NEW FACTORS AFFECTING THE EPIDEMIOLOGY OF BOVINE OSTERTAGIASIS

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

by

IHSAN MAHDI AL-SAQUR
B.V.M.S (Baghdad University)
M.Sc. (Baghdad University)

Department of Veterinary Parasitology,
University of Glasgow.
July, 1982.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ACKNOWLEDGEMENTS

Words cannot express my deepest feelings and thanks. However, I owe a debt of gratitude to Professor J. Armour who encouraged me throughout the study and who initially provided me with the necessary facilities. The stimulating atmosphere created by him was invaluable to me personally and essential to the enthusiastic cooperation between the scientific and technical staff in the Department.

I am also grateful to Professor G.M. Urquhart who allowed me to study in his Department and for his constructive criticism.

Appreciation is also due to Ken Bairden for tireless and knowledgeable help and whose spirit gives the work a special taste.

My sincere thanks go to Dr.M.M.Murray, Dr. A.M. Dunn and Dr.F.W. Jennings for their continuous encouragement, assistance and consideration throughout and I would like to express my gratitude to Dr. G. Gettinby for his statistical help.

My thanks extend to Mr.S. Brown, Mr.C. Hartley, Mr. P. Tiebosch, Miss J. Henderson and Mrs.C. Connelly for their help. I should also extend my thanks to Mr.A. Finnie, Mr.A. May and Mr.G. Cameron in the Photography Department and to Mrs.M. Mason in the Pathology Department. To Mrs.M. Smith and Mrs.B. Gillies thanks for typing the thesis.

I am most grateful to the University of Baghdad for their financial support.

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

CONTENTS

CHAPTER 1

Literature Review

CHAPTER 2

Materials and Methods

CHAPTER 3

SECTION 1: A population study of free-living L_3 of bovine Ostertagia spp. over a twelve month period.

SECTION 2: Availability of Ostertagia spp. to grazing calves after resting for 20 months.

CHAPTER 4

SECTION 1: Observations on the infectivity and pathogenicity of three isolates of *Ostertagia* spp. in calves.

SECTION 2: The interaction of quantitated infections of *O.ostertagi* and and *O. leptospicularis*.

CHAPTER 5

SECTION 1: 1980 Experiment.

SECTION 2: 1981 Experiment.

LIST OF PLATES

LIST OF PLATES (contd.)

LIST OF FIGURES

LIST OF TABLES

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Before the second half of the nineteenth century, the study of Veterinary Parasitology had been largely neglected in Great Britain (Foster, 1965). The first significant contribution to the literature was made by T.S. Cobbold in 1864, who published a general review of the subject. He noted how "remarkably numerous" nematode parasites were in several "herbivorous animals" and, in a subsequent publication, listed those present in "the ox" (Cobbold, 1873).

However, there was little clinical description of the disease caused by these parasites until the beginning of the twentieth century, when reports on outbreaks of parasitic gastritis began to appear (Gardener, 1911; Ackert and Muldoon, 1920). Since then, occurrence of the disease in cattle has been widespread, judging by reports from Northern England (Stewart and Crofton, 1941), Southern England (Bruford and Fincham, 1945), Scotland (Martin, Thomas and Urquhart, 1957) and Northern Ireland (Gracey, 1960). More recently field studies (Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart, 1965a,b; Michel, 1969a) have confirmed that ostertagiasis or parasitic gastritis, as it is often called is the most important gastro-intestinal helminthiasis of cattle in Britain. It is also recognised as being very important in France (Raynaud, Landren and Jolivet, 1971), Belgium (Pouplard, 1968), Netherlands (Borgsteede, 1977), Denmark (Henriksen, Jorgensen, Nansen, Serjrsen and Klansen, 1975) and Germany (Burger, Eckert, Wetzel and Michael, 1966) and it may also cause severe problems in countries with a sub-tropical climate provided there is winter rainfall (Anderson, 1971; Williams and Knox, 1976; Craig, 1979).

Although several species of Ostertagia are found in cattle, it is O. ostertagi which is associated with most recorded clinical outbreaks, but occasionally other members of the sub-family Ostertagiinae, namely, Skrjabinagia bisonis and O. leptospicularis, have been incriminated

as the species responsible for the disease (Worley and Sharman, 1966; Bissett, 1980a).

Life-cycle of Ostertagia ostertagi

The life-cycle may be conveniently divided into the free-living and parasitic phases. The free-living phase has been studied in detail by workers at the Ministry of Agriculture Laboratories at Weybridge, England (Rose, 1961; 1962; Michel, 1969a) and their findings may be summarised as follows:

Fertilised adult female worms, which are located on the surface of the abomasal mucosa, lay eggs, 70-84 u long and 40-50 u wide, which are passed out in the faeces at the morula stage. Hatching is influenced by temperature, moisture and oxygen tension, and may occur in as short a time as 24 hours. The emerging first-stage larvae (L_1), which measure between 300 u and 350 u in length, actively feed on coliform bacteria for a period of about twelve hours.

This is followed by a lethargic period, at the end of which occurs the first ecdysis or moult. The resulting second stage larvae (L_2) feed and develop and after a further moult become infective third stages (L_3). This moult is incomplete since the cuticle of the L_2 is retained as a sheath providing protection against adverse environmental conditions. Development of O. ostertagi from the egg to the L_3 takes place within the dungpat, and is influenced by climatic factors, temperature being of particular importance. Subsequent migration of the infective larvae from the faecal pat on to herbage will only take place in the presence of a continuous film of moisture such as that created by heavy rainfall.

Although the parasitic life-cycle of O. ostertagi had been described in part by Stadelmann (1891) and Stödter (1901), Threlkeld (1946) was the first to make a systematic study of the parasite in experimentally infected animals. Since then several other workers

have carried out similar experimental infections (Porter and Cauthen, 1946; Douvres, 1956; Ritchie, Anderson, Armour, Jarrett, Jennings and Urquhart, 1966; Murray, Jennings and Armour, 1970).

From the results of these studies the parasitic life-cycle may be summarised as follows.

Following ingestion by the calf, L_3 exsheath in the rumen and pass into the abomasum, entering the glands in the gastric mucosa by the second day. The larvae then undergo a third moult and reach the early fourth stage (L_4) by the fourth day. Although some larvae begin a fourth moult around day 7 it is not until 10 days post ingestion that most of the larvae have reached this stage. Development within the gastric glands continues and by day 12, nearly all the larvae are at the immature adult, or fifth stage (L_5). Adult O. ostertagi, 6-9 mm long, then begin to emerge from the mucosal glands around day 17 by which time the female worms are starting to ovulate. As a result, eggs may appear in the faeces from day 17 post-infection.

The life-cycle is outlined in Plate 1.

Although development of O. ostertagi from the infective to the adult stage in the host usually takes about three weeks, sometimes this may be extended to several months as a result of the ingested larvae becoming arrested in their development at the early fourth larval stage (EL_4). In temperate climates this seems to occur primarily in larvae ingested during autumn and winter.

This aspect of the life-cycle was highlighted by Martin, et al (1957) who described outbreaks of what they termed atypical parasitic gastritis in young cattle in Britain which had been housed for several months following their first grazing season. At post-mortem these cattle had large worm burdens of mature, developing and early fourth larval stages (EL_4) of O. ostertagi despite the fact that they had not grazed for several months.

The reasons for such outbreaks have been the subject of

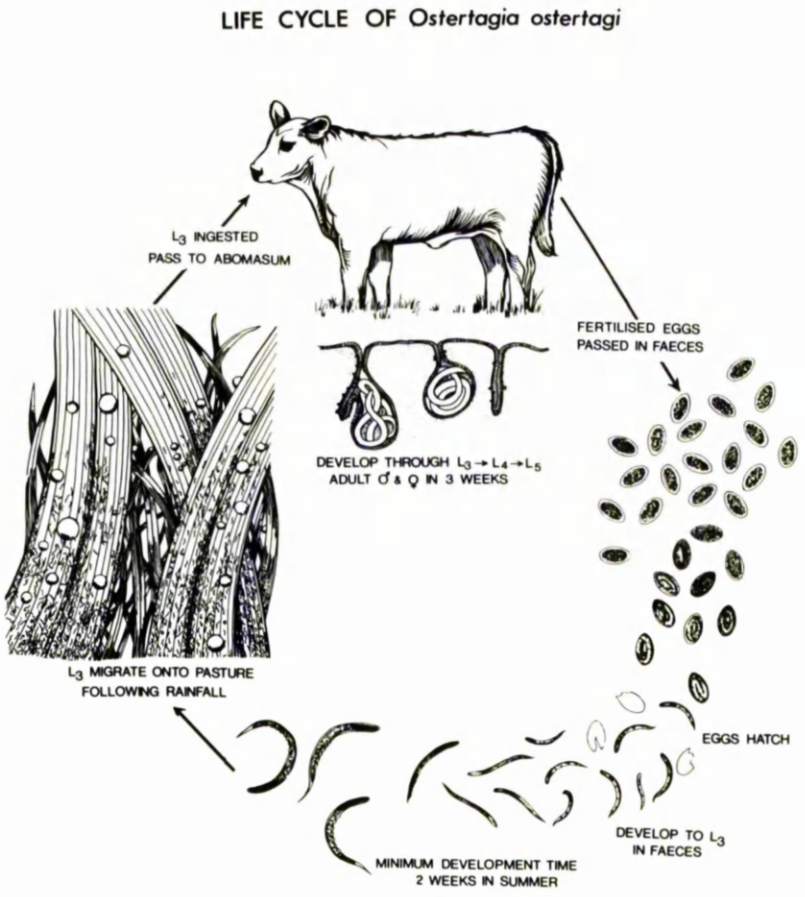


Plate 1. Life-cycle of Ostertagia ostertagi

much speculation and this has undoubtedly provided the main stimulus for the considerable amount of research which has been carried out since that publication. Studies on bovine ostertagiasis have largely concentrated on four main areas, namely, the clinical disease, its pathogenesis, the epidemiology including host immunity and arrested larval development and finally the treatment and control of the disease and it is proposed to review the literature pertaining to those major developments.

CHAPTER 1

LITERATURE REVIEW ON IMPORTANT ASPECTS OF OSTERTAGIASIS

THE CLINICAL DISEASE

Following the description by Martin and his colleagues (Martin et al, 1957) of atypical parasitic gastritis, a detailed field study of suspected outbreaks of ostertagiasis was carried out in South-West Scotland and Northern England (Anderson et al, 1965b). Apart from a detailed history of each outbreak, clinically affected and in some cases non-affected cattle were obtained for post-mortem. From the results of this study it was apparent that the disease occurred in two main clinical forms. The first, named Type I by the authors and similar to the classical form described for several decades, was seen in young cattle during the summer, principally between July and October; the other called Type II occurred in the late winter or early spring (March to May) usually in cattle housed after their first grazing season and was similar to that previously described as atypical parasitic gastritis by Martin et al (1957). Both forms of the disease were characterised by weight loss and severe diarrhoea, though in the Type II cases the diarrhoea was sometimes intermittent. A moderate anaemia and hypoalbuminaemia were present in many of the Type II cases, often accompanied by submandibular oedema. The authors also described a third type in which clinical signs were absent or mild in character and which they called the pre-Type II disease. In this stage the vast majority of Ostertagia worms were at the EL_4 stage, apparently arrested in their development. This description of the pre-Type II condition was obtained from examination of the clinically non-affected animals in herds where Type II disease was occurring or had occurred and since it appeared that it was the resumption of development by EL_4 stages which gave rise to the Type II condition, the name pre-Type II was coined.

Since this original description of two distinct clinical types of ostertagiasis many outbreaks of both forms have been reported from several European countries, and from Australasia and North and South America. In some countries for example, in Australia, New Zealand and France, the Type II outbreaks were often seen in cows, predominantly heifers at first calving (Hotson, 1967; Wedderburn, 1970; Raynaud and Bouchet, 1976). Since then, outbreaks of both Type I and Type II have also been noted in beef herds both in young animals after weaning and in adult animals, usually, but not always, after calving (Selman, Reid, Armour and Jennings, 1976). Although the terms Type I and Type II are widely used some British authors prefer the use of the terms summer and winter ostertagiasis respectively. However, since the Type I disease occurs in late winter and spring and the Type II from mid-summer to early-autumn in countries in the Southern Hemisphere, these seasonal terms do seem rather parochial.

PATHOGENESIS

The principal lesions of ostertagiasis are white, raised, umbilicated nodules on the surface of the abomasum; sometimes these are accompanied by hyperaemia and oedema and in heavy infections where coalescence of the nodules has occurred a morocco leather type appearance develops. These lesions have been described by many authors e.g. Ackert and Muldoon (1920); Martin, et al (1957) and Osborne, Batte and Bell (1960). However, it was not until the mid-1960's that the sequential development of these lesions and the pathogenic effects which they had on the host were first elucidated. From studies which involved the sequential necropsy of animals given a fixed larval dose and in some instances fitted with abomasal cannulae to facilitate collection of abomasal secretions (Ritchie et al, 1966; Jennings, Armour, Lawson and Roberts, 1966; Murray, et al, 1970) it is apparent that there are two distinct

phases of the life-cycle. The first occurs in the period immediately after infection when the larvae are developing in the gastric glands and any cellular changes are confined to the parasitised glands; there are no significant alterations in the biochemical values of either the abomasal fluid or the blood at this time and the clinical signs are not detectable. The second phase occurs from about 18 days after infection when the young adult worms begin to emerge from the gastric glands and marked cellular changes appear. Microscopically, there is hyperplasia and loss of cellular differentiation, particularly of the hydrochloric acid (HCL) producing parietal cells. This happens not only in the parasitised gastric glands but also in the surrounding glands. From electron microscope studies it was shown that the distended parasitised gland stretches the surrounding glands and this probably stimulates the multiplication of new cells in order to maintain the continuity of the epithelium. At the same time, the parietal cells become incorporated in this stretched epithelium, these parietal cells cannot be seen by the light microscope but in the electron microscope are seen to have lost their microvillary structure which is necessary for the secretion of hydrochloric acid. In heavy infections when the area of the affected gastric mucosa becomes confluent the pH of the abomasal fluid rises markedly, in certain cases to more than 7, which is well beyond the level required for normal digestion (less than 4.5). A marked increase in the numbers of viable bacteria also occurs at these high levels of abomasal pH. Undifferentiated and hyperplastic mucosa is also abnormally permeable to macromolecules due to the junctional complexes of many of the cells having broken down.

In summary, the principal consequences of these structural and biochemical changes are:

1. An elevation of the pH of the abomasal fluid from 2 up to 7 (Jennings, et al, 1966) resulting in a failure to activate pepsinogen secreted by the zymogen cells to pepsin - failure to denature proteins in the abomasum and a loss of bacteriostatic effect, with a resultant increase in the numbers of bacteria in the abomasum.
2. An enhanced permeability to macromolecules resulting in elevated plasma pepsinogen levels (the pepsinogen which is secreted into the gastric lumen and not activated enters the circulation via the open epithelial cell junctions) and in severely damaged mucosae, plasma proteins, particularly albumin can leak from the circulation into the lumen of the stomach via the open epithelial cell junctions causing hypoalbuminaemia.

Any loss of protein macromolecules is usually accompanied by loss of electrolyte, mainly sodium and chloride, and the onset of diarrhoea increases this loss of electrolytes. Continued loss may lead to increased hypoalbuminaemia, retention of fluid and the development of oedema. This can result in an alteration in the ratio of fluid to lean body mass (tissue protein) which may give a false impression of the animal's weight (Halliday, Dalton, Anderson and Mulligan, 1965). Where animals have a considerable portion of their apparent weight composed of retained fluid and not as tissue protein the real weight loss an animal with ostertagiasis suffers cannot therefore be accurately assessed by simply weighing the animal.

In heavy infections the clinical consequences of these changes are:

1. Loss of appetite - the cause of this not being known.

2. Impaired abomasal digestion, because pepsin activity is negligible above a pH of 4.5 leading to weight loss.
3. Diarrhoea: the cause of the diarrhoea is also unknown; however, since its onset and duration closely follow the elevation of abomasal pH it is possibly related to the increase of viable bacteria present in the abomasal fluid.

The assumption that the structural damage and biochemical changes caused by Ostertagia worms impair protein digestion has been questioned by Dargie (1981). He argues that while impaired abomasal protein digestion results form reduced HCl secretion, extra-gastric digestion may occur further down the alimentary tract, as it has been shown that large amounts of protein infused directly into the ileum of normal sheep are almost totally digested and substantial proportions of the nitrogen are then retained. This is probably correct in lower levels of infection, the so-called sub-clinical ostertagiasis, where it seems that the main effect of the parasite is to cause disturbances in appetite and post-absorptive metabolism, as has been clearly demonstrated in both sheep and cattle(Coop and Angus, 1981; Randall and Gibbs, 1981) and by Parkins, Bairden and Armour (1982) in calves recovering from ostertagiasis. In these studies the major differences occurred in nitrogen. The disturbances in post-absorptive metabolism were exacerbated by a poor level of nutrition and the excessive leakage of plasma into the gut from the damaged gastro-intestinal mucosa.

However, it is likely that in severe clinical disease where there is impaired abomasal digestion accompanied by diarrhoea and possibly and increase in gut motility, the rapid passage of ingesta with and elevated pH into the intestine could reduce secretion of pancreatic and intestinal enzymes responsible for extra-gastric protein digestion and so result in an overall impaired digestion.

EPIDEMIOLOGY

Epidemiology can be defined as the study of factors affecting the occurrence of disease in a population. There have been more studies on the epidemiology of ostertagiasis than on any other aspect and these have centred on three main areas, namely:

Population dynamics of the free-living larval stages

Arrested larval development

The acquisition of immunity by cattle

and it is convenient to consider the major advances under these three headings.

Population Dynamics of Free-living Larval Stages

A most important development in epidemiological knowledge came in the 1960's when it was clearly demonstrated by Michel (1966) that the numbers of free-living infective L_3 of *O. ostertagi* fluctuated seasonally on the herbage. In Britain, and indeed in Western Europe, the numbers of these larval stages increase markedly at some time during the second half of the recognised grazing season i.e. July onwards and then decline during the winter to reach low levels by the following spring and virtually to zero by June. This pattern appears to be a constant one and is accounted for by two important factors:

1. That the increase in pasture numbers of L_3 from mid-summer through autumn originates from infection deposited as eggs on the pasture by animals grazing during the spring or early-summer. For many years the development of the L_3 was thought to proceed quickly once the mean day/night temperature exceeded 10°C and that several cycles of free-living development then occurred. Michel showed that this concept was incorrect and that L_3 from the eggs of *O. ostertagi* deposited in faeces on the

herbage during the months of April, May and June first appeared sometime during July or August; thereafter the period taken for the eggs to develop to L_3 begins to lengthen again and little or no development occurs from September onwards. Michel therefore postulated that one or at most two generations of Ostertagia spp. occur annually, at least in Western Europe, and that the important contamination period is the spring or early-summer.

2. Once present on the pasture in the summer or autumn the L_3 are capable of survival until the following spring i.e. sufficiently long to infect the next years crop of calves.

   The combination of larval mortality and the dilution of surviving L_3 by the rapid growth of pasture in the spring results in larval numbers being extremely low by mid-summer.

Since Michel's original observations there have been a plethora of articles on the seasonal development of O. ostertagi larvae in various parts of the world. In other European studies the results confirmed what Michel found in England and these have been reviewed by, for example, in West Germany, Burger et al, 1966; Belgium, Pouplard, 1968; The Netherlands, Borgsteede, 1977; Poland, Malczewski, 1970; France, Raynaud et al, 1971; Sweden, Nilsson and Sorelius, 1973; Denmark, Henriksen, et al, 1975; Switzerland, Eisenegger and Eckert, 1975 and Norway, Tharadlsen, 1970. Meanwhile in Canada, Smith, 1974; in the USA, Malczewski, Westcott, Spratling and Gorham, 1975; Yazwinski and Gibbs, 1975; Williams and Knox, 1976; Craig, 1979 and in Australia, Anderson, 1971 and Smeal, Fraser and Robinson, 1980 have all demonstrated the seasonality of infection with clearly

defined important times of contamination and development of infective larval populations on the pasture with only a limited number of annual cycles.

Recently, it has been reported that the numbers of Ostertagia L_3 have increased on pastures which have not been contaminated in the spring and early summer; in some instances the numbers of larvae appearing on the pasture have been sufficient to cause clinical disease (Armour, Al Saqur, Bairden, Duncan and Urquhart, 1980a).

The source of these larvae is not definitely known but it has been suggested that they may have originated from a reservoir of larvae in the soil and indeed larvae were recovered from soil samples to a depth of 10 cm (Bairden, 1980; Armour et al, 1980a).

It is not yet clear how or why the larvae migrate within soil or on herbage but Gronvøld (1979) and Oakley (1981) have recently recovered viable O. ostertagi infective larvae and bovine lungworm larvae from earthworms and so it may be that the patterns of Ostertagia L_3 in the soil could be linked to earthworm activity. Whether this accumulation of Ostertagia L_3 in the soil is a new or even a widespread phenomenon or whether it only occurs in certain soil types such as occur in Northern England and South-West Scotland, where the studies were carried out, is not yet known. However, these L_3 populations in the soil do represent a possible danger to the functioning of the control schemes outlined below.

Despite the recent observation that L_3 can appear unexpectedly in the herbage the pattern observed by Michel (1969a) is generally regarded as the norm in temperate countries, although the magnitude of seasonal change and the precise timing can be altered by various factors which influence the development, dissemination, survival and availability of L_3. These can conveniently be discussed under the headings of environmental

and management factors.

Environmental Factors

Changes in seasonal climate can influence availability and survival of larvae by altering the microclimate and microhabitats in which they live.

As mentioned previously the soil may act as an important reservoir of nematode larvae during the winter and spring in temperate countries, which apparently re-emerge onto the pasture and cause disease the following summer (Bairden, Parkins and Armour, 1979; Duncan, Armour, Bairden, Urquhart and Jorgensen, 1979). The micro-climate in which these larvae possibly survive will be affected by the prevailing weather conditions and their effect on soil moisture. Thus the amount and type of precipitation together with evaporation, either directly from the soil surface or indirectly by means of plant transpiration, will determine the level of soil moisture (Levine, 1978).

Furthermore, soil structure may significantly affect larval migration, either by modifying water retention and drainage, or by influencing the growth and composition of the herbage (Furman, 1944a; Persson, 1974a).

