0	1.54
117	0	0	6400	6400	-
118	400	0	300	700	-
119	400	0	0	400	1.06
120	0	0	0	0	1.82
121	0	0	0	0	1.00
Mean	**370**	**100**	**2210**	**2680**	**1.96**

Appendix 7 (continued)

October

No.	L4	L5	Adult	Total	iU tyrosine
122	0	0	1400	1400	-
123	400	0	600	1000	-
124	400	0	0	4000	-
125	800	0	2400	3200	0.46
126	0	0	800	800	1.56
127	9600	0	19200	29200	1.71
128	4000	400	1200	5200	-
129	800	0	400	1200	-
130	800	0	0	800	-
131	0	0	0	0	-
132	400	0	2200	2600	-
133	0	0	0	0	-
Mean	**1400**	**33**	**2564**	**4117**	**1.24**

November

No.	L4	L5	Adults	Total	iU tyrosine
134	0	0	0	0	2.84
135	1300	0	0	1300	1.81
136	0	0	0	0	1.29
137	0	0	300	300	3.21
138	0	0	200	200	-
139	0	0	0	0	0.88
140	300	500	400	1200	0.56
141	800	0	0	800	2.34
142	10200	600	9400	20200	0.83
143	0	0	0	0	0.49
144	0	0	0	0	1.41
145	0	0	0	0	1.70
146	0	0	400	400	4.81
147	200	0	0	200	1.70
Mean	**914**	**79**	**764**	**1757**	**1.83**

December

No.	L4	L5	Adults	Total	iU tyrosine
148	0	0	0	0	1.19
149	0	0	400	400	1.48
150	4800	0	0	4800	1.12
151	0	0	100	100	-
152	400	0	0	400	-
Mean	**1040**	**0**	**100**	**1140**	**1.26**

Appendix 7 (continued)

January

No.	L4	L5	Adults	Total	iU tyrosine
153	5600	0	3000	8600	1.13
154	116000	0	800	116800	0.85
155	2000	0	200	2200	0.69
156	60800	800	5200	66800	2.41
157	392000	0	1500	393500	-
158	600	0	1000	1600	-
159	0	0	0	0	-
160	1000	0	0	1000	-
161	400	0	400	800	-
162	183800	1000	4200	189000	-
163	75600	1000	2400	21551	1.14
164	1200	0	0	1200	0.53
165	4400	0	200	4600	1.12
166	1600	0	200	1800	3.58
167	0	0	0	0	0.78
Mean	**52503**	**187**	**1273**	**53963**	**1.35**

February

No.	L4	L5	Adults	Total	iU tyrosine
168	25000	1400	9600	36000	0.92
169	400	0	0	400	-
170	0	0	0	0	-
171	0	0	0	0	1.19
172	3800	0	2000	5800	1.20
173	20400	4000	24600	49000	1.53
174	0	0	0	0	1.17
175	0	0	200	200	1.25
176	21800	0	1400	23200	1.67
177	7400	0	800	8200	0.44
178	800	0	200	1000	-
179	2800	400	600	3800	-
180	1400	450	3350	5200	-
181	600	0	0	600	-
182	0	0	0	0	-
183	226400	1000	19000	246400	2.97
184	1000	200	2200	3400	2.70
185	2800	400	2400	5600	1.89
186	4800	400	2400	7600	0.74
187	72400	1600	3800	77800	3.28
Mean	**19590**	**492**	**3627**	**23710**	**1.61**

Appendix 7 (continued)

March

No.	L4	L5	Adults	Total	iU tyrosine
188	5600	0	800	6400	0.97
189	5200	400	0	5600	2.94
190	7600	800	1300	9700	0.70
191	600	0	1200	1800	-
192	7800	800	6000	14600	-
193	3600	0	1000	4600	1.10
194	5600	0	7800	13400	-
195	1600	0	200	1800	1.24
196	1000	400	200	1600	2.51
197	17000	0	400	17400	1.31
198	0	0	0	0	1.42
199	13600	0	1600	15200	1.15
200	2600	1200	3200	7000	-
201	22000	200	8800	31000	0.88
Mean	**6700**	**271**	**2321**	**9292**	**1.42**

Appendix 8 Individual and mean monthly *Ostertagia* L4 recoveries by Baermannisation and Digestion