The layer or mat composed largerly of dead plant material, which forms between the herbage and the soil, particularly on older permanent pastures, may impair evaporation from the soil surface, and act as a reservoir of moisture even in the absence of rain. This, together with pockets of trapped air, helps to buffer the larvae against changes in temperature, and thus favour their development and survival.

The composition and length of herbage may influence the intake of larvae by stock at pasture (Taylor, 1938). Furman (1944b) found that clover favoured the development and survival of O.

circumcincta compared with lucerne or rye grass, while Tarshis (1958) noted that the lateral migration of cattle nematodes was less on clover than grass-like pastures. In contrast, Rose (1956) was unable to demonstrate any difference in the longevity of nematode larvae kept on either clover or rye grass pastures. Any variation in the length of herbage may affect nematode larvae by modifying their microclimate (Michel and Rose, 1954). Taller grass apparently favours their survival (Michel and Rose, loc. cit.), although its effect on the vertical migration of trichostrongylid larvae is not known (Silangwa and Todd, 1964; Skinner and Todd, 1980).

Host faeces offer considerable protection to many parasite free-living stages including those of Ostertagia spp. enabling them to exist under conditions that would otherwise prove fatal. The development and survival of these stages is largely dependent upon temperature, humidity (Rose, 1956, 1961, 1963; Silverman and Campbell, 1958; Ciordia and Bizzell, 1963; Crofton, 1963) and oxygen tension (Silverman and Campbell, loc. cit.; Rose, 1961). In Britain the development of eggs continues throughout much of the year although it is unlikely to be completed during winter when temperatures are lower. However, a proportion of the eggs may survive in the pat and resume their development when the temperature rises (Rose, 1961) providing a source of infection the following spring. The dungpat also protects eggs and pre-infective larvae against the effects of drought, to which they are susceptible, since the moisture content at the centre of the pat remains high even in the absence of rain (Roberts, O'Sullivan and Riek, 1952). Other factors regulating development and survival within the dungpat include the form and consistency of the faeces (Rose, 1961, 1962; Williams and Bilkovitch, 1971) which may be influenced by host diet (Gordon, 1967b), the rate of pat disintegration (Christie,

1963), dung-beetle activity (Reinecke, 1960; Bryan, 1973; Fincher, 1973, 1975), and certain management practices such as harrowing (Rose, 1962).

To ensure infection of the final host, dissemination of infective larvae onto the pasture is vital, since ruminants normally avoid grazing faecally-contaminated grass, or the lush herbage growing nearby (Rose, 1956; Crofton, 1958b). Thus in the absence of wet conditions, the L_3 remain congregated with, or adjacent to, the faeces and because of the so called "ring of repugnance" effect contact between host and parasite is minimal. Temperature and humidity are again important, larvae being more active at higher temperatures than low (Sturrock, 1965) and requiring a continuous film of moisture for migration (Roberts et al, 1952; Rose, 1956, 1961, 1963; Durie, 1961; Wallace, 1961; Crofton, 1963). Consequently, wet weather tends to favour migration from the dungpat, while drier conditions either prolong or delay it (Rose, 1956, 1961, 1962, 1963; Skinner and Todd, 1980). Furthermore, wet weather may facilitate the dissemination of larvae by increasing the rate of pat breakdown (Michel and Parfitt, 1956), a factor which Michel (1969a) regarded as being more important than larval migration. However, in the absence of such conditions, the faecal pat may act as a reservoir of infection for several months, until a change in the weather makes dissemination possible (Rose, 1961, 1962, 1963). Even under favourable conditions infective larvae move only short distances from faecal pats (Dinaburg, 1944; Furman, 1944b; Rose, 1961, 1963, 1970; Sturrock, 1965; Skinner and Todd, 1980). As a result, other methods of dissemination assume importance such as the feet of animals (Taylor, 1938; Michel and Parfitt, 1956), earthworms (Gronvøld, 1979; Oakley, 1981), psychodid flies (Jacobs, Todd, Dunn and Walker, 1968), fungi (Robinson, 1962)

and harrowing or mowing (Rose, 1962).

Survival of the free-living stages on pasture is important, not only to ensure continued development, but also to overcome any delay in contact with the final host (Michel, 1969a). The susceptibility of these stages to adverse weather conditions varies, the least resistant being the unembryonated egg and the pre-infective larvae, which are particularly vulnerable to desiccation and freezing (Rose, 1956, 1961; Andersen, Wang and Levine, 1966). The embryonated egg (Silverman and Campbell, 1958) and the infective larva (Furman, 1944a; Rose, 1961, 1962; Andersen et al, loc. cit.; Donald, 1968) are better able to withstand such conditions, especially if the latter develops within the egg, for example, Nematodirus helvetianus (Rose, 1966). Both these stages possess envelopes capable of keeping out water and filtering out some light (Levine, 1978).

Management Factors

The migration of larvae onto pasture and in turn their availability to grazing animals is also influenced by stock management.

An increase in stocking density not only raises the level of pasture contamination, as already mentioned, but also forces the animals to graze the herbage more closely (Taylor, 1930). This increases the availability of infection to the stock since the majority of larvae are found at the base of the plants (Taylor, loc. cit.; Rose, 1961, 1965). A higher stocking density may also result in a change in grazing behaviour, compelling animals to graze closer to dungpats, thereby taking in greater numbers of infective larvae (Nansen, Jorgensen, Hansen and Segrsen, 1978). At the same time however, this decrease in sward height may adversely affect the microclimate of the larvae and lead to increased mortality and lowered translation (Michel and Rose, 1954).

For many years rotational grazing has been recommended as a method for improving grassland utilisation and minimising the dangers of parasitic gastro-enteritis in ruminants (Gibson and Everett, 1968; Armour, 1978a). Conflicting results have, however, been obtained as to the benefits of this system (Michel, 1969a) and in some instances it has even increased the level of pasture infestation when compared with other systems, such as set-stocking (Armour, 1980). This has been attributed to the fact that development of infection in pasture and the mortality of infective larvae are much slower processes than was previously thought and also that the lush pasture produced a more suitable environment for survival.

Furthermore, certain pasture improvement methods have been shown to influence the level of infection on the herbage. Furman (1944b) found that the development and survival of O. circumcincta L_3 were enhanced on pastures that were irrigated, whilst Ciordia, Ernst, Stuedemann, Wilkinson and McCampbell (1977) and Ciordia, Stuedemann, Ernst, McCampbell and Wilkinson (1980) showed that moderate levels of nitrogen fertilisation lowered the burden of infection. This was probably due to a dilution effect of the stimulated growth of herbage on larval numbers.

The availability of larvae on a pasture may also be affected by the date of parturition of a herd. For example, single-suckled beef calves born in the spring and summer will commence to graze only when the overwintering herbage infestation has fallen to a low level and so there is a minimal risk of disease. Autumn-born suckled calves, however, will be virtually weaned by spring or early summer and be ingesting larger quantities of grass may pick up greater numbers of overwintering larvae. The risk of parasitic gastroenteritis is therefore increased in these animals, especially if they separated at weaning and are not grazed alongside immune stock which in effect reduce the rate at which parasite numbers are built up on the herbage (Michel, Lancaster and Hong, 1972a).

Arrested Larval Development

Although the term arrested development will be used here, the synonyms, inhibited larval development and hypobiosis are also frequently used. For present purposes it may be defined as the temporary cessation of development of a nematode at a precise point in its early parasitic life; in the case of Ostertagia spp. this is the EL_4 stage; it must however be distinguished from normally developing larval stages.

Three acceptable criteria for determining the occurrence of true larval arrest have been defined by Michel (1974) as follows:

(a) the finding of a large number of larval stages at precisely the same stage in grazing animals where there has been no recent uptake of larvae

(b) the same finding in animals withheld from possible infection for a period of time longer than that required to reach the stage of arrest

(c) the occurrence of a bimodal size distribution in worm populations from hosts not exposed to a corresponding pattern of infection.

Although interest in arrested larval development is now world-wide, the phenomenon was not considered to be of great importance until Martin et al (1957) published their stimulating article on outbreaks of atypical parasitic gastritis in housed cattle. As mentioned previously, these authors suggested that these outbreaks were due to the maturation of EL_4 stages which had been ingested as L_3 during the previous grazing season and then arrested in their development at the EL_4 stage, presumably due to an immunity acquired by the host as a result of exposure to larval challenge during the grazing season. This theory, that the arrestment of O. ostertagi larvae was due to host immunity, persisted for several years and indeed is still regarded as a possible cause of arrestment, particularly

in older cattle. However, in 1965a,b Anderson et al, initiated a series of experiments which suggested that host immunity was not the major influence in inducing O. ostertagi larva to become arrested in development. There were four key experiments carried out by this group of workers at Glasgow University (reviewed by Armour, 1978b).

In the first, which was primarily an epidemiological study replicated on two farms over a 2 year period, 10 helminth-naive "permanent" calves aged about 3 months were grazed from May to October on fields known to be contaminated with O. ostertagi larvae; they remained on the same fields unless the onset of clinical disease necessitated their earlier removal and necropsy. To monitor the seasonal fluctuations and availability of O. ostertagi L_3, groups of two helminth-naive calves ("tracers") of the same age as the "permanents" were grazed at each farm during successive fortnights throughout the grazing season; these tracers were then housed for a week and necropsied.

When the O. ostertagi burdens of the permanents were analysed at post-mortem they showed that:

When the worm burdens of the calves necropsied before and after October 1st were compared, there was a marked increase in the percentage of EL_4 stages in those necropsied after October 1st. This result was not unexpected in view of previous field observations but when a similar seasonal trend recurred in the proportion of EL_4 stages present in the tracer calves this was completely unexpected. When these results were published (Anderson et al, 1965a,b) the authors suggested that physiological changes in either the host or parasite during the autumn were responsible for the arrested development of larvae.

To overcome the possible criticism that the increase in numbers of arrested EL_4 stages in the autumn tracers might be due

to an exaggerated antigenic stimulus from the ingestion of high numbers of larvae on the pasture, even within the 14 day grazing period, a second experiment was carried out in the following year on which the periods grazed by the tracers were varied to 1, 14 and 28 days. The proportions of arrested larvae were similar in all the tracers and therefore independent of the length of time grazed and presumably of the level of larval challenge.

In the third experiment, several attempts were made to induce arrested larval development by subjecting L_3 of O. ostertagi to the environmental conditions of a European late autumn and inoculating these larvae to helminth-naive calves. All these attempts initially failed to induce a significant degree of arrest in the inoculated larvae. The isolate of L_3 used in these experiments had been experimentally passaged in calves on numerous occasions since it was first isolated at the Ministry of Agriculture laboratories in Weybridge in the early 1960's so it was decided to repeat the experiments using a fresh field isolate obtained from calves in the Glasgow area. The results of "autumn conditioning" these larvae and inoculating them to naive calves clearly showed that a large proportion of "conditioned larvae" became arrested in development; they also showed that simple storage at $4^{o}C$ produced the same effect and that if cold or autumn "conditioned larvae" are then subjected to spring conditions then they appeared to have lost the propensity to become arrested in development (Armour and Bruce, 1974).

The fourth and final experiment in the Glasgow studies was designed to assess if the host had any role in inducing seasonal larval arrest or stimulating the eventual development of these arrested larvae. Freshly harvested L_3 of a recent field isolate or those conditioned for several weeks at $4^{o}C$ were administered to calves kept in large animal climatic chambers in which the temperature, humidity and light

were programmed to simulate a spring environment; only the calves given the cold conditioned larvae had burdens containing significant proportions of arrested larvae and the proportion of arrested larvae were similar to those present in calves kept under normal winter conditions at the Glasgow University farm. These results emphasised the relationship between the larval condition prior to ingestion by the calf and the proportion of larvae which became arrested in development.

The maturation of arrested larvae was studied in calves in which large numbers of arrested EL_4 stages had been produced following the inoculation of cold conditioned larvae from a recently isolated field strain and the removal of susceptible adults by an anthelmintic. By serial slaughter of such calves it was demonstrated that the majority of larvae did not resume development until 4 months later. This experiment was completed during the winter and when it was repeated in the spring the period of maturation was again 4 months although the rate of maturation was faster. The spontaneous and synchronous development of the arrested larvae on the Glasgow experiments together with the ability of temperature variations to induce arrest prompted the Glasgow group to suggest that the whole phenomenon of larval arrest and maturation in O. ostertagi resembled diapause in insects, particularly as the ultra-structural appearance of the larvae indicated a reduction in secretory and neuromuscular activity.

Studies in several other laboratories in the Northern Hemisphere have also shown that arrest of O. ostertagi ingested during autumn grazing is a common occurrence e.g. in Northern Ireland (Ross, 1965), in Poland (Malczewski, 1970), in Canada (Smith, 1973), in Switzerland (Eisengger and Eckert, 1975), in Sweden (Nilsson and Sorelius, 1973), in England (Michel, 1974).

Michel and his colleagues have also been able to confirm

the Glasgow findings in relation to the induction of arrested development by prior exposure of O. ostertagi L_3 to cold conditioning (Michel, 1974). The one area of dissent is in relation to factors affecting maturation of arrested larvae. As mentioned previously, Armour and Bruce (1974) found that arrested larvae matured after a period of 4 months in a spontaneous and synchronous fashion.

Using a Glasgow isolate, Michel's group were unable to confirm these findings under both experimental and field conditions (Michel, Lancaster and Hong, 1976a,b). Indeed, from the analysis of O. ostertagi worm burdens in calves necropsied sequentially after both experimental and natural infections they consider that there is a regular turnover of Ostertagia populations in the host and that maturation is most definitely not spontaneous or synchronous although they concede that a higher proportion mature in spring; the reasons for the differences between the Glasgow and Weybridge data are not known but may in part depend on obviously dissimilar climatic influences.

In other areas of the world arrested O. ostertagi EL_4 accumulate at different seasons. For example, in the Southern Hemisphere such as temperate areas of Argentina and Australia they accumulate in spring (Hotson, 1967; Anderson, 1971) whereas in New Zealand the highest levels are recorded in autumn (Brunsdon, 1972). In Southern parts of the USA such as Lousiana and Texas, EL_4 stages of O. ostertagi are most common in late winter and early spring (Williams, 1980; Craig, 1980).

In contrast to the findings of Armour and Bruce (1974), in the above countries all experimental attempts at inducing larval arrest of O. ostertagi by subjecting them to cold conditioning have failed. This is not really surprising since the onset of the phenomenon occurs principally in spring or under conditions when the temperature is rising and dry conditions are prevalent.

The finding by Smeal et al (1980) that isolates of O. ostertagi from different areas of Australia display a varying propensity to become arrested in development has provided the opportunity to compare such isolates under differing environments. In an elegant experiment Smeal and Donald (1981) have recently studied two isolates of O. ostertagi, one from the Northern Tablelands of New South Wales which was prone to arrested development and another from the Coastal Region which showed a lower propensity for arrestment. When the respective isolates were transferred to the opposite environment they became arrested in development to the same degree as in their original environment suggesting that the propensity for arrestment was genetically determined and independent of an environmental stimulus such as cold temperatures. In summary, it appears that when arrested development of O. ostertagi occurs during a particular season of the year, it is a heritable trait of the larva itself and may occur in response to various adverse environmental stimuli, one of which in the Northern Hemisphere appears to be declining temperatures or cold conditions.

Host Immunity

Although comparatively few studies have been made on the acquisition of immunity by the host in ostertagiasis, three important findings have emerged.

The first is that an absolute age immunity against O. ostertagi does not operate. The lack of such an immunity is evident from both field and experimental studies. Thus in the United States outbreaks of clinical ostertagiasis have been described in adult cattle moved from areas where the causal parasite does not occur, to areas where outbreaks are common in young stock (Bailey and Herlich, 1953). In Britain, clinical ostertagiasis has also been described in groups of adult beef cows introduced into dairy farms where the disease is endemic; in each instance these beef cows originated

from farms with no history of ostertagiasis (Selman et al, 1976). Experimental studies in the United States and Britain have also demonstrated that while adult cattle were more resistant to the debilitating effects of experimentally induced ostertagiasis (Herlich, 1960) and the developmental period of the worms was slower and the egg production markedly lowered (Armour, 1967) considerable numbers of worms developed and the pathological changes were similar to young naive calves. It is possible of course that adult cattle will acquire an immunity more rapidly than young stock but this aspect has not yet been examined.

The second important finding is that young cattle acquire an immunity relatively slowly and outbreaks occur frequently on animals after 3 to 4 months exposure. Studies in Britain have shown that calves at the end of their first full grazing season of approximately 5 months i.e. May-October were immune to experimental challenge infection (Ross and Dow, 1965 and Armour, 1967) but this immunity had waned considerably following a winter period of housing i.e. by the following April (Armour, 1967). More recently, studies in young cattle over two consecutive summer grazing seasons with an intervening period of winter housing have clearly shown that while cattle at the end of the first season had high O. ostertagi burdens (>50,000), those which grazed for a second season had a mean burden of less than 10,000 O. ostertagi and markedly lower faecal egg counts (Armour, Bairden, Duncan, Jennings and Parkins, 1979). It seems that while young cattle acquire a good immunity by the second grazing season sufficient burdens were established to maintain contamination of the pasture albeit at a reduced level.

Experimental attempts to induce an immunity in young cattle using X-ray attenuated larvae (Armour, 1967; Burger,Ekert, Chevalier, Rahman and Königsmann, 1968) have been unsuccessful which is not surprising in view of the field situation.

The third area of interest is that where a reasonable degree of immunity has become established after two grazing seasons, the state is not absolute, particularly in the heifer cow around calving and during early lactation. This was clearly shown by Michel, Lancaster and Hong (1979) who found that dairy heifers were highly susceptible to experimental infections with O. ostertagi at this time. Immunity appears to be restored towards the end of the first lactation but low burdens are often present in older cows as shown by the results of several surveys. Some workers consider that those low burdens can cause a loss of productivity but this requires further investigation. Sometimes individual dairy cows accumulate large burdens of arrested larvae which mature at the time of parturition causing severe clinical ostertagiasis (Hotson, 1967; Wedderburn, 1970; Raynaud and Bouchet, 1976). The reason for these outbreaks is not clear but has been generally ascribed by the authors to a breakdown in immunity caused by the stress of calving; why these particular cows should have accumulated such high burdens of arrested EL_4 is not known.

TREATMENT AND CONTROL

Treatment

Outbreaks of Type I ostertagiasis respond well to treatment with broad-spectrum anthelmintics (Armour, 1974) for within 48 hours, abomasal function and appetite return to normal (Armour, Jennings, Kirkpatrick, Malczewski, Murray and Urquhart, 1967c).

In contrast, cases of Type II diseases have, until recently, been more difficult to treat since even repeated dosing had little effect on the arrested larvae deep in the abomasal glands (Armour, 1974). Moreover, attempts to stimulate their resumed development by hormone therapy, and thereby render the larvae more susceptible to the available anthelmintics, have given conflicting results

(Armour, Jennings, Reid and Selman, 1975; Cawley and Lewis, 1975; Cummins and Callinan, 1979).

Fortunately, recent work with some of the latest oral benzimidazoles, notably fenbendazole (Duncan, Armour, Bairden, Jennings and Urquhart, 1976; Duncan, Armour and Bairden, 1978), oxfendazole (Armour, Duncan and Reid, 1978; Kistner, Wyse and Averkin, 1979) and albendazole (Williams, Knox, Sheehan and Fuselier, 1977; Downey, 1978), has been promising, with efficacies of over 90 per cent demonstrated against inhibited Ostertagia larvae. However, variations in activity of two of the drugs, fenbendazole (Lancaster and Hong, 1977; Elliot, 1977; Lancaster, Hong and Michel, 1981; Williams, Knox, Bauman, Snider and Hoerner, 1981a) and albendazole (Williams, Knox, Baumann, Snider and Hoerner, 1981b) have been reported against these stages, reasons for which are discussed later.

Even more encouraging results have been obtained with a new group of antiparasitic agents, the avermectins, which are produced as a fermentation metabolite of the actinomycete, Streptomyces avermitilis (Burg, Miller, Baker, Birnbaum, Currie, Hartman, Knog, Monaghan, Olson, Putter, Tunas, Wallick, Stapley, Owia and Omura, 1979). A chemically modified injectable derivative, ivermectin, has been shown to possess high efficacy (over 99 per cent) against arrested Ostertagia larvae (Armour, Bairden and Preston, 1980b; Yazwinski, Williams, Greenway and Tilley, 1981) and is likely to be a valuable tool in the control of Type II ostertagiasis.

Sources of variation in anthelmintic efficacy

The efficacy of anthelmintic therapy in the treatment of ostertagiasis was considered by Kelly, Gordon and Whitlock (1976) to be influenced by parasite-, host- and drug-related factors.

The parasite may affect anthelmintic efficacy either by developing drug resistance or by undergoing arrested larval development.

Resistance was defined by Kelly and Hall (1979a) as a significant

increase in the ability of individual parasites within a population to tolerate doses of a compound which would prove lethal to the majority of individuals in a normal population of the same species. Selection towards resistance can be achieved by breeding from adult worms that survive a discriminating dose of the drug.

Anthelmintic resistance amongst sheep nematodes has been widely reported since Drudge, Leland, Wyant and Elam (1954) first isolated a strain of Haemonchus contortus resistant to phenothiazine in the United States. The occurrence of resistance to the later broad spectrum anthelmintics, including thiabendazole, levamisole and morantel, has been reviewed by Kelly and Hall (1979b).

In contrast, resistance among cattle parasites has only been reported in the last five years. Recent studies in Australia have demonstrated inconsistent results following treatment with levamisole and thiabendazole, with efficacies as low as 49 and 56 per cent, respectively, against adult Ostertagia spp., and even lower efficacies against larval stages. Although fenbendazole and oxfendazole were much more effective in controlling worm burdens in the same studies, a few animals still harboured large numbers of Ostertagia spp., particularly early fourth stage larvae, after treatment (Anderson, 1977; Anderson and Lord, 1979). Similar results have been obtained in studies in the United States with fenbendazole (Williams et al, 1981a; Craig and Bell, 1978) and albendazole (Williams et al, 1981b).

While helminth populations are undergoing genetic change in response to anthelmintic selection, there is also a simultaneous selection for genes that enhance the survival potential of resistant worms (Kelly and Hall, 1979b). Physiological changes that have been reported in resistant parasite strains include an increase in infectivity (Drudge, Leland and Wyart, 1957; Kelly, Whitlock, Thompson, Hall, Martin and Le Jambre, 1978), pathogenicity (Kelly,

et al, loc.cit.). Other changes associated with resistance and discussed by Kelly and Hall (1979b) include the development of side and/or cross-resistance and significant reductions in the inhibition of parasite fumarate reductase by benzimidazole drugs (Prichard, 1970, 1973) and in the uptake of the anthelmintic fenbendazole by the parasite (Prichard, Donald, Dash and Hennessy, 1978a). Resistant strains may also exhibit increased levels of arrested larval development (Le Jambre, Southcott and Dash, 1978) and greater development and survival of eggs and infective larvae on pasture (Kelly et al, 1978).

The chemotherapeutic significance of arrested development lies in the fact that inhibited or hypobiotic larvae are relatively insusceptible to anthelmintics known to be effective against worms developing at the "normal" rate. Using radioisotope labelled thiabendazole, Prichard et al (1978a) showed that the relative anthelmintic tolerance of inhibited Ostertagia ostertagi was not due to a lower uptake of the drug, but might be related to the duration of contact. This hypothesis was confirmed by Prichard, Kelly and Thompson (1978b) who infused thiabendazole over 36 hours and were able to remove 90 per cent of the arrested larvae. They suggested that the larvae were able to withstand a temporary energy deprivation as a result of treatment, but not a sustained one. Accordingly, anthelmintics with a relatively long half-life in the host, for example fenbendazole and oxfendazole, are far more effective against inhibited larvae, than those with much shorter half-lives, such as thiabendazole.

Host factors likely to influence the efficacy of anthelmintic treatment include the oesophageal or reticular groove reflex, and the self-cure phenomenon. Early investigation of erratic anthelmintic activity showed that the oesophageal groove reflex was often responsible for reduced activity against some sheep nematodes (Gordon, 1962, 1963). More recent work with radioisotope labelled thiabendazole

and fenbendazole showed that high plasma levels of the active compounds were reached more rapidly following intra-abomasal administration, though their persistence was much shorter, than when the two drugs were given intraruminally (Prichard et al, 1978b). Furthermore, ruminal by-pass has been suggested (Duncan, Armour, Bairden, Jennings and Urquhart, 1977) as a cause of the variable action of fenbendazole on arrested O. ostertagi larvae in cattle (Duncan et al, 1976; Elliot, 1977; Lancaster and Hong, 1977). A similar hypothesis was proposed for the variable efficacy of albendazole against early fourth-stage larvae (Williams et al, 1981b).

Certain physical characteristics of anthelmintics have been shown to affect efficacy, notably particle size and dose volume. The effect of particle size of the active component on the efficacy of insoluble or sparingly soluble drugs has been reviewed briefly by Kelly et al (1978). Enhanced anthelmintic activity following a reduction in particle size has been demonstrated with phenothiazine (Gordon, 1940, 1956; Whitten, 1956; Thomas and Elliot, 1957; Kingsbury, 1958), mebendazole (Kelly, Chevis and Goodman, 1975).

The effect of dose volume on anthelmintic efficacy was demonstrated by Clunies Ross (1936) who found that a greater proportion of sheep will swallow a drench directly into the abomasum when the dose volume exceeds 10 ml. However, with the trend towards smaller dose volumes with modern anthelmintics this is likely to become less important (Kelly et al, 1976).

Control

Control of ostertagiasis is applied mainly to yearling stock in the herd; it has been attempted in a number of ways e.g. strategic chemotherapy involving the use of anthelmintics at selected times, various systems of grazing management or a combination of both.

The fact that the annual pattern of increase and decrease

of numbers of L_3 on herbage at varying times of the year usually occurs in a regular pattern, has provided the philosophy for most of these control measures. For example, in the Northern Hemisphere and particularly in Western Europe, the so called Weybridge dose and move system (Michel, 1969a) relies primarily on two factors; first, that L_3 acquired by young cattle turned out to graze in the spring are seldom sufficiently numerous to have a significant effect on these cattle although they can result in considerable numbers of eggs in the faeces being deposited on the pasture within 3-4 weeks; secondly, it takes until mid-July for these newly deposited eggs to become L_3 so that a move prior to that time to pasture ungrazed by cattle since the previous autumn should result in stock becoming only lightly infected. If this move were accompanied by effective anthelmintic treatment the stock should remain virtually worm free during the remainder of the grazing season. The logical conclusion from the use of such systems would be that if widely used they should lead to the eradication of ostertagiasis but it is pertinent to note the comment of Spedding (1969) that whilst the eradication of helminth disease is possible the eradication of helminths is not.

Grazing management systems used to combat bovine ostertagiasis involve either rotational grazing of cattle, alternate grazing of cattle with different host species or integrated rotational grazing of different age groups of cattle. The straightforward rotational system involving only cattle has proved to be less effective than set stocking of cattle on pasture (Levine and Clark, 1961; Michel, 1969b). It appears that in the continuous grazing systems the sparse nature of the herbage results in the larval stages being denuded of grass cover with increased exposure to the environment and consequently they succumb more readily to the effects of adverse

conditions. In contrast, the more luxuriant herbage cover on rotational systems affords protection and encourages larval survival.

Better control has been achieved by methods which involve either grazing mixed host species together (Arundel and Hamilton, 1975) or alternate grazing with two different host species (Southcott and Barger, 1975; Rutter, 1975). Both of these systems depend for their success on the host specificity of Ostertagia spp. By using systems in which the different host species alternate between fields or integrate in a mixed grazing system the non-receptive host can ingest L_3 harmful to the other without deleterious effect. This has the effect of reducing the contamination of pasture and so the numbers of potentially infective stages available to each species.

Improved liveweight gains have also been reported when susceptible dairy calves were rotationally grazed on permanent pastures and followed by replacement heifers in their second or third grazing season (Leaver, 1970) (Nagle, Brophy, Capprey and Ô'Nuallain, 1980). The success of this system known as the leader/ follower depends on the careful management of the paddocks grazed by the calves and on the assumption that the two to three year old dairy heifers are immune to infection with O. ostertagi. Thus the calves are only permitted to graze the upper leafy part of the herbage before being moved onto the next paddock thus avoiding the mass of L_3 thought to be concentrated in the lower quartile of the herbage (Crofton, 1954) and subsequently ingested by the incoming immune heifers. However, the immunity expressed by the heifers will be dependent on their previous exposure to the parasite, since age per se does not guaranteee a high immunity. A possible weakness of the system is that even when heifers have acquired a good resistance this may wane in the period around the first calving, and the worm burdens which develop may be sufficiently

large to affect production (Michel et al, 1979). An alternative to using heifers is to use dry cows (Downey and Fallon, 1973) but it may not always prove possible to have sufficient dry cows available throughout the season to maintain the ratio of cows to calves.

The well-known husbandry system of zero grazing, in which cattle are housed and fed indoors with cut grass, is used in mainland Europe, but only to a limited extent in Britain. This system has an obvious application in ostertagiasis control, and an adaptation has been described by Borgsteede (1977). His calves were grass-fed indoors only from April to June, and were then turned out to graze on the well-documented premise that the mortality in overwintered L_3 would have resulted in almost negligible pasture infectivity by then; though these calves did acquire some infection, with consequent pasture contamination, the levels of L_3 which appeared on the herbage were insignificant compared with those on control pastures.

Finally, though anthelmintic treatment of young cattle set-stocked on permanent pasture has been shown to be economically beneficial (Cornwell, Jones and Pott, 1973b)regular reinfection occurs and several treatments are necessary to maintain production levels; this is undesirable from the management viewpoint, particularly so when labour costs are increasing annually. To obviate these problems several authors have recommended that treatments should be concentrated in the early part of the grazing season (Pott, Jones and Cornwell, 1974; Armour, 1978a; Herd, 1980). In such a regime they have demonstrated that by limiting pasture contamination to a sufficiently low level in the spring and early summer the expected increase in L_3 numbers from mid-July onwards was considerably reduced and economically significant weight gains were achieved. In the Southern Hemisphere, the same principles have been used for the control of ostertagiasis and the reservation of safe pasture

at times of the year when levels of infection are known to be increasing has been the method widely advocated in areas such as Australasia.

Clearly there is now a wide range of effective anthelmintics for the treatment of ostertagiasis and several options are available for its prevention. The problem lies in disseminating the correct information at farm level and deciding on which system is most suitable and cost efficient for the enterprise in question.

The choice will depend on various factors including:

1. the availability of host species for alternate grazing
2. the degree of intensification which permits the reservation of adequate acreage of safe grazing

It should however be possible to construct a control programme to fit the systems employed on a particular farm.

Introduction to Field and Experimental Studies

From the literature review it is clear that further studies are necessary on two factors which could adversely affect the implementation of control schemes for ostertagiasis. These are 1) the existence of a mobile reservoir of larval infection in the soil which is capable of migrating onto the herbage of pastures ungrazed for several months and thus generally considered to be safe; 2) the development of strains or isolates of Ostertagia spp., possibly induced by drug selection, which are of a very high infectivity and therefore pathogenicity and could cause problems of production losses when the level of larvae on the pasture was below that usually associated with disease.

In this thesis both these factors are investigated. First, the Ostertagia spp. larval populations in the herbage and soil of a rested pasture (i.e. free from grazing livestock for 20 months) are monitored over a 12 month period and their availability assessed after a further 8 months i.e. a total of 20 months. Secondly, the infectivity, fecundicity and pathogenisis of three different isolates of bovine Ostertagia spp. are compared under both experimental and field conditions. Finally, the possible significance of the results are discussed in relation to current control measures.

CHAPTER 2

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

The calves used in the experiments were male calves either of the Friesian or Ayrshire breed. All animals were reared under helminth-free conditions and to facilitate handling, the calves were castrated.

Management

In experiments carried out indoors, the calves were penned individually and bedded on clean straw which was replaced every 2 days; they were offered 1 kg. of concentrates and 3 kg. of hay daily, and water was available ad libitum. In the outdoor experiments the calves were grazed on plots, situated within the grounds of Glasgow University, which had been grazed regularly by ruminants for a number of years. The area of each grazing plot was approximately 0.33 hectares and separation of adjacent plots was achieved by means of a double wire mesh fence with 1.5 metres between fences. When necessary, supplementary feeding consisting of 1 to 3 kg. of hay per day was given towards the end of the grazing season i.e. late September/October.

Clinical Examination

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

BIOCHEMICAL TECHNIQUES

Plasma pepsinogen estimation

Blood samples for pepsinogen estimation were taken directly from the jugular vein into heparinised vacutainer tubes (Becton - Dickinson Ltd., York House, Empire Way, Wembley, England) and centrifuged at 2000 revolutions per minute (rpm) for 20 minutes. The plasma

obtained was then processed to determine the level of pepsinogen present, the method used being that described by Edwards, Jepson and Wood, 1960 . In this technique the plasma is incubated with bovine serum albumin (BSA) at pH 2.0 for 24 hours and the phenolic amino acids (tyrosine) liberated estimated using the Folin-Ciocalteau reaction. Corrections are made for the normal (i.e. non-incubated content of tyrosine substances and also for the release of these substances from BSA when incubated alone. The detailed procedure used is given in Appendix A.

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

PARASITOLOGICAL TECHNIQUES

a) Faecal Analysis

Two techniques were used to examine faeces for the presence of helminth eggs, namely:

1) The Clayton Lane method

2) A modified McMaster flotation (Gordon and Whitlock, 1939).

Although the Clayton Lane method is the more accurate, government legislation (Health and Safety at Work Act, 1981) recently introduced now precludes its use on the grounds of safety. As a result, only the modified McMaster technique was used in the later experiments.

Clayton Lane method

A flotation technique similar to that devised by Gordon and Whitlock (1939) was used to detect the presence of trichostrongyle eggs in faecal samples collected for examination. In this technique 4.5 g. of faeces were homogenised with 40.5 ml water and the resulting suspension passed through an 8" diameter 100 mesh screen resting in a round bottomed aluminium bowl. This sieve retained the larger particles of debris but allowed the passage of nematode eggs (size

range 70-165 u.). After thorough mixing, 18 ml. of filtrate were withdrawn into a Clayton Lane tube (i.e. a flat bottomed 18 ml. volume test tube) and centrifuged at 1500 rpm for 5 minutes. The supernatants from the tubes were then discarded and the remaining faecal mass broken up by rotary agitation (Whirlimixer, Griffin, Wembley, Middlesex, England). The tube was then filled to its former level with saturated salt solution and after mixing, sufficient of the suspension was transferred quickly by pipette to fill both chambers of a McMaster slide (Gelman Hawksley Ltd., Harrowden, Northampton, England). The number of eggs under both total areas of the slide were counted and the result multiplied by 10 to give the number of eggs per gram (epg) of faeces according to the following calculation:

4.5 grams of faeces plus 40.5 ml. of water gives a concentration of 1 gram / 10ml.

The total volume of both chambers equals 0.96 ml.

Therefore the number of eggs present x 10 is approximately equal to the number of eggs in 10 ml. which is equivalent to the number of eggs per gram.

If less than two eggs were seen, saturated salt solution was added to the tube to create a small positive meniscus. The top of the tube was then sealed with a thick coverglass and spun at 900-1000 rpm for two minutes. The coverglass was then placed on a clean slide and examined microscopically, each egg seen representing 1 epg of faeces. This examination was carried out as soon as possible after the McMaster count.

Modified McMaster Method

In this technique 3 g. of faeces were homogenised with 42 ml. of water and the resultant suspension passed through a 250 micron sieve (Endecotts Test Sieves Ltd., Morden, London). After thorugh mixing of the filtrate, 15 ml. were withdrawn into each of two flat

bottomed centrifuge tubes (capacity 15 ml) and the latter centrifuged at 2000 rpm for two minutes. The supernatants from both tubes were then discarded and the remaining faecal mass broken up by rotary agitation. One tube was then filled to its former level with saturated salt solution and after inverting 6 times a volume of the suspension, sufficient to fill both chambers, was quickly transferred by pipette to a McMaster slide. The number of eggs under the etched areas of the slide were counted and the result multiplied by 50 to give the number of eggs per gram of faeces according to the following calculation:

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

Volume under one square equals 0.15 ml.

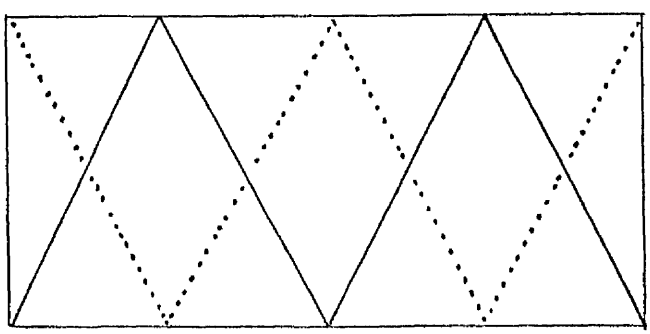
No. of eggs seen in one square x 100 = No. of eggs/gram

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

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

b) Analysis of herbage for the presence of trichostrongyle L_3

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



——————— Route 1

..... Route 2

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

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

The grass washings were allowed to sediment for a minimum of six hours, the supernatant drawn off and the sediments bulked. After filtration through a double milk filter (Maxa Filters, A. McCaskie, Stirling, Scotland) the larvae retained were recovered using a Baermann apparatus, consisting of a glass funnel closed at the stem with a length of rubber tubing and clip and filled with warm water (Plate 2). A 150 micron aperture sieve supported the milk filter, thus allowing the motile larvae to migrate to the warmer side of the temperature gradient. Twelve hours later, 200 ml of fluid was withdrawn from the neck of the funnel and reduced by a process of sedimentation and centrifugation to a final volume of 10 ml. The larvae in 1 ml. were differentiated and counted. The criteria for larval identification were those detailed in Technical Bulletin No. 18 (Ministry of Agriculture, Fisheries and Food) and used by Keith (1953) i.e. body length, prolongation of the second larval sheath beyond the tail of the third stage larva and the

Plate 2. Baermann apparatus used to recover trichostrongyle larvae from herbage and soil.

presence of refractile structures.

c) Analysis of soil for the presence of trichostrongyle L_3

The recovery of trichostrongyle larvae from soil was achieved using the technique described by Bairden (1980) and can be summarised as follows:

Fifty to one hundred and twenty-five soil cores were collected using a modified bulb planter (Plate 3) the side slots of which enabled the core to be divided inside the sampler, thus minimising contamination between layers. The cores, as far as possible uniformly sized (i.e. 15 cms. in length by 6 cms. diameter) were subdivided into four sections (Plate 4). A - herbage, B - root-mat (i.e. top 0.5 cm. layer), C - upper soil (7.0 cm. depth) and D - lower soil (greater than 7.0 cm. depth) and material from each section bulked. All aggregates were weighed and processed as follows:

Each sample was added to 20 litres of warm water, to which 5 ml. Lissapol detergent had been added, and allowed to soak for at least 12 hours with intermittent agitation. The mixture was then stirred and after standing for approximately 30 seconds, during which time the heavier soil particles and stones etc. sank to the bottom of the container, the supernatant was passed through a one metre square of nylon cloth, supported by a conical wire mesh in the form of a filter (Plate 5).

By hosing with large volumes of water the larvae were further cleaned and this stirring, sedimentation and filtration process repeated four times for each sample. The material retained by the filter was further concentrated using a perspex column/sieve shown in Plate 6. After baermann extraction for 12 hours, 200 ml. was drawn off from the funnel and concentrated by sedimentation and centrifugation to a final volume of 1 ml. The larvae were identified using the same criteria as for identification of those

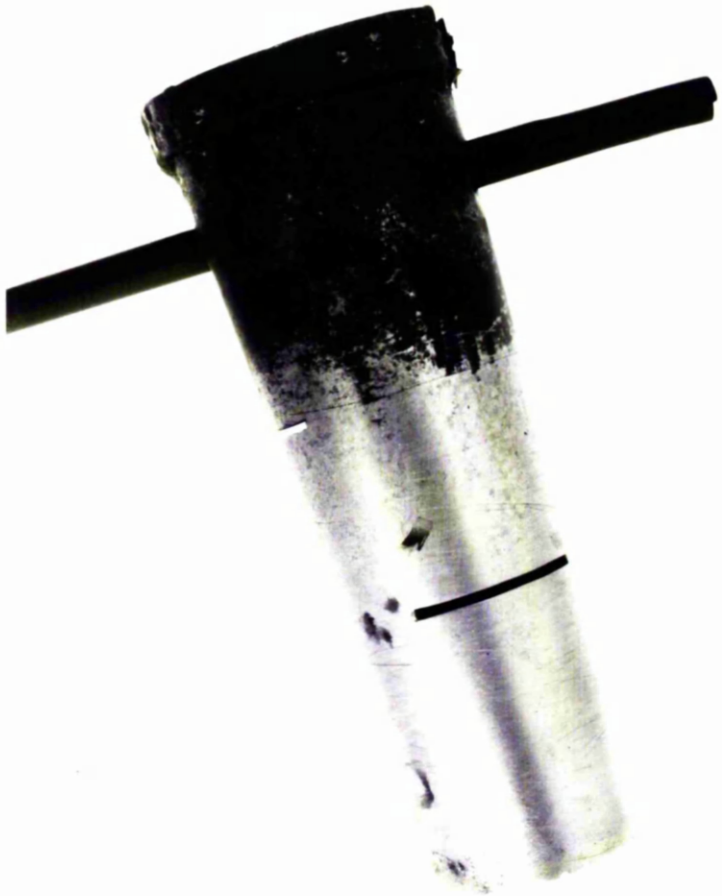


Plate 3. Soil sampler used to remove soil cores.

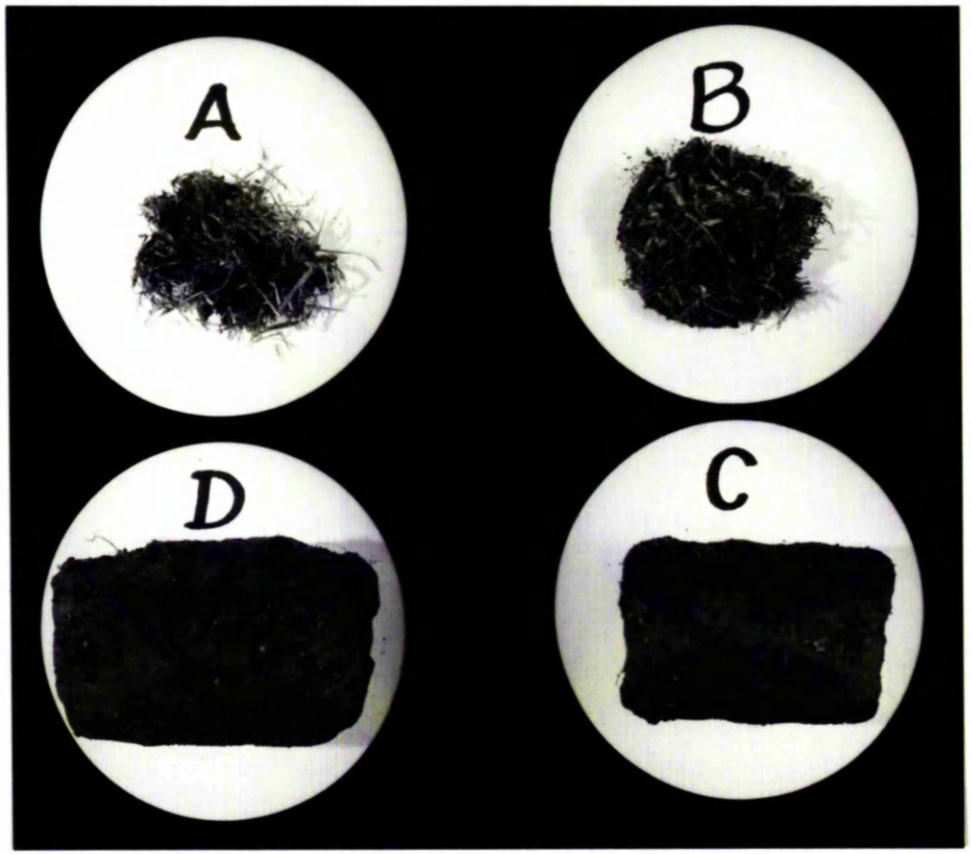


Plate 4. Soil core sections: A-Herbage, B-Root mat (0.5cm), C-Upper soil (0-7cm) and D-Lower soil (7-14cm).