April

No.	Digestion	Baermannisation
1	0	1200
2	4000	1600
3	400	200
4	800	2000
5	3200	9200
6	33200	41200
7	0	0
8	800	6000
9	12800	16800
10	192600	25600
11	6200	7200
12	3600	5200
13	1200	0
14	0	800
15	0	0
16	0	0
17	200	0
18	0	200
19	0	0
Mean	**13631**	**6168**

May

No.	Digestion	Baermannisation
20	4400	2200
21	800	2400
22	1000	600
23	600	400
24	4600	4200
25	0	1200
26	0	1800
27	2400	0
28	3800	8400
29	0	0
30	0	1400
31	0	0
32	5200	3200
33	0	0
34	0	0
35	1000	1000
36	0	0
37	10200	66400
38	0	0
39	0	0
40	1400	2400
41	0	0
42	0	0
Mean	**1539**	**4156**

Appendix 8 (continued)

June

No.	Digestion	Baermannisation
43	800	1000
44	2000	0
45	0	200
46	13000	5400
47	4400	7000
48	7800	6600
49	0	0
50	0	0
51	2200	200
52	2800	2200
53	1200	1600
54	800	200
55	800	2000
56	0	400
57	4000	4000
Mean	**2653**	**2053**

July

No.	Digestion	Baermannisation
58	0	1000
59	200	600
60	4200	5600
61	200	200
62	0	0
63	0	200
64	0	600
65	0	0
66	0	0
67	400	600
68	0	0
69	0	0
70	0	0
71	0	0
72	600	400
73	400	600
74	0	400
75	8800	9600
76	2200	1400
77	800	2800
78	1600	1000
79	0	800
80	200	200
81	0	0
Mean	**817**	**1083**

Appendix 8 (continued)

August

No.	Digestion	Baermannisation
82	200	0
83	200	0
84	1400	600
85	400	0
86	0	0
87	400	600
88	200	0
89	400	400
90	600	200
91	0	0
92	0	0
93	0	0
94	0	0
95	0	0
96	200	0
97	200	800
98	200	1200
99	1600	6200
100	0	200
101	0	200
Mean	**300**	**520**

September

No.	Digestion	Baermannisation
102	2800	3000
103	0	0
104	0	0
105	0	0
106	0	400
107	0	0
108	400	400
109	0	400
110	200	1600
111	1600	400
112	1200	400
113	0	0
114	0	0
115	0	0
116	400	400
117	400	0
118	0	0
119	0	0
120	0	0
121	0	0
Mean	**350**	**350**

Appendix 8 (continued)

October

No.	Digestion	Baermannisation
122	0	0
123	400	200
124	800	0
125	0	400
126	9600	800
127	4000	800
128	800	0
129	800	2200
130	0	0
131	400	0
132	0	0
133	0	0
Mean	**1400**	**360**

November

No.	Digestion	Baermannisation
134	0	200
135	1200	1000
136	0	0
137	0	0
138	0	0
139	0	0
140	0	600
141	800	0
142	8400	7200
143	0	0
144	0	0
145	0	0
146	0	0
147	200	0
Mean	**757**	**643**

December

No.	Digestion	Baermannisation
148	4800	11200
149	0	0
150	400	200
151	0	0
152	0	0
Mean	**1040**	**2280**

Appendix 8 (continued)

January

No.	Digestion	Baermannisation
153	5600	800
154	11600	66000
155	2000	800
156	60600	66000
157	39200	41600
158	600	800
159	0	0
160	1000	800
161	400	0
162	171200	103600
163	63600	66400
164	1200	3600
165	4400	4000
166	1600	1200
167	0	0
Mean	**22835**	**19505**

February

No.	Digestion	Baermannisation
168	24800	24800
169	400	1600
170	0	0
171	0	1000
172	3600	1600
173	13600	21000
174	0	0
175	0	0
176	21800	21000
177	6000	5200
178	800	1600
179	2800	2800
180	1400	2600
181	600	800
182	0	200
183	78200	232000
184	1000	2000
185	2400	3300
186	2400	2400
187	44800	15200
Mean	**10567**	**17772**

Appendix 8 (continued)

March

No.	Digestion	Baermannisation
188	5600	3200
189	5200	4000
190	7600	9400
191	600	1800
192	5600	8200
193	3600	3200
194	5200	4400
195	1600	600
196	800	2000
197	11600	12000
198	0	400
199	13600	18400
200	1600	1200
201	22000	30000
Mean	**7357**	**7057**