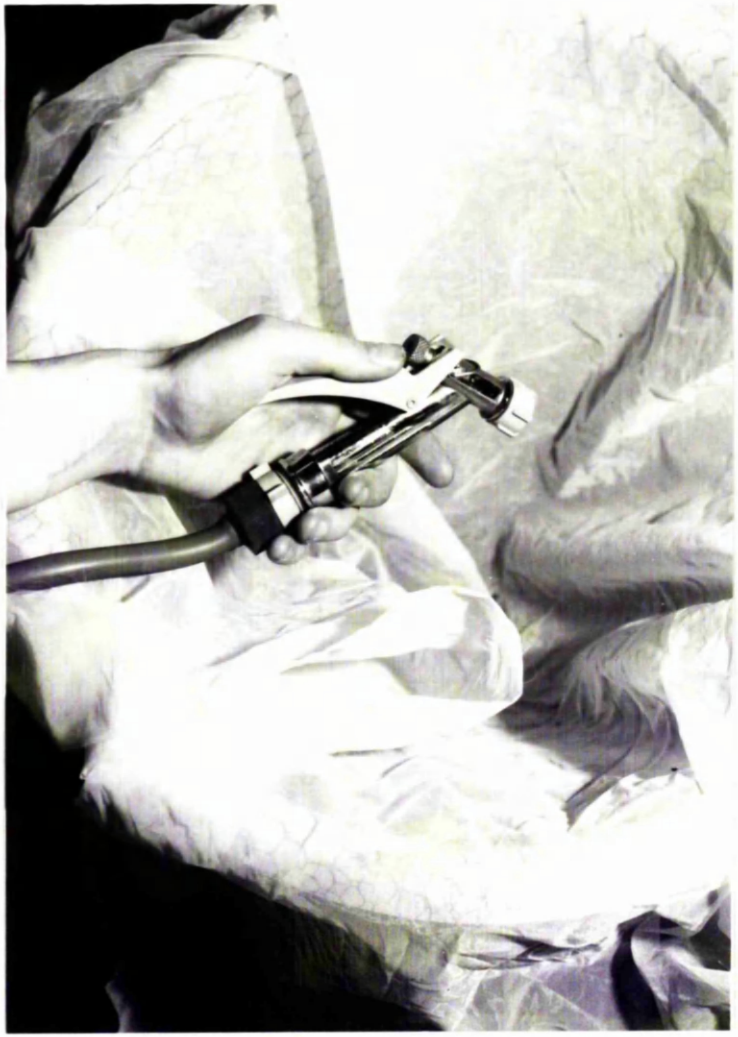


Plate 5. Nylon mesh used for larval filtration.

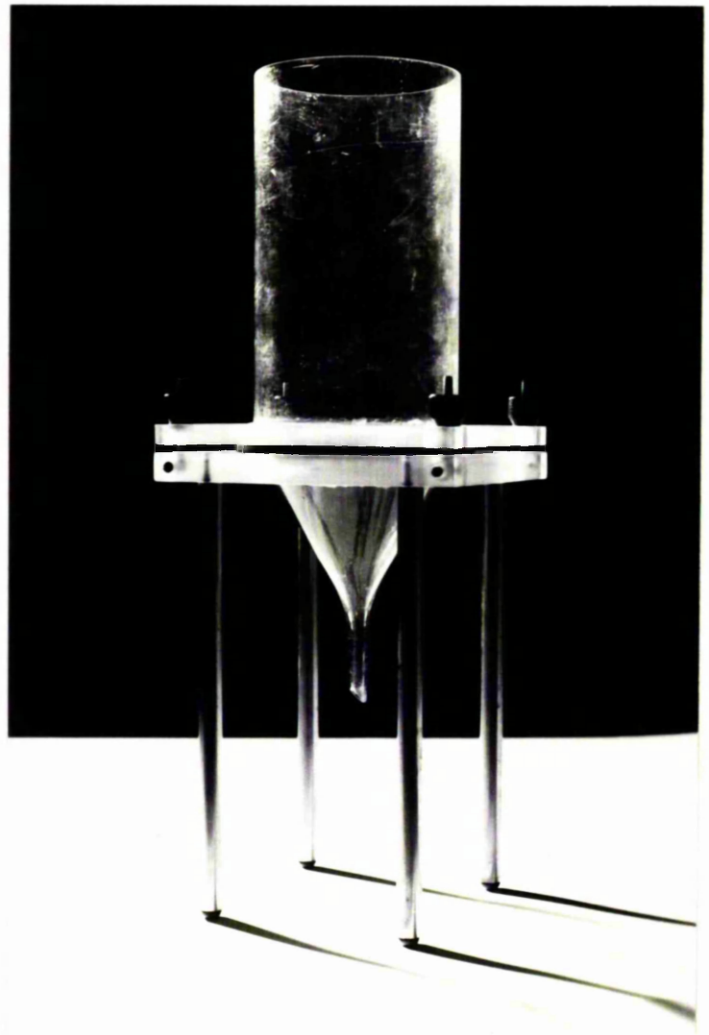


Plate 6. Larval concentration apparatus.

recovered from herbage.

d) Preparation of larval inocula

Faeces containing Ostertagia spp. eggs were collected from an experimentally infected calf using a bag and harness system as shown in Plate 7. These were subsequently mixed with Vermiculite, 100 g. aliquots placed in screwtop glass jars and incubated at 23°C for 14 days. The infective larvae were recovered by standard Baerman techniques as described previously.

Larval inocula were prepared by counting the number of larvae present in 40 x 0.025 ml. aliquots. A minimum total number of 400 larvae were counted and to facilitate examination the larval dilution was so arranged that the number of L_3 per 0.025 ml. aliquot did not exceed 30. Once the number of L_3 present in 1 ml. was known the volume necessary to provide the required inoculum was pipetted out and made up to a volume of approximately 20 ml. prior to dosing the calves. Throughout the whole counting procedure emphasis was placed on regular agitation of the suspension to prevent clumping.

e) Necropsy procedure

In the outdoor experiments, except when their clinical condition necessitated earlier slaughter, the calves were housed for at least seven days prior to post-mortem. A captive bolt pistol was used to kill the calves which were then immediately bled out. After opening the abdomen, the pyloric sphincter was ligatured and the gastro-intestinal tract then removed from the body cavity. The large intestine and its contents were discarded as L_3 of Oesophagostomum radiatum were absent from all the pasture samples and Trichuris species eggs from all of the faecal samples examined.

The abomasum and small intestine were separated (in the indoor experiment only the abomasa were examined). A sample of

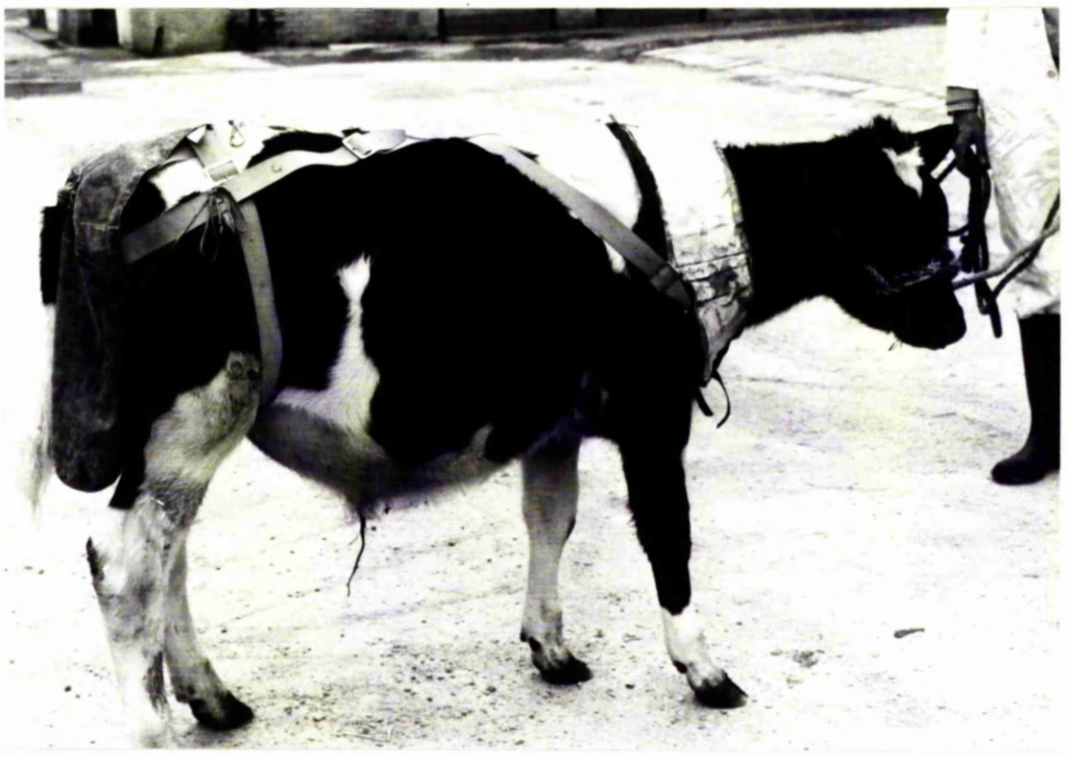


Plate 7. Harness system used to collect faeces.

abomasal contents was taken as soon as the abomasum was opened and a Radiometer pH meter type PHM 26 c (Electronic Measuring Instruments Ltd., Copenhagen, Denmark) was used to determine the pH levels.

The contents were washed into buckets and the volume made up to a standard 4 litres (except where an unusually large amount of material was present when a greater volume of water was required) and duplicate samples of 200 ml. withdrawn and formalised for subsequent examination. The abomasal mucosa was scraped off and digested in three times its volume of a pepsin/hydrochloric acid mixture for six hours at 42°C. The digested mixture was then made up to 4 litres and 200 ml. samples withdrawn as before. The parasites present in 10 x 4 ml. aliquots were counted and classified as adult male or female, developing fourth or fifth larval stages or early fourth stage larvae (EL_4) depending on bursal or vulvar development, the presence of a sheath projection and size respectively. In the case of Ostertagia spp. the EL_4 were considered to be arrested in development since this stage is reached in four days and the animals were maintained free from further infection for a minimum of seven days.

Two hundred male worms from each calf were mounted in Berlese's medium for more precise identification.

In the field experiment, where lungworm larvae were known to be present on the herbage, the lungs were processed using a modification of the Inderbitzen (1976) perfusion technique. In essence the procedure was as follows:

The pericardial sac was incised and reflected to expose the pulmonary artery in which a 2 cm. incision was made. Rubber tubing was introduced into the artery and fixed in situ by double ligatures (Plate 8). The remaining great vessels were tied off and water from a mains supply allowed to enter via the pulmonary

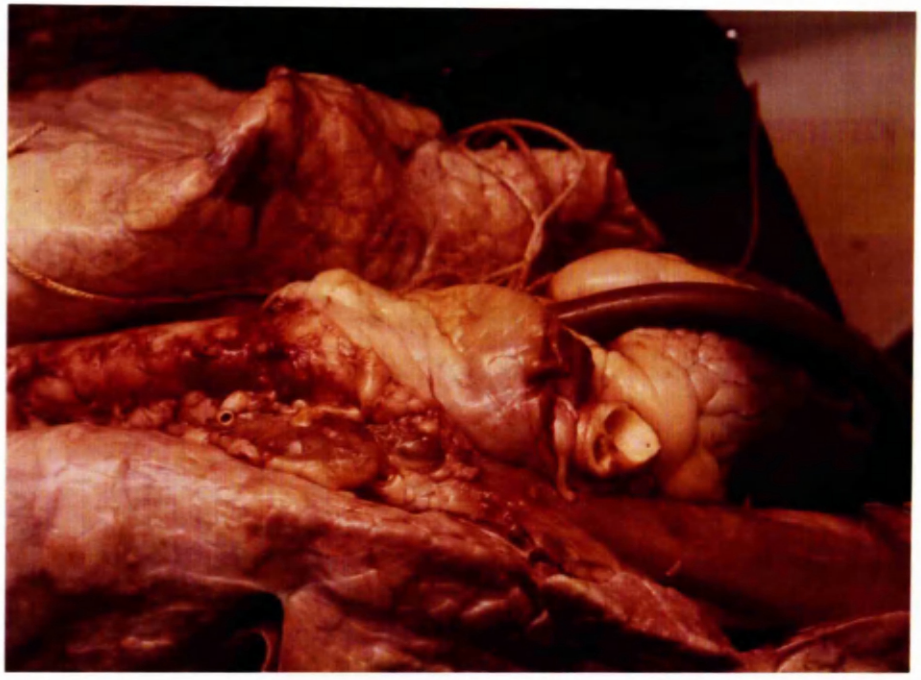


Plate 8. Rubber tubing fixed in situ in pulmonary artery.

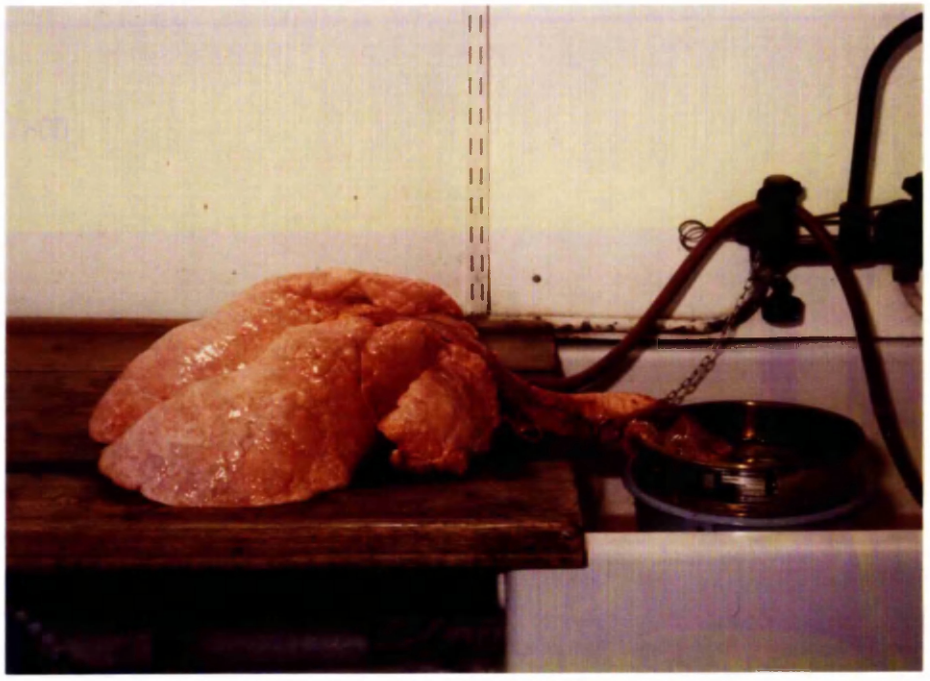


Plate 9. Sieving of pulmonary washings.

artery (Plate 9). Twenty litres of washings were collected from the lungs and concentrated by passing through a 37 u aperture sieve. Parasites recovered in this way were allowed to relax overnight at 4°C, counted and differentiated on the basis of size and stage of development i.e. size range 1-2 cm and the absence of any definite internal structure (4th larval stages), size range 3-5 cm and a noticeably increasing developmental pattern, particularly in the uterine and bursal regions(5th larval stages), size range 5-10 cm.,presence of eggs in females and fully developed bursal regions in males. (mature parasites).

HISTOPATHOLOGY

Tissue samples were taken from the pyloric and fundic areas of the abomasum. These were fixed in either 10% formalin, for routine microscopic study of the histological structure and eosinophils, or Carnoy's fluid for mast cells, mucus and immunoglobulin-containing cells.

Formalin-fixed tissues were processed in a standard Histokinette cycle and vacuum embedded in paraffin wax. Sections cut at 6 u were examined after the following histochemical stains:

1. Haematoxylin and Eosin.
2. Haematoxylin and Azo-Eosin.

Carnoy-fixed tissues were dehydrated and cleared in three changes each of 100% methanol and xylene at 4°C and vacuum embedded in wax. Sections cut at 6 u were examined after staining with:

1. Astra-blue/Safarin O.
2. Alcian blue/Periodic Schiff (PAS).

METEOROLOGICAL OBSERVATIONS

For the outdoor experiments wet and dry temperatures were recorded at ground level using a mechanically operated thermograph (Negretti and Zambra Ltd., Ayesbury, England) and weekly rainfall

measured using a Symons rain gauge, the result being expressed in millimetres.

General Statistical Methods

Where data were compared that could not be shown to be normally distributed, non-parametric tests were used. In particular the Mann-Whitney test was employed to compare one treatment group against another or a control group. Correlation co-efficients were used to obtain a measure of correlation between variables.

Unless otherwise stated, the tests were carried out at the 5% significance level. All statistical methods used can be found in Steel and Torrie (1980).

CHAPTER 3

STUDIES ON THE SURVIVAL AND AVAILABILITY OF BOVINE *Ostertagia* SPP. INFECTIVE LARVAE ON HERBAGE AND IN SOIL OF RESTED PASTURE.

1) A population study of free-Living L_3 of bovine *Ostertagia* spp. over a 12 month period.
2) Availability of *Ostertagia* spp. to grazing calves after resting for 20 months.

1) A Population Study of Free-Living L_3 of Bovine Ostertagia spp. over a 12 month period.

INTRODUCTION

Though the larval populations of plant-parasitic nematodes in soil have been the subject of much useful study, there has been little equivalent research on the infective larvae of animal parasites. Soil scrapings have been shown by Tripathi (1974), Levine and Todd (1975), Callinan (1978, 1979), Young and Trojstman (1980) and Skinner and Todd (1980) to carry trichostrongylid L_3, but in only one case (Fincher and Stewart, 1979) have the dynamics of these larvae been examined, when it was shown that the L_3 of O. ostertagi and Cooperia oncophora can not only survive deep in soil, but will then show positive movement towards the surface.

The possibility that larvae moving onto herbage from a reservoir site in the soil may have been the cause of disease was indicated in some outbreaks of dictyocauliasis and ostertagiasis in cattle in S.W. Scotland (Duncan et al, 1979; Bairden, et al, 1979; Armour et al, 1980a). A preliminary study by Bairden (1980) in that area showed that L_3 of bovine Ostertagia spp. could persist in the soil of ungrazed cattle pasture at 10 cm. depth throughout the year. The latter finding had clear epidemiological implications, and this experimental study reports the results of a fuller investigation of the dynamics of infective Ostertagia spp. larval populations in their environment of soil and overlying herbage.

EXPERIMENTAL DESIGN

Location and Grazing History

The experimental area was a 0.33 hectare paddock of permanent grazing protected by woodland on three sides and on the fourth by a field grazed solely by sheep which were treated fortnightly

with fenbendazole* to minimise contamination. The soil in the area is of a medium loam type. In 1979, as in previous years, the field was grazed from May to October by five susceptible calves. In 1980, to simulate the effect of grazing on herbage length, the pasture was topped prior to August and lightly grazed by parasite-free sheep in September.

Herbage sampling

From May 1979 through to September 1980, grass samples from 400 loci within the paddock were collected each week then bulked and processed by a method similar to that used by Parfitt (1955) and described on page 42 ; larval counts were expressed as Ostertagia spp. L_3 (OL_3) per kilogram of dried herbage (Kdh). Weekly rainfall and temperature figures were recorded using a rain gauge and a mechanical thermograph placed on a Stevenson screen.

Soil sampling

From October 1979 through September 1980, 15 cm. soil cores including a herbage component were removed each month with the exception of December and February when snow or very hard frost made sampling virtually impossible. Sampling locations were chosen at random within the paddock. Five soil cores in close proximity (within an area of 1 square metre) were taken using a modified bulb planter. These cores represented a single sampling unit and in the first two months of collection i.e. October and November 1979, totals of 10 and 16 sampling units were taken respectively. In subsequent months the total was increased to 25 sampling units. On the few occasions when larvae were absent from some of the core segments further core samples to a depth of 25 cm. were collected.

Core processing

Each of the cores was divided into four strata: herbage (A),

*Panacur (Hoescht, Milton Keynes, Bucks., England)

root mat approximately,0.5 cm. (B), 0-7 cms. depth (C) and 7-14 cms. depth (D). For each sampling unit the aggregates of each layer were processed separately as described on page 45.

To assess the viability of OL_3 recovered from soil 100 such larvae were inoculated into a helminth-free calf and the worms established recovered at post-mortem 21 days later.

STATISTICAL METHODS

Presentation of data

For each month, counts of the number of larvae in each stratum of a sampling unit were used to determine the mean, $\bar{x}$, and standard deviation, s, of larvae per stratum per sampling unit. From these, using a conversion factor based on the number of core areas within a hectare, it was possible to estimate the total larval population per hectare within each strata, T, and the standard deviation of this estimate, S, using the standard statistical formulae:

$$T = N\bar{x} \text{ and } S = N\frac{s}{n}\sqrt{1-f}$$

N, the conversion factor, is the number of sampling units available in a one hectare field and n is the number of sampling units examined each month.

A sampling unit consisted of five soil cores in close proximity. Since each core was of cross-sectional area 9 cm^2 then the area of each sampling unit was 45 cm^2. The total number of sampling units available in one hectare, N, is given by:

$$N = \frac{\text{area of one hectare}}{\text{cross-sectional area of sampling unit}}$$

$$= \frac{10^8 cm^2}{45 \text{ II} cm^2}$$

$$= 707264$$

In practice, it was found that the sampling fraction, f = n/N, was so small it could be ignored.

Analysis

In the early stages of the experiment an analysis was made of the variability of the larval counts in order to best predict the number of sampling units needed in subsequent months. An analysis using the mean and s.d. of larval counts per sampling unit for the month of November indicated that the sample mean using 16 sampling units was only accurate to within 50% of the true larval count with 95% confidence. Consequently, it was decided to improve the estimation by increasing the number of sampling units to 25. To minimise damage to pasture and expedite processing of the sampling units this number could not be exceeded.

Two other statistical methods were employed in the analysis of the data. For each stratum, data on the number of sampling units giving positive larval counts were tabulated in a 2 x 10 contingency table and the X^2 test (Stoodley, Lewis and Stainton, 1980) for association between month of the year and the frequency of larval counts performed. Similarly, larvae recovered from each stratum for different months of the year were analysed using a 4 x 10 contingency table and the X^2 test. This tested for an association between month of the year and the proportion of larvae in each stratum. In the analyses, unusually large deviations between the observed larval counts and those expected under the hypothesis of no association were identified.

However, to test if larval counts obtained in each stratum varied from month to month a X^2 goodness-of-fit test was used. This was carried out excluding the months of October and November as a different number of sampling units had been used in each of these months.

Unless otherwise stated, statistical analyses were carried out at 1% significance level.

RESULTS

Herbage Larval Counts

The pattern of OL_3 in herbage samples obtained using conventional techniques for recovery is shown in Figure 1 together with data on rainfall and temperature. From May 1979 through to October 1979 this pattern conformed to that described by Armour (1980) over a 5 year period for calf pastures in S.W. Scotland i.e. a marked increase in the numbers of OL_3 from July reaching a maximum in September. Thereafter, when the paddock was not grazed the numbers also conformed to the expected annual pattern until the spring of 1980 in that a significant reduction occurred during the winter months and pasture larval levels were reduced to zero in February. However, although the pasture was not grazed from October 1979, OL_3 reappeared in March and were present in most of the weekly samples thereafter. The highest numbers recorded were 940/Kdh in April and 1400/Kdh in August. Larvae were absent from three successive samples in May i.e. immediately following and during a spell of low rainfall in April and May.(Fig.1).

Larvae from Soil and Herbage Cores

Using the soil sampling technique, the mean numbers of OL_3 recovered from each stratum in 10 different months during the year are shown in Table 1 together with the s.d. and number of samples. The individual numbers are shown in Appendices B and C and estimated totals of $OL_3 \times 10^6$ per hectare are shown in Table 2 with the SD of the estimate. Large variations in the larval numbers occurred in each stratum at different times of the year though it should be noted that the general pattern in the herbage layer conformed to that found by the more conventional sampling techniques (Figure 1). Thus a consistent decrease in OL_3 numbers occurred from October through to March in all strata except for the lower soil level

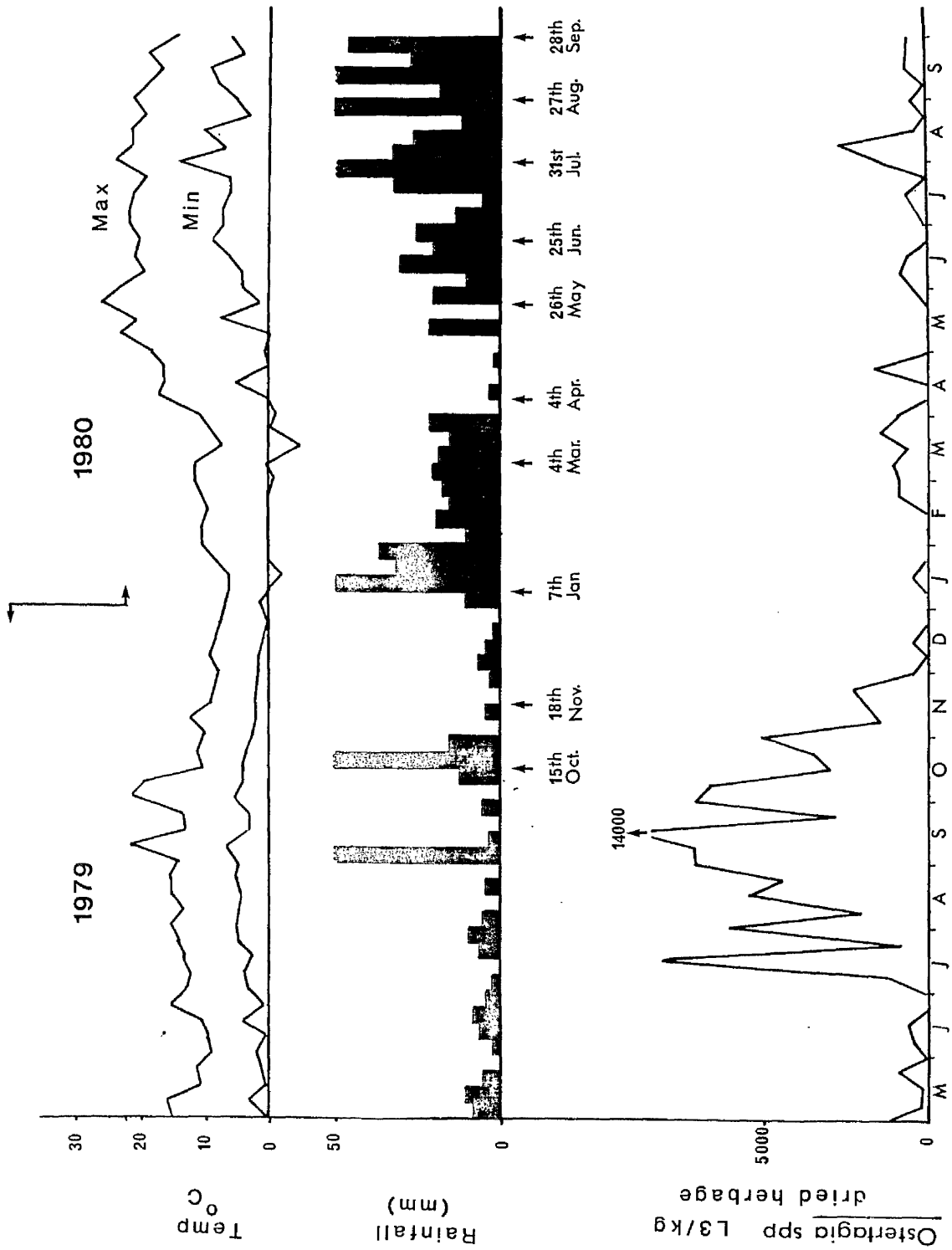


Figure 1. Weekly climatic data 1979-1980 and numbers of Ostertagia spp. L_3 per kg. dried herbage.

Table 1. Mean $\bar{x}$, and s.d., s, of bovine Ostertagia spp. infective larval counts from n sampling units in different strata of herbage and soil.

Stratum		Oct	Nov	Jan	Mar	Apr	May	Jun	Jul	Aug	Sept
A	$\bar{x}$	48.9	5.69	5.72	0.36	8.00	0.12	0.72	2.88	0.40	0.25
(herbage)	s	34.1	6.51	7.20	0.70	20.3	0.33	2.46	6.57	0.91	0.66
	n	10	16	25	25	25	25	25	25	25	25
B	$\bar{x}$	19.9	9.50	3.64	0.28	0.88	0	0.52	0.36	9.20	1.00
(root	s	12.5	16.9	6.95	0.46	1.98	0	0.87	0.57	35.5	2.63
mat)	n	10	16	25	25	25	25	25	25	25	25
C	$\bar{x}$	22.2	21.3	11.0	0.68	20.0	0.08	0.96	21.0	1.76	1.04
(0 - 7	s	21.2	19.4	17.2	1.52	46.3	0.28	1.93	63.2	5.35	4.19
cm soil)	n	10	16	25	25	25	25	25	25	25	25
D	$\bar{x}$	10.1	4.37	8.40	0.20	7.52	0	2.56	1.84	0.92	0.40
(7 - 15	s	14.5	7.04	17.1	0.41	34.7	0	9.91	5.49	1.82	0.82
cm soil)	n	10	16	25	25	25	25	25	25	25	25

Table 2. Estimated total, T, and s.d. of the estimate, S, of bovine *Ostertagia* spp. infective larval populations, expressed in units of 10^6 per hectare.

Stratum		Oct	Nov	Jan	Mar	Apr	May	Jun	Jul	Aug	Sept
A	T	34.2	4.0	4.0	0.25	5.6	0.08	0.50	2.0	0.28	0.17
(herbage)	S	7.6	1.1	1.0	0.10	2.8	0.05	0.34	0.92	0.13	0.09
B	T	13.9	6.6	2.5	0.20	0.62	0	0.36	0.25	6.4	0.70
(root	S	2.8	2.9	0.9	0.06	0.28	0	0.12	0.08	5.0	0.37
mat)											
C	T	15.5	14.9	7.7	0.48	14.0	0.06	0.67	14.7	1.23	0.73
(0 - 7	S	4.7	3.4	2.4	0.21	6.5	0.04	0.27	8.9	0.75	0.58
cm soil)											
D	T	7.1	3.1	5.9	0.14	5.3	0	1.80	1.3	0.64	0.28
(7 - 15	S	3.2	1.2	2.4	0.06	4.9	0	1.39	0.77	0.25	0.11
cm soil)											

in January. In April the numbers of OL_3 present in all strata increased and herbage levels sampled by the traditional technique also increased at this time. In May, a sharp fall in larval numbers occurred at all levels and indeed larvae were absent in some. The numbers of OL_3 remained low in June but in July and August they again increased markedly in upper soil, root mat and herbage; an increase in the herbage larval population (Fig. 1) also occurred at this time. In September, a marked fall again took place at all levels. Analysis of the number of OL_3 recovered in separate strata from month to month indicated that exceptionally high counts were found in Stratum A in October, January and April. Similarly high counts were found in Stratum B in October, November, January and August. In Stratum C high numbers of OL_3 were observed in October, November, January, April and July. Finally, in Stratum D high counts were recorded in October, January and April. In all strata very low numbers of OL_3 were recorded in March and May although counts as high as 9×10^6 and 7×10^6 per hectare respectively estimated from the samples taken from a depth of greater than 15 cm.

Statistical Analysis of Larval Populations in Cores

A significant association occurred between month of the year and the proportion of OL_3 in individual strata. Details pertaining to this analysis are given in Table 3. The observed larval counts within each stratum are contrasted with counts which might be expected were there no association between month of the year and the distribution of larvae in the strata. Large deviations between the observed and expected OL_3 counts occurred in October, January, April, June, July and August. The proportions of OL_3 in Stratum A in October and Stratum D in January were significantly higher. By April, the proportion in Stratum B was significantly low yet by June the proportion in Stratum D was exceptionally high. A reappearance of OL_3 in Strata C and B occurred in July and August respectively,

Table 3. Observed and expected larval counts in different strata of herbage and soil used in the statistical analysis.

Stratum		Oct.	Nov.	Jan.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.
A	Obs.	490	91	143	9	200	3	18	72	10	6
(herbage)	Exp.	235	152	167	9	211	1	28	152	71	16
B	Obs.	199	152	91	7	22	0	13	9	230	25
(root mat)	Exp.	169	109	120	7	152	1	20	109	51	10
C	Obs.	222	341	276	7	501	2	24	526	44	26
(0 - 7 cm	Exp.	446	288	317	17	402	2	52	288	135	30
soil)											
D	Obs.	101	70	210	6	188	0	64	46	23	10
(7 - 15 cm	Exp.	162	105	116	6	146	1	19	104	50	11

when the proportion of larvae was exceptionally high.

Table 4 shows the number of positive larval counts recorded in the different strata throughout the year. Significant differences were observed as follows: a high percentage of positives in Stratum A in October, April and July and a low percentage in May and June. In Stratum B a high percentage of positives occurred in October and August and a low number in May. In Stratum C a high percentage of positives was recorded in October and November and a low number in May. Finally, in Stratum D the number of positives was high in October and low in March, May and September.

Worm Counts

The calf which received approximately 100 OL_3 three weeks previously had 37 Ostertagia spp. worms at post-mortem indicating that the OL_3 were of a high viability.

DISCUSSION

The above results are based on the examination of herbage and soil core samples collected on a single day within each month and do not therefore represent a continuous assessment of the larval populations in soil. Nevertheless, several interesting points emerge. Firstly, OL_3 were present on the herbage and in the soil to a depth of at least 15 cms. for a minimum of 12 months after livestock had been removed from the area of grazing sampled. Since the final stage of the technique used for recovering the larvae depends on their motility the OL_3 enumerated were viable; their infectivity was confirmed by inoculation of a parasite-free calf.

Secondly, at certain times of sampling significant differences occurred in the numbers and proportion of OL_3 present at various levels in the soil cores. The general trend and statistical analysis

Table 4. Percentage of sampling units giving positive bovine *Ostertagia* spp. infective larval counts in each stratum.

Stratum	Oct	Nov	Jan	Mar	Apr	May	Jun	Jul	Aug	Sept
A (herbage)	100	50	44	24	56	12	12	48	20	16
B (root mat)	90	37.5	28	28	32	0	32	32	56	20
C (0 - 7 cm soil)	90	87.5	52	28	52	8	44	52	44	20
D (7 - 15 cm soil)	60	31.3	40	20	28	0	44	28	32	24

indicate a downward movement of larvae during late autumn and winter, a trend which was reversed in April when large populations reappeared in the upper layers only to virtually disappear in May. Larvae reappeared in the cores in June and there was an indication of a steady upward movement from lower levels during July and August. One statistic which is particularly interesting is that the absolute numbers of OL_3 (Table 1) present in the upper soil larvae in July and the root mat in August had returned to similar levels as those recorded in the previous November. These changes occurred in the absence of any fresh contamination. Another relevant statistic is evident from the data in Table 4, namely, that while approximately 50 per cent of the soil samples were positive for OL_3 in November, decreasing to only 8 per cent in May, the percentage of positives had returned to approximately 40 per cent in July and August.

Some association between climatic changes and fluctuations in the OL_3 population is apparent. Thus a reduction in numbers between October and November coincided with heavy rainfall and decreasing temperatures (Fig. 1). The reappearance of significant populations in the upper strata in April paralleled an increase in temperature and moist conditions while the drop in numbers in May followed a period of dry weather. It is possible that the depletion in larval numbers in the autumn could be due to the percolating rain water carrying the larvae downwards in the soil and beyond the level sampled. The reason for the reappearance of larvae in the upper strata in April is not known but the presence of such large numbers cannot be satisfactorily explained by sampling error; it may be linked to earthworm activity and migration since earthworms have recently been shown to act as transport hosts for OL_3 (Gronvøld, 1979) and Dictyocaulus viviparus L_3 (Oakley, 1981). Alternatively, these larvae might have originated via eggs from disintegrating

faeces which were flushed into the soil by rain water and developed into larvae in response to the increasing temperatures in April.

In May, the dry conditions appear to have proved lethal to the OL_3 although the detection of some larvae below 15 cm. suggests they may have migrated downwards possibly in search of moisture. Certainly, the subsequent reappearance of OL_3 in the upper strata in July and August, coincided with the re-establishment of moist conditions in the upper soil. Perhaps the OL_3 became desiccated and non-motile during the dry conditions in May and survived by entering a state of anhydrobiosis (Ellenby, 1968); it is unlikely that such desiccated larvae would be recovered using the techniques employed. When moist conditions returned the larvae would be rehydrated and their motility restored. Clearly, more frequent and detailed examination must be undertaken if the seasonal pattern of OL_3 in the soil is to be properly understood. The interaction between earthworm populations, water table, soil type and other trophic influences on larval movement also requires further investigation.

2) Availability of Ostertagia spp. to grazing calves after resting for 20 months.

INTRODUCTION

Since the work of Michel (1969a)established the existence of an annual pattern of bovine trichstrongyle larval populations on pasture, it has generally been assumed that an estimation of the numbers of infective trichostrongyle larvae available to grazing ruminants are best obtained by the use of pasture sampling techniques. While these are certainly the most economical methods available they may not be the most accurate since there are obvious differences between animal grazing patterns and "homo sapiens" collection patterns. It may therefore be inaccurate to extrapolate from the results of herbage larval examination to the numbers of larvae either being ingested by or available to, grazing animals.

An alternative method of determining the numbers of such larval stages on pasture at a given time is the one pioneered by Durie (1962) which involved the use of helminth naive "tracer" calves (animals turned out to graze for a period,usually of 2-3 weeks,to monitor the seasonal fluctuation of nematode larvae). With this system the normal grazing pattern is maintained and at slaughter the worm burdens established can be accurately assessed. This technique is, however, not without its problems. Firstly, compared to the estimation of larval numbers on herbage it is a very expensive exercise involving the purchase and rearing of calves and secondly, since there is an expulsion of most nematodes after a limited period in the host so the "tracer" can be used for a relatively short period which again increases the cost. Furthermore, the numbers established do not necessarily reflect the numbers of larvae ingested or on the pasture.

A combination of the two methods would be better or perhaps as has been suggested by the Australian workers, Heath, Southcott and May (1970) the use of animals with an eosophageal fistula may prove to be an ideal compromise. It is interesting that in preliminary work in this laboratory Armour (pers. comm.) found that the larval intake of sheep with oesophageal fistulae was greater compared to pasture samples collected in the normal way.

In the current study the availability of Ostertagia species infective larvae on a pasture which was not grazed for the previous 20 months was measured by the random pasture sampling technique described previously. In addition, two permanent calves were grazed continuously during the experimental period and infection in these was assessed by faecal examination and examination of their worm burden at post-mortem.

EXPERIMENTAL DESIGN

Two helminth-free Ayrshire X calves (Nos. 10 and 43) aged 8-10 weeks were set-stocked from May 1st to September 15th, 1981 on the 0.33 hectare paddock from which the soil cores, referred to previously in section 1 of this chapter, were collected. Since the area to be grazed by these animals was in excess of their requirement, parasite-free lambs were introduced at intervals to prevent overgrowth of the herbage which could possibly create a dilution effect on the herbage analysis for larvae. At the termination of the experiment the calves were housed for 1 week prior to slaughter.

OBSERVATIONS

Each week the calves were clinically examined and faecal samples collected for examination by flotation McMaster and Baermann techniques. Weekly pasture samples and, at post-mortem the abomasa

and lungs, were processed as described under materials and methods.

RESULTS

Clinical Signs

A mild cough was observed towards the end of the experiment (week 20) in one of the calves (No.10); otherwise no adverse clinical signs were noticed.

Parasitological Findings

Faecal Egg Counts

Individual and mean faecal egg and larval counts (epg and lpg respectively) are detailed in Table 5. Trichostrongyle eggs were first detected from calf No. 10 after 3 weeks grazing while the faeces of animal No. 43 remained negative until the fourth week when 100 epg were present. Thereafter the numbers of eggs per gram detected ranged from 0 to 300 in both calves this latter figure being recorded on the 9th September (week 20). First stage D. viviparus larvae were recovered from the faeces of calf No. 10 and the faecal larval counts ranged from 0 to 300 lpg, the maximum recovery being recorded on the 15th September. One week prior to this, the faeces of calf No. 10 had 100 D. viviparus larvae (September 8th). Apart from these two positive counts the faeces of both calves were continuously negative for D. viviparus larvae.

Pasture Larval Counts

Ostertagia spp. L_3 were only recovered on four occasions namely on May 11th (909 L_3 / Kg.), August 17th (465 / Kg.), September 21st. (1379 / Kg.) and September 29th.(1333 / Kg.). At no time were D. viviparus L_3 recovered from the pasture samples.

Worm Burdens

From Table 6, which records the individual worm counts at post-mortem, it can be seen that both calves were infected with Ostertagia species, animal No. 10 having a total population of 850, while the burden recovered form calf No. 43 was 2400. the parasites present

Table 5. Individual and mean faecal egg and larval counts (epg and (lpg) of grazing pasture rested for 20 months.

	Week	Epg (mean) 10	Epg (mean) 43	Lpg (mean) 10	Lpg (mean) 43
May 1st	1	0	0 (0)	0	0 (0)
	2	0	0 (0)	0	0 (0)
	3	50	0 (25)	0	0 (0)
	4	50	100 (75)	0	0 (0)
	5	0	100 (50)	0	0 (0)
June 1st	6	100	50 (75)	0	0 (0)
	7	50	0 (25)	0	0 (0)
	8	50	0 (25)	0	0 (0)
	9	0	0 (0)	0	0 (0)
	10	0	0 (0)	0	0 (0)
July 7th	11	50	0 (25)	0	0 (0)
	12	0	0 (0)	0	0 (0)
	13	50	50 (50)	0	0 (0)
	14	0	0 (0)	0	0 (0)
Aug. 3rd	15	50	0 (25)	0	0 (0)
	16	0	0 (0)	0	0 (0)
	17	50	100 (75)	0	0 (0)
	18	50	100 (75)	0	0 (0)
Sept.1st	19	300	100 (200)	100	0 (50)
	20	100	150 (125)	300	0 (150)

Table 6. Worm burdens and abomasal pH of calves grazing pasture rested for 20 months.

Calf No.	Abomasal pH	Abomasal worm burdens Adults of individual spp. (Percentage total adult population) Total	Ostertagia sp	S. lyrata	L_4	EL_4	Lungworm burdens (D. viviparus) Total	Adult	L_4
10	3.8	850	819 (94.4)	31 (3.6)	-	-	2018	1904	114
43	4.4	2400	2203 (91.8)	197 (8.2)	-	-	-	-	-

were either adult or developing larval stages with no arrested larvae being observed. Species composition was similar to that commonly observed being 92% and 96% O. ostertagia and 8% and 4% S. lyrata in calf numbers 43 and 10 respectively.

D. viviparus parasites were only present in calf No. 10 A total of 2018 lungworms were present and consisted of 1904 adults plus 114 immature stages.

Pathology

The pathological changes were mild in the abomasa of both calves and reflected the low Ostertagia spp. worm burdens. Pneumonic lesions were observed in the lungs of calf No. 10 due to the presence of D. viviparus and were characterised by severe emphysema as well as consolidation. Lymphoid nodules as described by Pirie, Doyle, Mc Intyre and Armour (1971),which are indicative of previous infection were present in calf No. 10 but not in No. 43 suggesting that this calf had not become infected.

DISCUSSION

Despite the absence of grazing ruminants from this area for 20 months, infective larvae of O. ostertagi were recovered from the herbage during early May and it is clear that sufficient numbers of L_3 were ingested by the two calves to produce a patent infection.

However, despite this establishment of infection in the calves, the expected seasonal increase of the free living L_3 did not materialise during the summer; thus on only three subsequent occasions were Ostertagia spp. L_3 recovered from pasture, the highest numbers being observed towards the end of the experimental period In September.

The reasons for this are not clear but may be a reflection of the low infectivity of the worms accruing from infections with the aged infective larvae which had probably originated from eggs deposited by the set-stocked calves in 1979. The low numbers of L_3 on pasture may also have been partly due to the rather dry weather in the summer of 1981 particularly in August.

A more likely possibility is that the low stocking rate of two calves on the 0.33 hectare would have resulted in only a low contamination of the total area and so the availability of L_3 both to the grazing calves and to the collections by the random sampling technique would be greatly reduced.

The importance of stocking rate during the important period of contamination in the spring has been emphasised by Armour (1978b) and demonstrated in the studies of Nansen et al, 1978. In the latter paper they observed that the larval contamination on the herbage is generally much higher close to dung pats than elsewhere on the pasture and it was therefore expected that the stocking rate might be an important factor influencing the aquisition of infection as when grass becomes scarce because of overstocking, animals are forced to graze closer to faecal pats and so they inevitably ingest more larvae. Nansen et al (1978) also found that the higher stocking rates reduced not only the availability of grass on the paddock, but also the size of the grass tufts surrounding the dung pats, since presumably the scarcity of alternative feed force the calves to crop within the "ring or repugnance". This phenomenon became very significant in the late summer when herbage larval counts were at their highest.

Although larvae of the lungworm D. viviparus were not recovered from any of the pasture samples, the recovery of L_1 from the faeces of calf No.10 and the presence of 2018 lungworm at its post-mortem clearly demonstrates that lungworm larvae as well as Ostertagia

spp. survived the 20 month period of pasture spelling. It further demonstrates how quickly high numbers of lungworm infective larvae can be available to the host. Whether the presence of lungworm larvae originated from the cycling of a low residual infection on pasture as Jorgensen's work (1981) would indicate or from a reservoir in soil as suggested by Duncan et al (1979), Gronvøld (1979), Armour et al (1980a)and Oakley (1981) is not known. However, these reasons are the likeliest sources since field to field transmission even by winds or flies as proposed by Jorgensen (1981) as a common cause of these infections is not very likely, since the nearest cattle pasture was 100 yards distant.

On reflection, it is perhaps unfortunate that a more critical examination of the herbage for the presence of Dictyocaulus spp. larvae was not undertaken. It is possible that by using the bile agar technique of Jorgensen (1975 a) the presence of infective larvae of this bovine respiratory parasite would have been detected although the results obtained by this technique in the Glasgow University laboratory are not as good as those obtained by Jorgensen (Bairden pers. comm.).

There are two other points of interest in relation to the lungworm infections, namely 1) that no clinical signs of "husk" were observed in one animal, despite 2018 lungworms being present at at post-mortem and 2) the absence of lungworms in the other animal.

With a lungworm burden of 2018, the absence of the clinical symptoms associated with dictyocauliasis is most unusual; one possible explanation is that the lungworms were from a strain of very low pathogenicity possibly influenced by the long free living exposure. The absence of lungworm infection in the other calf might suggest that it had become immune. However, the absence of lymphoid nodules in this calf at post-mortem makes it seem more

likely that it had never been infected emphasising again the ubiquitous nature of D. viviparus free living larvae.

It is also interesting to speculate whether revitalisation of aged infective stages of bovine D. viviparus and Ostertagia spp. might occur following passage through these calves and that the expected epidemiological features of ostertagiasis and parasitic bronchitis might occur in the following season.

It is generally recognised that if a pasture is rested for the first half of the normal grazing season (i.e. May to July) the overwintered nematode larvae from the previous season will have succumbed to the changing environmental conditions, in the spring, and that the pasture would then be virtually clean. It is clear from the results of this study this is not always the case and they therefore complement the findings of the previous section dealing with the persistence of larval populations in the soil.

CHAPTER 4

EXPERIMENTAL STUDIES ON THE INFECTIVITY AND PATHOGENICITY OF DIFFERENT ISOLATES OF *Ostertagia* SPP.

1) Observations on the infectivity and pathogenicity of three isolates of *Ostertagia* spp. in calves.

2) The interaction of quantitated infections of *O. ostertagi* and *O. leptospicularis*.

1. Observations on the Infectivity and Pathogenicity of three Isolates of Ostertagia spp. in calves.

INTRODUCTION

Bovine ostertagiasis in Great Britain, as mentioned in the general introduction is caused by species of the genus Ostertagia and of its close relative Skrjabinagia, often collectively termed Ostertagia spp. Experimental studies have principally used infective larvae of O. ostertagi which were first isolated at the Central Veterinary Laboratory, Weybridge in the late 1950s and have since been experimentally passaged an unknown number of times. Some studies using this isolate have concentrated on the biological aspects of infection, e.g., Michel (1963) and Ross (1963), while others were primarily concerned with the replication of the disease as seen in the field (Ritchie et al 1966, Anderson, Armour, Eadie, Jarrett, Jennings, Ritchie and Urquhart 1966). In the latter the clinical and pathological changes previously reported from the field (Anderson et al 1965b) were successfully reproduced and therefore no cognisance was taken of the fact that the larvae used had undergone numerous laboratory passages.

However, it was later noted (Armour, Jennings and Urquhart, 1967a) that the Weybridge isolate (hereafter termed isolate W) had a low propensity for becoming arrested in development compared with a field strain isolated from a farm near Glasgow (isolate GA). Subsequent experiments confirmed this observation (Armour, Jennings and Urquhart, 1969a) Since then published discussion on biological differences between isolates of O. ostertagi has been in abeyance.

Recently, it was observed that calves from another farm near Glasgow harboured a mean adult Ostertagia spp.

burden in excess of 40,000 yet displayed no obvious clinical signs of ostertagiasis such as weight loss and diarrhoea; this was surprising since the studies of Anderson, Armour, Jennings, Ritchie and Urquhart, 1969) showed that this level of adult worm burden was sufficient to cause severe morbidity and mortality in calves of a similar size and age. Infective larvae from this source were isolated following faecal culture and coded GB.

In the same year, severe clinical ostertagiasis occurred in calves grazing on another farm; the mean Ostertagia spp. burden of these calves was also in excess of 40,000 but the lesions post-mortem were particularly severe with gross hyperaemia and oedema. Infective larvae from this population were also isolated following faecal culture and coded GC.

It was decided to compare these two latter isolates, GB and GC, using experimental infections to assess whether the differences observed in the field were due to a variation in infectivity or whether there was a real difference in pathogenicity. Since previous experimental infections in our laboratory have been made with either the W or GA isolates the former was included for comparison.

EXPERIMENTAL DESIGN

Fifteen Ayrshire male calves aged 8 to 10 weeks were divided on a weight basis into three groups of five calves each. The isolates of Ostertagia spp. infective larvae were then allocated as follows, the numeral after the letter code indicating the number of passages since isolation. These passages were necessary to remove Cooperia spp. contaminants by selective anthelmintic therapy with haloxon (Loxon; Wellcome) and augment the numbers of Ostertagia spp. infective larvae.

Group 1: W (number of passages unknown)

Group 2: GB3

Group 3: GC3

On day 0 and using the appropriate isolate, one calf in each group received 25, 50, 75, 100 or 200 x 10^3 infective larvae. All calves were killed on day 21 following inoculation.

OBSERVATION

Clinical. The calves were examined daily and particular attention was paid to appetite and faecal consistency.

Faecal examination. Faeces were examined for the presence of nematode eggs before the experiment and on days 0, 7, 14 and daily from day 17 to 21 by flotation methods. When eggs were present these were counted using the Clayton Lane method and expressed as eggs per gram (epg).

Plasma pepsinogen estimation. On days 0, 7, 14, 17 and 21 blood was collected from the jugular vein into tubes containing heparin. After centrifugation the plasma was removed for determination of plasma pepsinogen levels by the method of Edwards et al (1960) (page 39) and these were expressed as international units (iu) of tyrosine.

Post-mortem examination. The gastrointestinal tract was removed and examined for the presence of nematodes by the method of Ritchie et al (1966) (page 48).

When the abomasum was opened a sample of contents was removed for estimation of the pH. Duplicate portions of abomasal tissue were taken for histological examination one being fixed in 10 per cent formol and the other in Carnoy's fluid. Sections were stained by haematoxylin and eosin.

The nematodes present were counted, identified and classified as adult, i.e. mature male and female worms or fifth larval stages (L_5), and fourth larval stages (L_4). Two hundred male worms from each calf

were mounted in Berlese's medium for more precise identification.

RESULTS

Clinical findings

Depression of appetite occurred from day 14 onwards in all of the calves given 200,000 larvae irrespective of isolate. Calves given 75,000 or more of the GC3 strain also showed appetite depression from this time.

From day 17, moderately soft faeces were noticed in the calves receiving the heaviest level of infection of all three isolates and also in the other calves given the GC3 strain. The calf given 75,000 of the GB3 isolate showed intermittent diarrhoea from day 3 onwards. The cause of this diarrhoea was not known.

Faecal examination

Faecal egg counts are detailed in Table 7. Eggs appeared in the faeces of all calves between days 18 and 21; on day 21 the mean epg of the calves given the W, GB3 and GC3 isolates were 874, 678 and 1842, respectively. Appendix D gives details of individual faecal egg counts.

Plasma pepsinogen estimations

Before the inoculation of the larvae, the plasma pepsinogen values of all calves were within the normal range for helminth free calves of this age, i.e. less than 1.0 iu. From day 10 some elevation in the levels of all calves was noticed, this being more marked in those given the heaviest level of infection. Thereafter, the level steadily increased until the termination of the experiment on day 21. The highest level recorded in each calf during this period is shown in Table 7. There was a very high degree of correlation between plasma pepsinogen on day 21 after infection and the total

Table 7. Biochemical and Parasitological findings at post-mortem of calves infected with different isolates of Ostertagia spp.

Group and isolate	Larval inoculum X 10^3	Abomasal pH	Plasma pepsinogen (i.u.)	Eggs per gram of faeces at Day 21
1	25	3.0	2.0	120
W	50	5.3	3.0	420
	75	6.4	4.0	1210
	100	5.5	5.2	300
	200	5.7	7.5	2320
2	25	3.6	1.6	1090
GB3	50	4.2	6.6	1470
	75	5.5	9.7	180
	100	6.8	4.8	460
	200	6.8	5.7	190
3	25	3.9	1.8	640
GC3	50	5.1	5.8	1460
	75	6.4	3.9	2020
	100	6.1	8.8	1590
	200	6.7	8.2	3500

worm burden in the case of the GC3 isolate (r = 0.8978) and also in the W isolate (r = 0.9128). However, no such correlation existed in the GB3 isolate (r = 0.5507). Removal of the data pertaining to the calf with intermittent diarrhoea from day 3 did not appreciably improve this correlation. Figure 2 illustrates the pattern of plasma pepsinogen levels. These are numerically detailed in Appendix E.

The pH of the abomasal contents is also given in Table 7 and these clearly increase in proportion to the number of adult worms present.

Post-mortem findings

Worm burdens. The individual worm burdens are also shown in Table 8. The percentage of each inoculum established with isolate W ranged from 16.7 to 37.4, these levels being inversely proportional to the number of larvae administered. Slightly higher establishment was obtained with the GB3 larvae, the percentages ranging from 28.3 to 38.3 but no obvious trend in relation to numbers of larvae administered was present. However, markedly higher numbers of worms were found in the calves given the GC3 isolate, the percentage establishment ranging from 33.9 to 67.9, the lowest being the figure relating to the heaviest inoculum. Examination of the worm burdens indicated that virtually all the worms present were mature adults or fifth larval stages and only low numbers of fourth stage larvae were found. However, although the stadial structure of the populations arising from the different isolates was similar it became apparent on detailed microscopical examination that the species composition was different.

The numbers of different Ostertagia spp. resulting from each isolate and estimated by examining 200 males from each worm population is also given in Table 8.

The worm populations from two of the isolates, W and GB3

Table 8. Post-mortem worm burdens of calves infected with different isolates of Ostertagia spp.

Group and isolate	Larval inoculum X 10^3	Total numbers	Percentage of inoculum est.	Abomasal worm burden Adult of individual species (percentage total adult population)			El_4	L_4
				O. leptospicularis	O. ostertagi	S. lyrata		
1	25	9350	37.4	0	8667(93)	683(7)	-	-
W	50	16750	33.5	0	15931(95)	819(5)	-	-
	75	15150	20.2	0	13788(91)	1362(9)	-	-
	100	16650	16.7	0	15312(92)	1338(8)	-	-
	200	36300	18.2	0	35937(99)	363(1)	-	-
2	25	7200	28.8	0	6455(90)	745(10)	-	-
GB3	50	17550	35.1	0	15795(90)	1755(10)	-	-
	75	28700	38.3	0	25222(88)	3478(12)	-	-
	100	31400	31.4	0	29086(93)	2314(7)	-	-
	200	62200	31.1	0	56765(91)	5435(9)	-	-
3	25	13800	55.2	4485(33)	9015(67)	0(0)	-	300
GC3	50	33050	66.1	7999(25)	23713(73)	588(2)	-	750
	75	47400	63.2	11368(24)	34930(75)	602(1)	-	500
	100	67850	67.9	20853(31)	45335(68)	662(1)	-	1000
	200	67750	33.9	16885(26)	48011(73)	604(1)	-	2250

consisted solely of O. ostertagi and S. lyrata (Plates 10 and 11). However, analysis of the worm population from the GC3 group showed that O. leptospicularis (Plate 12) was present in addition to the other two species in a proportion of about one in three of the total. Although the numbers of O.ostertagi administered were clearly less (because of the presence of the O. leptospicularis component) the absolute numbers established were in general higher than in calves receiving corresponding inocula consisting primarily of O. ostertagi in the other groups.

Pathological Changes.

Macroscopically and histologically the characteristics of bovine ostertagiasis as described by Ritchie et al (1966) and Murray et al (1970) were evident in all of the calves. In those given the W and GB3 isolates, the severity and extent of these lesions was proportional to the numbers of larvae administered. However, in the calves given the GC3 isolate the lesions produced were much more severe and extensive than in similar levels of infection with the other isolates although they were of a similar nature. Thus hyperplasia, marked congestion and oedema were present even when lower numbers of larvae were used for infection; as was the presence of superficial cytolysis in association with emerged parasites and eggs. In addition, some of the nodular lesions were quite distinct from the equivalent nodule of O. ostertagi in the following ways:

1. Many gastric glands still contained large mature parasites 21 days after infection and these were causing considerable distension of the inhabited glands.

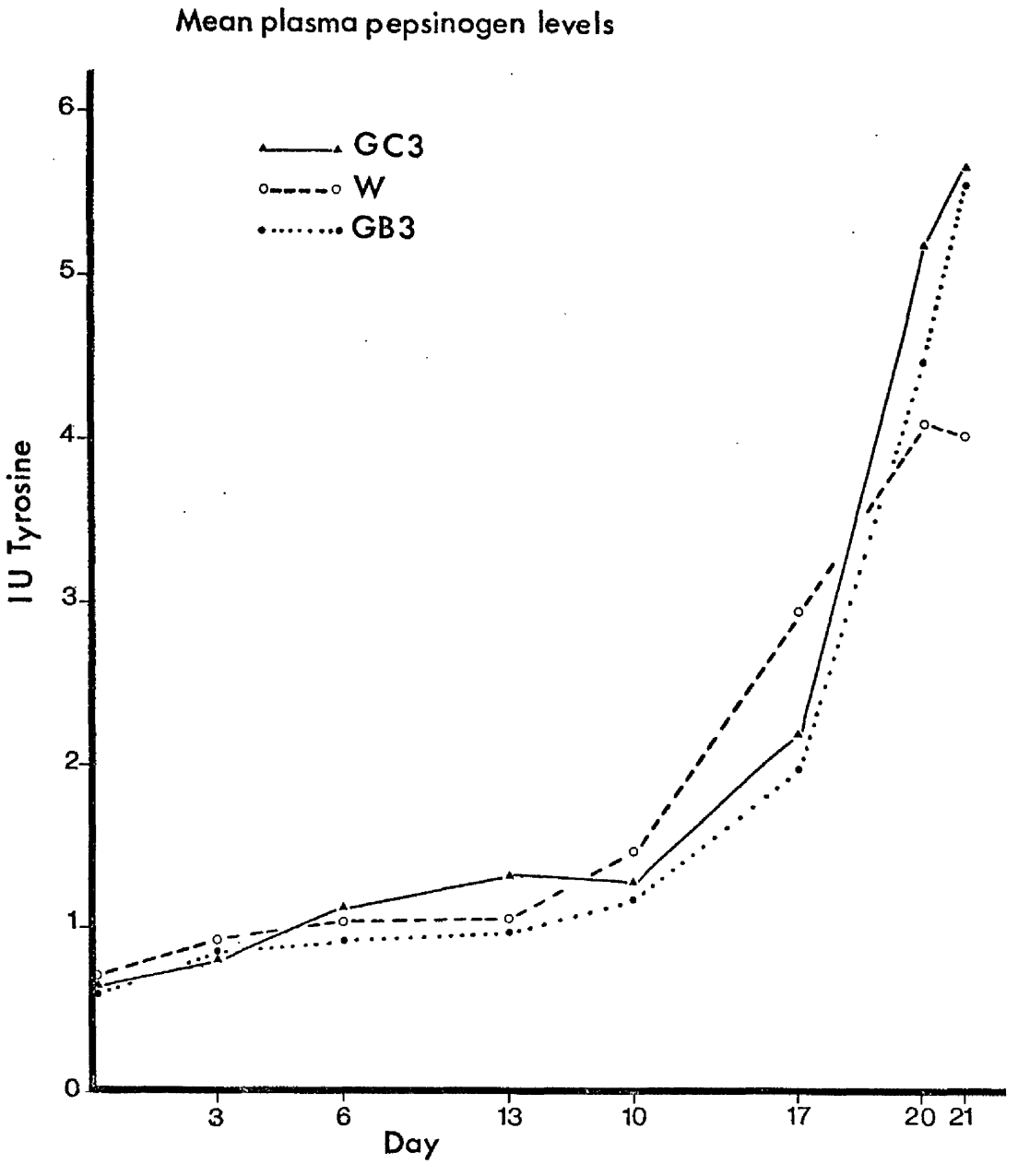


Figure 2. Mean plasma pepsinogen values following infection with three isolates of Ostertagia spp.

Plate 10. Copulatory spicules of O.ostertagi x 150.

Plate 11. Copulatory spicules of S. lyrata x 150.

Plate 12. Copulatory spicules of O. leptospicularis x 150.

2. Adult female parasites and eggs were found in several glands (Plate 13) while in others the parasites had emerged leaving only eggs in the glands (Plate 14). In some cases the gastric glands were distended with necrotic debris surrounding the eggs (Plate 15).
3. The glands surrounding the parasitised ones were lined by rapidly dividing low cuboidal or tall columnar cells, the latter containing mucus. Also, occasional degenerate parietal cells were found. The extent of the hyperplasia and the number of surrounding glands lined by poorly differentiated epithelium was much greater than has been seen with an O. ostertagi nodule (Murray et al 1970). Possibly this was a result of the presence of the fully grown adult female worm within the gland. The degree of dedifferentiation in glands surrounding those in which parasites were still present resembled that reported with O. circumcincta in sheep (Armour, Jarrett and Jennings 1966).

Intense reactivity also occurred in the lamina propria. In addition to marked congestion and oedema, granulomatous lesions also occurred. It appeared that eggs were breaking through the stretched and damaged mucosa of the parasitised glands and stimulating a marked reaction in the surrounding lamina propria with infiltration of neutrophils and connective tissue deposition. In addition, eggs were found free in the submucosa and in the lymphatics of the submucosa (Plate 16).

There was also a diffuse infiltration of mononuclear cells, predominantly plasma cells, especially in the upper part of the lamina propria. Sometimes in the vicinity of this plasma cell infiltrate, superficial cytolysis associated with parasites and eggs occurred. Marked infiltration of eosinophils and focal areas of neutrophils occurred in the vicinity of parasitised glands. Occasional lymphocytic follicles were present in the deep lamina propria sometimes penetrating into the submucosa.

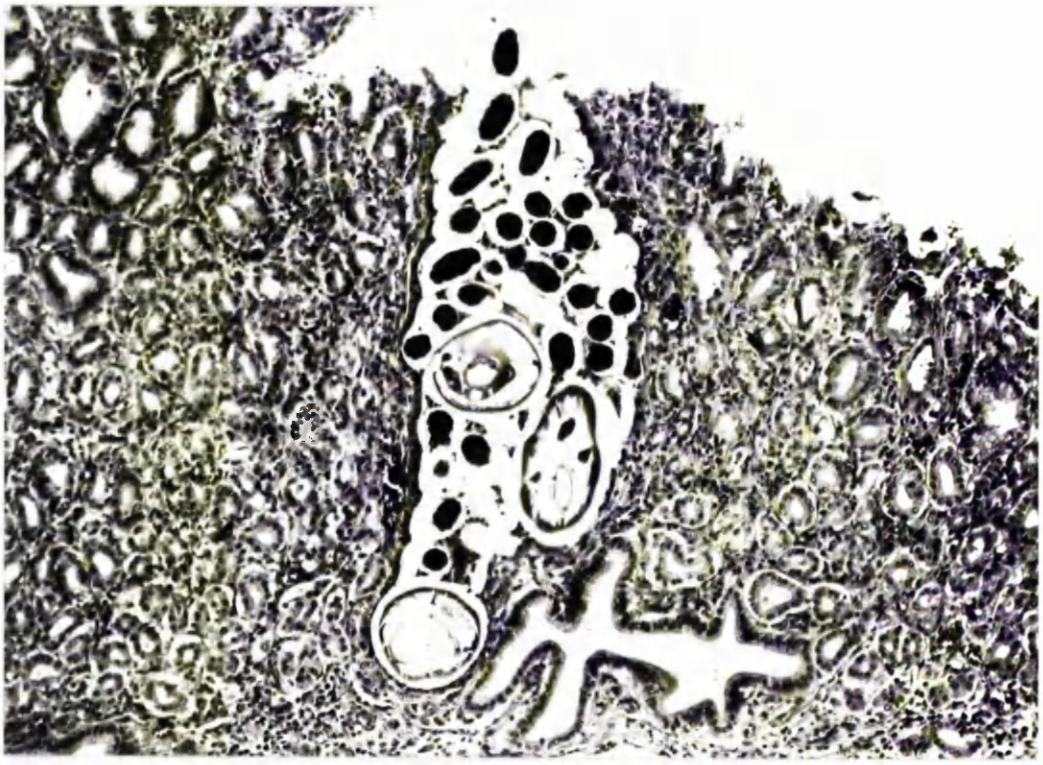


Plate 13. Adult female Ostertagia spp. with mature ova in gastric gland x 150.

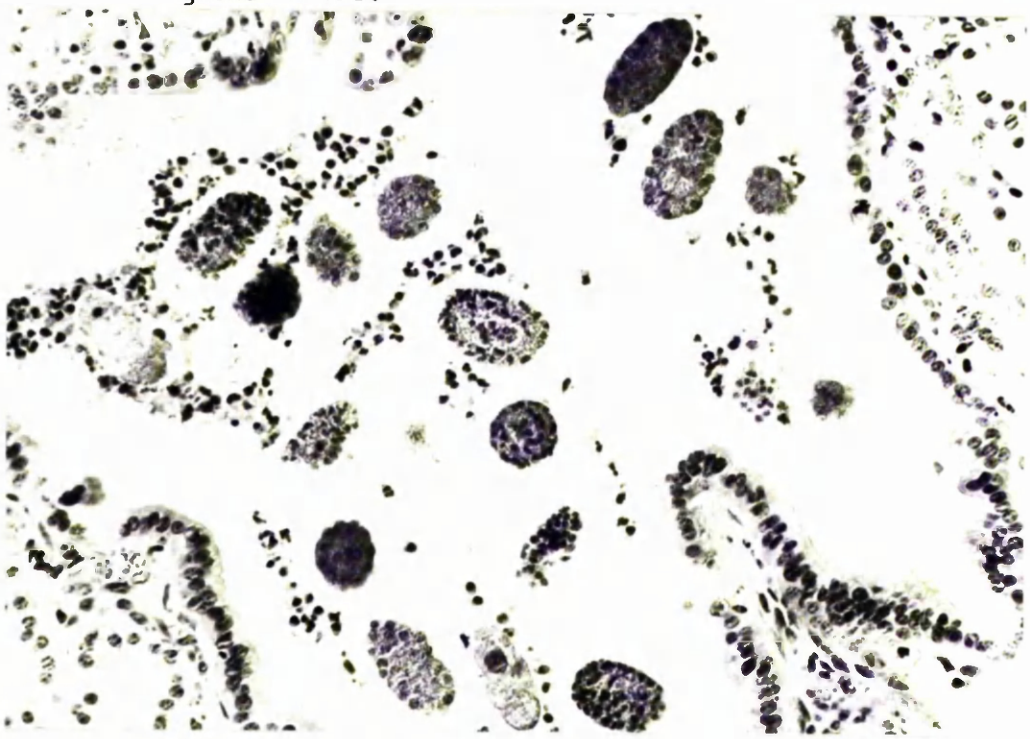


Plate 14. Distended gastric gland containing developing Ostertagia spp. egg x 300.

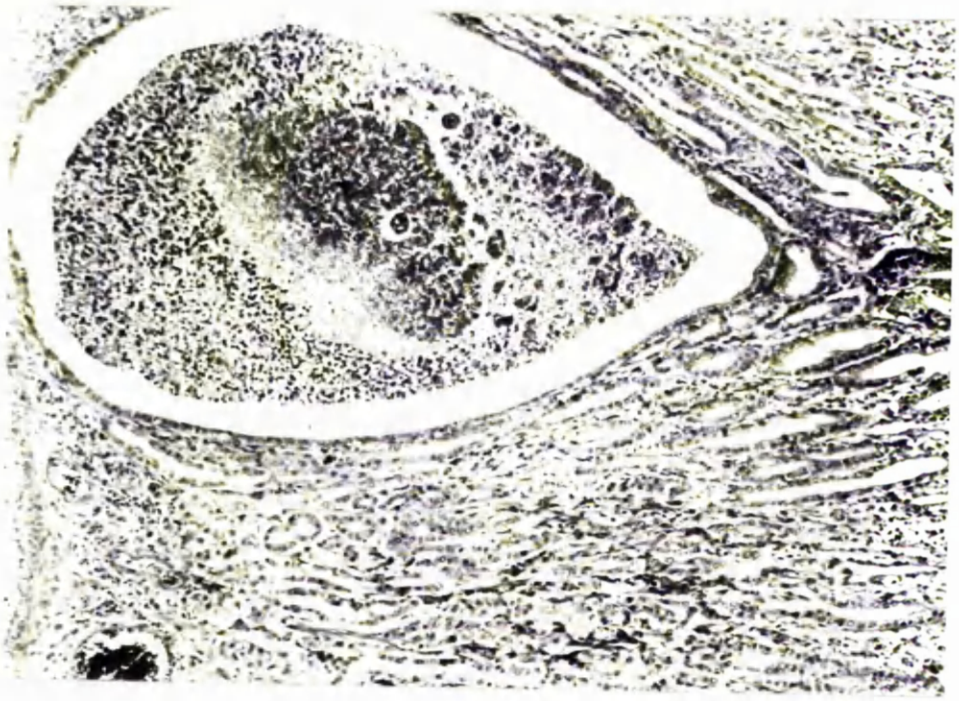


Plate 15. Gastric glands distended with necrotic debris surrounding eggs of Ostertagia spp. x 82.5.

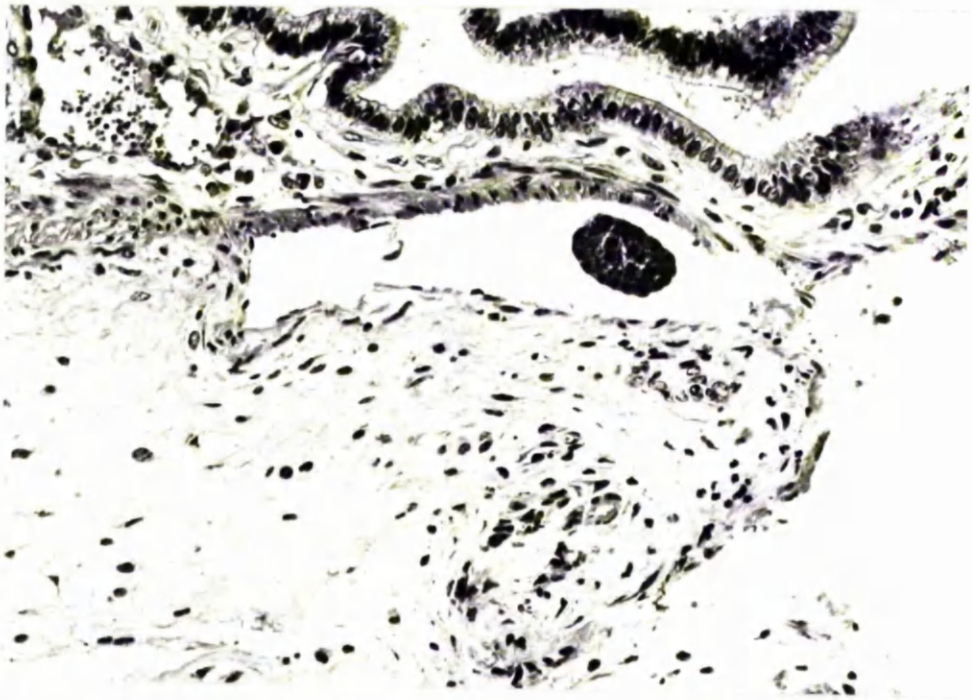


Plate 16. Ostertagia spp. egg in lymphatics x 300.

DISCUSSION

Several interesting points emerge from the results of this experiment. First, the varying infectivity of the different isolates, measured by numbers of Ostertagia spp. worms established, is particularly intriguing. The GC3 isolate was the most highly infective with the percentage of inoculum established ranging from 33.9 to 67.9 per cent with a mean of 57.3 whereas the percentage establishment of the GB3 and W isolates ranged from 28.8 to 38.3 (mean 32.9) and 16.7 to 37.4 (mean 25.2) respectively. Although O. ostertagi was the dominant species present in the worm burdens established from the three isolates there were considerable differences in the prevalence of the other species present. Thus the GC3 worm burdens consisted of approximately 71 per cent O. ostertagi, 28 per cent O. leptospicularis and 1 per cent S. lyrata whereas the GB3 burdens were made up of 91 per cent O. ostertagi and 9 per cent S. lyrata and the burdens from the W isolate were 94 per cent O. ostertagi and 6 per cent S. lyrata.

A second point is the increased severity of the clinical and pathological changes produced after infection with larvae of the GC3 isolate. In particular, the soft faeces, the severe hyperplasia, marked loss of functional cells in the non-parasitised glands and the presence of mature worms and normal ova in the gastric glands and submucosa is a quite different result from that produced by the other two isolates and previously reported by Ritchie et al (1966) and Murray et al (1970) following infection with O. ostertagi. Although it appears that these differences can be directly related to the increased total numbers of worms established, i.e. the greater infectivity of the GC3 isolate, the fact that equally high levels of abomasal pH and plasma pepsinogen (both accepted markers of abomasal damage) occurred in the calves receiving the other inocula suggests

that the severe reaction, both clinically and pathologically, of the GC3 group of calves must therefore be attributable to certain differences within the worm populations. Since O. leptospicularis was not present in the populations resulting from the GB3 and W isolates it is reasonable to ascribe the additional severity of the changes to this parasite. Also, it is possible that the clinical effect of these changes was not fully realised since the calves were sacrificed on day 21 just before the period when severe clinical signs usually occur in bovine ostertagiasis (Jennings et al, 1966). However, this date was chosen since I wished to evaluate infectivity and it is known that a loss of established worms commonly occurs between 21 and 28 days after inoculation with pure cultures of O. ostertagi (Ritchie et al, 1966).

The finding of mature females and ova within the glands leads to speculation on how fertilisation takes place as it has been generally accepted that each gland contains a single worm of either sex. It must therefore be supposed that fertilisation in the case of O. leptospicularis takes place before emergence.

The reason for the high numbers of worms becoming established from the mixed isolate GC3 is unknown but it is possible that the presence of two or more different species at the same time alters their ability to become established. One or both of the species may exert an immunosuppressive effect allowing a much greater establishment of parasites. In this context, it should be noted that the total numbers of O. ostertagi established in the mixed (GC3) infection are with one exception (the 200,000 GB3 inoculum) higher than those established from the corresponding inoculum of the other two isolates. Although the actual percentage of O. ostertagi established from the GC3 inoculum cannot be computed, since as yet the infective larvae of the different cattle Ostertagia spp. are

not readily differentiated, it must be considerably higher than that from the other inocula.

Interaction between gastrointestinal nematode species has not been reported previously to my knowledge, though Herlich (1959) and Ross, Purcell, Todd and Dow (1968) have demonstrated an increased establishment of abomasal nematodes from different genera (O. ostertagi and Trichostrongylus axei) when mixed infections were administered. Kates and Turner (1953) also noted enhanced infectivity of Nematodirus spathiger and T. colubriformis infective larvae when these intestinal genera were inoculated simultaneously.

The origin of the O. leptospicularis was presumably roe deer since a single animal has occasionally been observed on the grazing from where this isolate was made but how it became established as a significant partner to O. ostertagi is not known. It is possible that O. leptospicularis is particularly fecund and so increases proportionally after a few generations, though if this were the reason it is odd that it has not appeared in high numbers previously. Alternatively, this species may be less susceptible to anthelmintics so that selection may happen when the general Ostertagia population is exposed to drug pressure. Interestingly, recent reports from New Zealand and Netherlands (Bissett, 1980a ; Borgsteede, 1981) have also incriminated O. leptospicularis as an important species in the causation of naturally occurring bovine ostertagiasis.

In view of the field observation that the GB3 isolate was not very pathogenic the infectivity of this isolate was expected to be very low. However, it proved to be slightly more infective than the W isolate and caused similar biochemical and pathological changes. It is of course possible that the three laboratory passages and anthelmintic treatments necessary to produce sufficient infective larvae and remove contaminants of C. oncophora affected its behaviour. However, the W isolate had a similar infectivity and

produced the same pathological changes as in the experiment of Ritchie et al (1966) suggesting that if any alteration in the host-parasite relationship of an O. ostertagi isolate occurs during laboratory passage it eventually stabilises.

In conclusion, the results of this experiment indicate that different isolates of Ostertagia spp. can vary in infectivity and pathogenicity. These differences appear to relate more to the interaction of different species than to the evolution of strains of the same species.

2. The Interaction of Quantitated Infections of O. ostertagi and O. leptospicularis

INTRODUCTION

In the previous section it was shown that the infectivity and consequently the pathogenicity of certain isolates of bovine Ostertagia spp. was higher when O. leptospicularis was one of the species present.

In these studies the proportion of O. ostertagi larvae present in the mixed inoculum was not known as only adult males can be positively identified and thus no estimate of percentage establishment of the two species could be made. In the present section, the results of an experiment designed to study the effect of inoculating known numbers of a pure isolate of O. leptospicularis infective larvae, an O. ostertagi isolate which contained only small numbers of S. lyrata and an equal mixture of these two isolates.

EXPERIMENTAL DESIGN

Fifteen Ayrshire male calves, aged 10 weeks, which had been reared under helminth-free conditions were allocated on a weight basis to 3 groups of 5 calves. The calves were penned individually to facilitate daily observations.

On day 0 and using the appropriate larval inoculum the calves in the 3 groups were each given 100,000 Ostertagia spp. infective larvae per os.

Preparation of Larval Isolates

The O. ostertagi isolate was that described in the previous section (isolate GB) and was obtained from calves reared on a farm near Glasgow. As it had been passaged four times under laboratory conditions it was given the code GB4 and consisted mainly of O. ostertagi with less than 10% S. lyrata (hereafter referred to as O. ostertagi isolate).

The O. leptospicularis larvae were obtained by passaging the GC3 isolate through sheep. Following one such passage the larvae inoculated into a helminth-naive calf produced a pure population of O. leptospicularis, the O. ostertagi and S. lyrata present apparently failing to establish as mature infections in the sheep.

The groups of calves received inocula as follows:

Group A: 100,000 O. ostertagi isolate.

Group B: 100,000 O. leptospicularis isolate.

Group C: 50,000 O. ostertagi + 50,000 O. leptospicularis

One calf from each group was sacrificed on days 10, 17, 21, 28 and 35.

OBSERVATIONS

The calves were examined daily, particular attention being paid to appetite and faecal consistency. Faecal samples were collected on days -2, -1, 0, 7, 14 and daily from days 17 to 35. When nematode eggs were detected in the faeces by the flotation method, counts were made using the modified McMaster method (Ministry of Agriculture Technical Bulletin No.18) and expressed as eggs per gram (epg).

At post-mortem examination the gastro-intestinal tract was removed, the abomasum isolated by ligatures, opened, and after tissue had been taken for histology, the fluid contents and washings of the mucosa were examined for Ostertagia spp. as described by Ritchie et al (1966). The worms present in the abomasal washings as well as those in the digests of the abomasal mucosa were classified as adult, i.e. mature male and female worms or fifth larval stages (L_5), and fourth larval stages (L_4). Differentiation into species was possible from the L_5 stage.

The samples of abomasal mucosa and of lymph nodes draining the abomasum were fixed in both 10% formol-saline and Carnoy's fluid prior to processing for histological examination.

RESULTS

Clinical

The calves of Group A and B showed neither a depression of appetite nor diarrhoea. On the other hand, from day 22 the 2 remaining calves in Group C developed a depressed appetite and continuous diarrhoea from day 25.

Parasitological data

Ostertagia spp. eggs were first detected on day 17 and were present in the remaining calves of all three groups. The number of eggs increased markedly between days 17 and 21 and then decreased until day 28. The highest counts were recorded in the Group C calves (mixed infection). Appendix F. shows the mean individual egg counts.

The individual worm burdens and the percentage of each inoculum established are shown in Table 9 together with the mean Ostertagia spp. faecal egg count for each group at the time of slaughter. The relative proportion of each species present in the mucosa (recovered from the mucosal digest) and in the lumen are given in Table 10.

The percentage of the inoculum established in the Group A calves ranged from 9.0% to 44.4% the maximum establishment being recorded at day 17 and the lowest at day 28. The worms were primarily O. ostertagi but included a small number of S. lyrata (4%).

In the Group B calves which had received pure O. leptospicularis the percentage establishment ranged from 23.6 to 48.9; this was higher than that of the Group A calves. Some reduction in the number of worms present occurred at day 21, but this was not as marked as in the Group A calves, despite an indication (Table 10) that a proportionally higher number of O. leptospicularis was already present in the lumen by day 21. Negligible numbers of L_4 were present in one calf.

Table 9. Post-mortem worm burdens of calves infected with single or mixed infections of O.ostertagi and O.leptospicularis.

Day necropsied	Group	Mean epg	Adults and L_5			L_4	Total worm burden	Percentage of Larvae Established		
			O.oster.	O.lepto	S.lyrata			% of total established	% O.lepto. established	% O.oster. established
D 10	A	0	-	-	-	-	13,250	13.3	-	13.3
	B	0	-	-	-	-	24,500	24.5	24.5	-
	C	0	-	-	-	-	44,900	44.9	-	-
D 17	A	38	41,061	-	3,339	-	44,400	44.4	-	41.1
	B	63	-	48,350	-	500	48,850	48.9	48.9	-
	C	25	33,237	31,295	2,868	200	67,600	67.6	62.6	66.5
D 21	A	683	16,202	-	948	-	17,150	17.2	-	16.2
	B	1250	-	47,450	-	-	47,450	47.5	47.5	-
	C	2967	23,813	27,879	3,908	600	56,200	56.2	55.7	47.6
D 28	A	400	7,779	-	1,221	-	9,000	9.0	-	7.8
	B	650	-	23,550	-	-	23,550	23.6	23.6	-
	C	900	10,259	19,955	1,836	-	32,050	32.1	39.9	20.5
D 35	A	650	9.779	-	521	-	10,300	10.3	-	9.8
	B	800	-	29,950	-	-	29,950	30.0	30.0	-
	C	700	9,080	24,030	790	-	33,900	33.9	48.1	18.2

A = 100,000 Ostertagia spp. GB4 isolate
B = 100,000 O. leptospicularis
C = 50,000 O. leptospicularis + 50,000 ostertagia spp. GB4 isolate

Table 10. Proportion of worm burdens in abomasal mucosa compared to lumen.

Group		Day 10	Day 17	Day 21	Day 28	Day 35
A	O. oster.	100	87.7	56.3	13.9	17.4
	S. lyrata	100	92.9	8.2	18.1	0
B	O. lepto.	100	96.9	34.6	6.4	2.3
C	O. oster.	100	96.3	89.1	6.2	8.4
	S. lyrata	100	97.0	69.3	0	19.0
	O. lepto.	100	88.0	38.3	11.3	4.1

By contrast with Groups A and B the proportions of the total inocula established in the calves of Group C were much higher (33.9% to 67.6%). As in the other groups the highest number of worms was present in the calf killed on day 17 with a reduction in numbers by day 28 and 35. The worm burden consisted primarily of O. ostertagi and O. leptospicularis together with a very low number of S. lyrata (4%). Negligible numbers of L_4 were present in the two calves.

Pathology

Typical lesions of ostertagiasis including nodules with umbilicated centres, hyperaemia and oedema were present in the abomasa of all the calves and were most severe in those of Group C (Plates 17, 18, 19). In the calves necropsied from this group on days 21, 28 and 35 coalescence of the nodules had taken place and some diptheresis was present (Plate 20). Histological examination showed changes similar to those described in the previous section where O. leptospicularis was present (Groups B and C) many gastric glands were still parasitised by mature worms on days 21, 28 and 35 (Plates 21, 22). At the same time, numerous eggs were found dilating gastric glands and, in certain locations, had breached the mucosal lining of the gland, an outcome which was unusually accompanied with haemorrhage and fibrinous exudation containing numerous neutrophils (Plate 23). These changes were also observed in the surrounding lamina propria and in the adjacent submucosa. The result was that in the glands in the vicinity of parasitised glands not only was there a loss of parietal and zymogenic cells with replacement by undifferentiated epithelium, but also, by days 28 and 35, the post-parasitised glands in O. leptospicularis infected animals were often surrounded by a diffusely fibrosed lamina propria lacking glands (Plate 24).

Marked reactivity occurred in the lymph nodes draining the abomasum in all three groups, but the extent of the response was

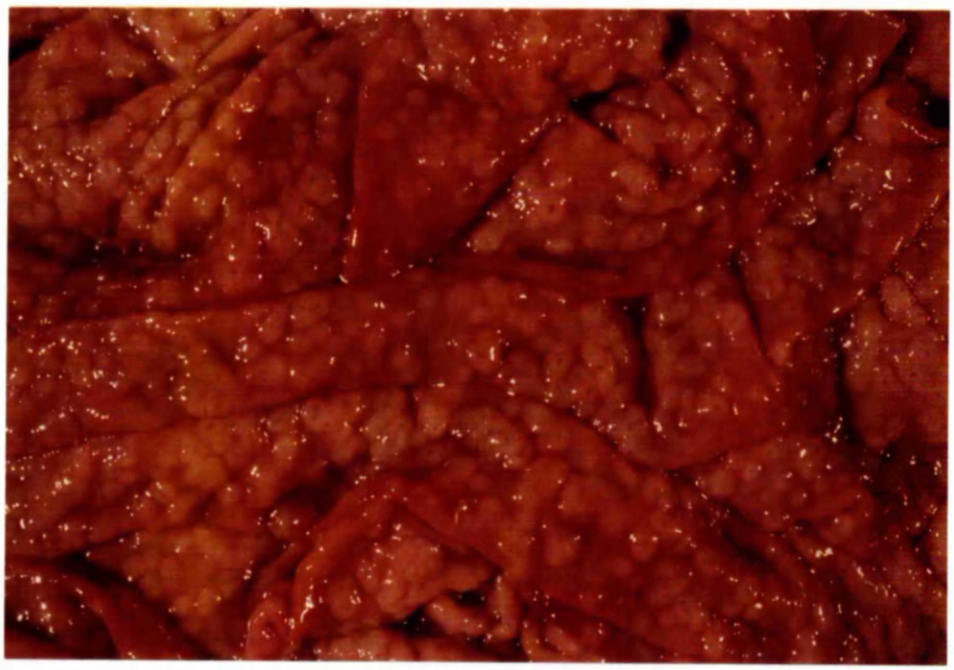


Plate 17. Abomasum of calf 17 days post infection with O.ostertagi

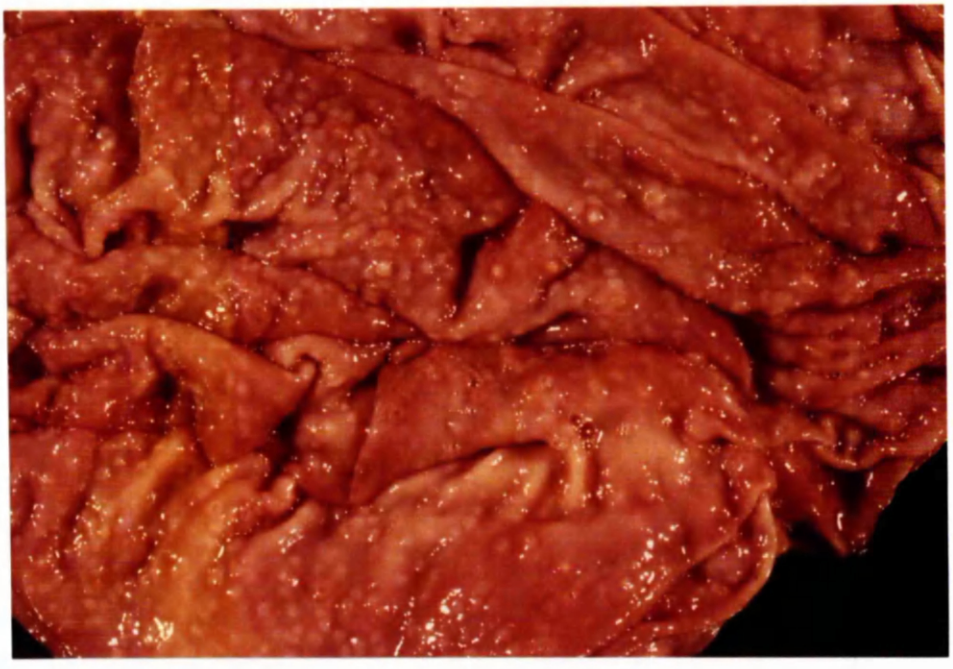


Plate 18. Abomasum of calf 17 days post infection with O. leptospicularis

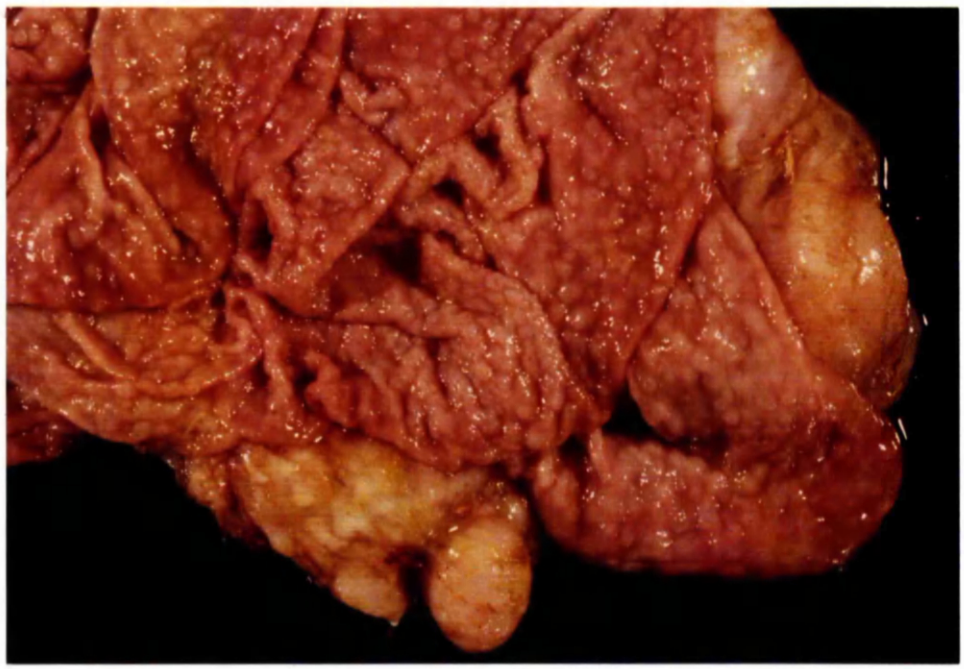


Plate 19. Abomasum of calf 17 days post infection with O.ostertagi plus O. leptospicularis.

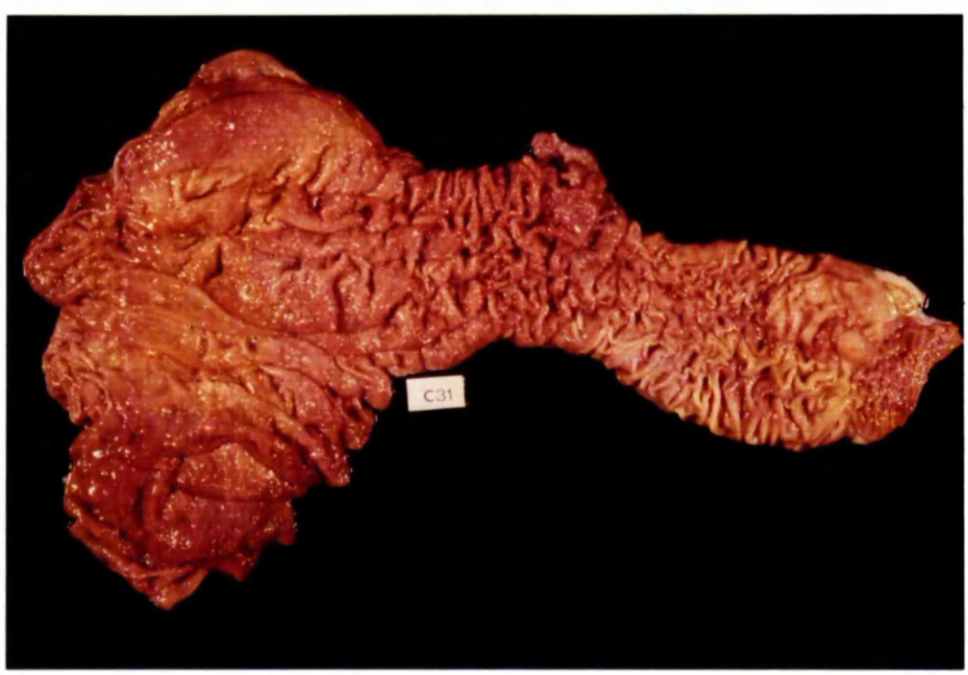


Plate 20. Abomasum of calf 35 days post infection with O.ostertagi plus O. leptospicularis.

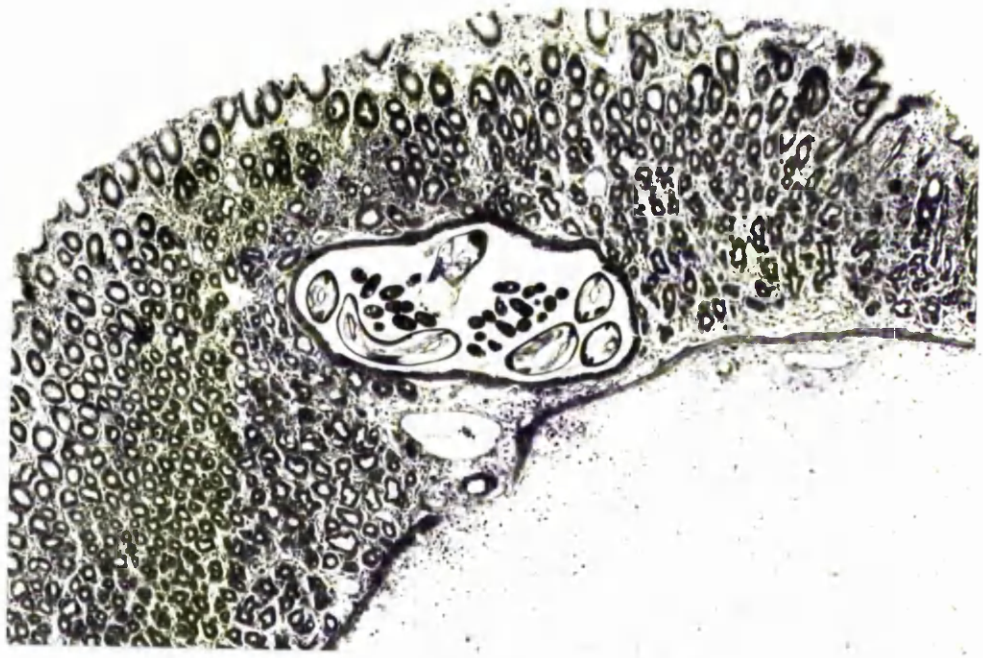


Plate 21. Mature O. leptospicularis female and eggs in gastric gland 21 days post infection x 100.

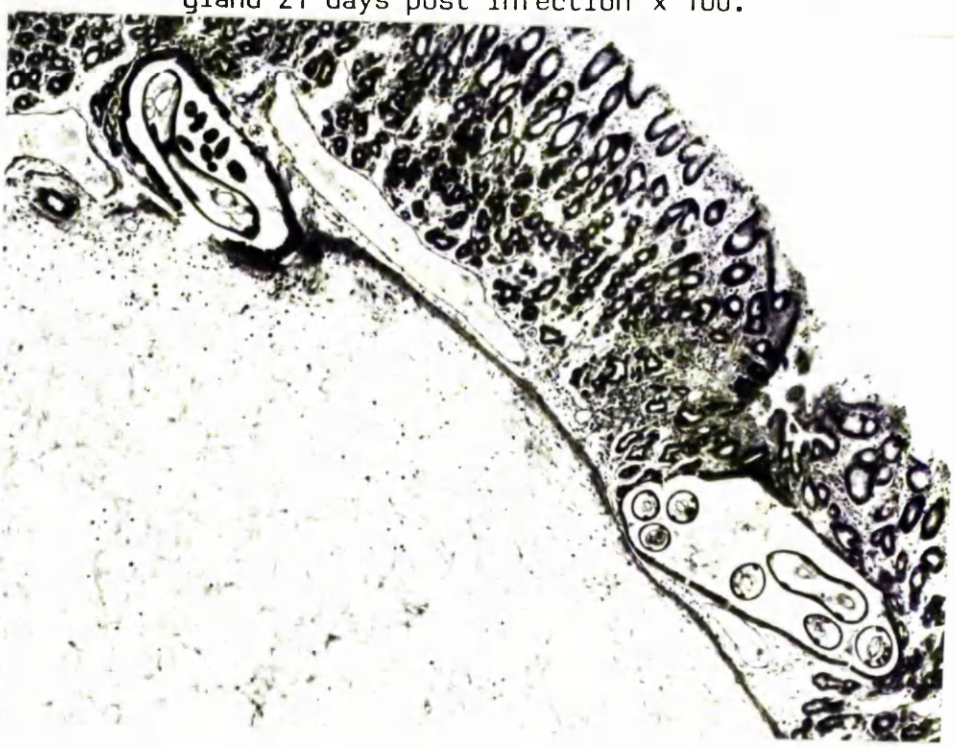


Plate 22. Gastric glands containing mature O.ostertagi, O. leptospicularis and eggs 21 days post infection x 80.

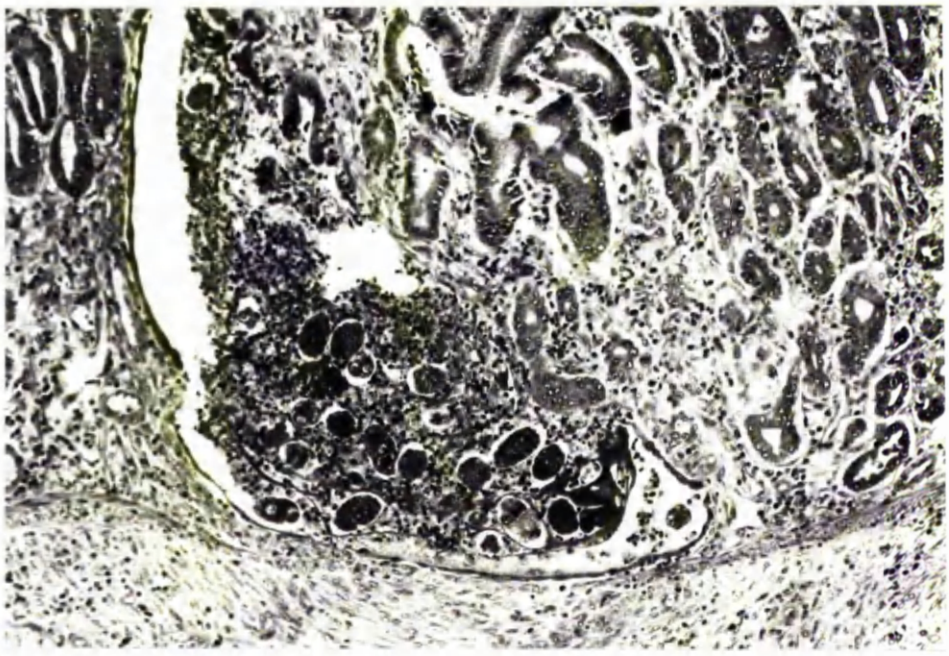


Plate 23. Post - parasitised gastric gland 21 days after infection with O. leptospicularis x 140.

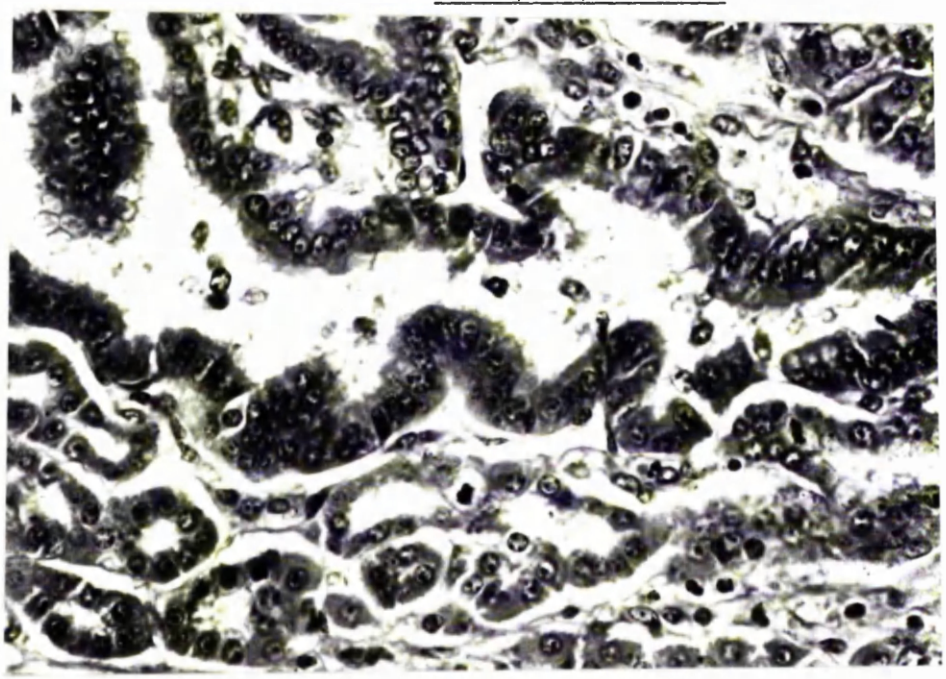


Plate 24. Post - parasitised gastric gland 28 days after Infection with O. leptospicularis x 300

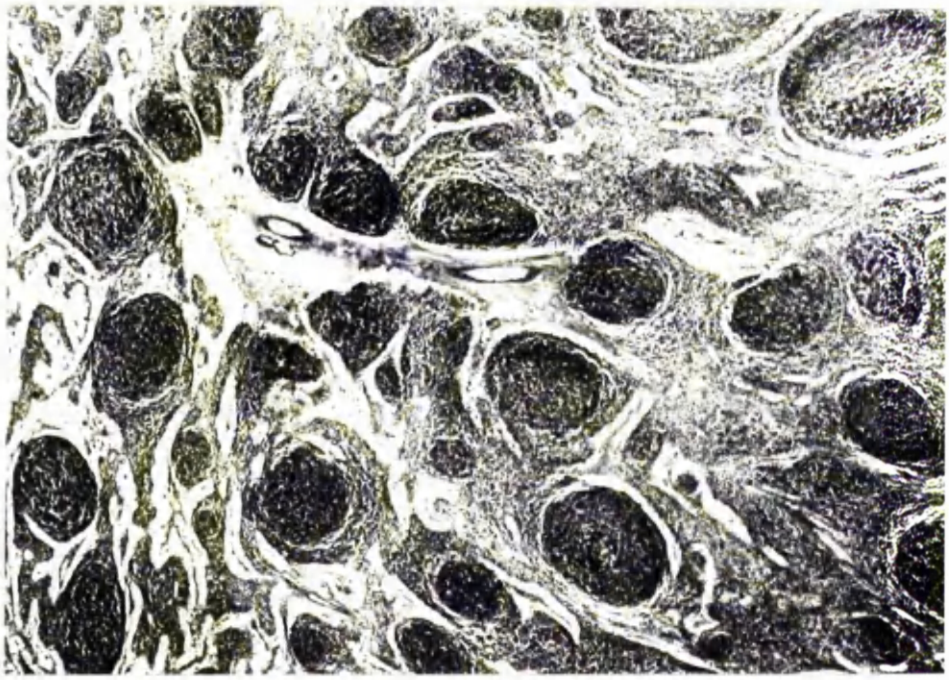


Plate 25. Abomasal lymph node 35 days after infection with O. leptopsicularis x 25.

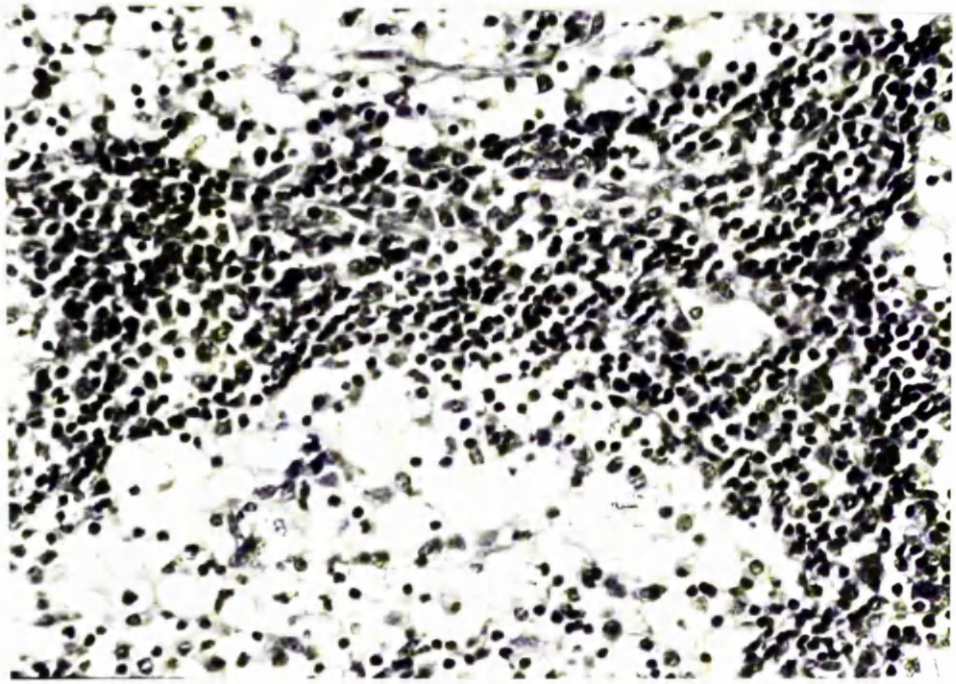


Plate 26. Medulla of abomasal lymph node 21 days after infection with O. leptospicularis x 310.

much greater in animals infected with O. leptospicularis. By day 10 after infection, an increase in the number, size and activity of lymphocytic follicles was obvious, with the follicles becoming distended by germinal centres. By day 21, the number of lymphocytic follicles with germinal centres had increased greatly throughout the nodes from the outer cortex (Plate 25) into the medullary cords, where, by day 35 numerous follicles were present.

Numerous lymphoblasts started to appear in the sinusoidal channels within the paracortex on day 10, although no other changes were observed in this location at this time or later. Also, by day 10, the medullary cords were becoming populated by cells which included small lymphocytes, lymphoblasts and eosinophils. At the same time, the sinuses were distended by oedema and contained macrophages, small lymphocytes and lymphoblasts. Thereafter, there was a progressive increase in plasma cells in the medullary cords (Plate 26) and, as a result, by days 21 through to 35, the medullary cords were markedly distended by both plasma cells and lymphocytic follicles. During this time, the adjacent sinuses continued to contain numerous macrophages (Plate26) and lymphoblasts and were dilated to the extent that the sinus reticulum cells traversing the sinusoids were often disrupted.

DISCUSSION

The results of this experiment confirm the results of the previous section, namely, that when inocula of bovine Ostertagia spp. which contain O. leptospicularis are given to parasite-free calves the percentage of worms established is markedly increased compared with inocula devoid of O. leptospicularis.

It is not possible to quantitate precisely the degree to which worm establishment was enhanced since some loss of worms clearly occurred in most calves necropsied after day 17. However, if the

numbers present in the calves of the different groups necropsied on days 10 and 17 are compared it is evident that the mean percentage establishment of the different groups was: Group A 28.8%, Group B 36.6%, Group C 56.2%. In other words, the simultaneous inoculation of equal numbers of O. leptospicularis and those of the other Ostertagia spp. (Group C) resulted in approximately double the numbers of worms becoming established compared with a quantitatively similar inoculum without the O. leptospicularis. The total numbers established were also much greater (ca 30%) than a pure inoculum of O. leptospicularis.

The absolute numbers of O. leptospicularis and O. ostertagi established by the mixed inocula were similar so clearly the enhanced establishment applies to both species. There was some evidence that a higher proportion of O. leptospicularis emerged from the glands from day 17 (Table 10) and therefore might be expected to be susceptible to earlier expulsion; this did not occur. Indeed the loss of O. ostertagi after emergence was much more prominent and coincided with evidence of marked immunological reactivity in the lymph nodes draining the abomasum.

The gastric lesions produced by O. leptospicularis, as in the previous section, are distinct from those of O. ostertagi. Thus, in O. leptospicularis infections even at day 21, many adult parasites were still within the mucosa resulting in marked distension of the parasitised glands. At the same time, the extent of the loss of functional cells from the gastric glands surrounding the parasitised glands would appear greater in O. leptospicularis infections, and, unlike O. ostertagi, eggs were deposited in the gastric glands and in the surrounding lamina propria resulting in intensive inflammatory changes. The present study offered an opportunity to construct a more complete picture of the consequences of an O. leptospicularis infection in cattle, as animals were sacrificed not only on day 21, as in the previous experiment but also on days 28 and 35.

As with O. ostertagi, the main long term consequence of an O. leptospicularis infection was loss of the functional parietal and zymogenic cells lining the gastric glands, but, in contrast to O. ostertagi, the diffuse inflammatory reactions produced by the deposition of eggs within the mucosa had resulted in severe and extensive fibrosis of the lamina propria.

While marked reactivity was observed in the lymph nodes draining the abomasum in all three infected groups, the response observed to O. leptospicularis infections was much greater. It may be that this difference reflects the greater mucosal damage inflicted by O. leptospicularis, although the possibility that O. leptospicularis was a more potent immunogen must also be considered. The dramatic increase in lymphocytic follicles with germinal centres, accompanied by the marked plasma cell response in the medullary cords and by lack of any significant reaction in the paracortex, would suggest that the immune response was mainly a B-cell one. The presence of eosinophils in the abomasal lymph nodes as early as day 10 after infection indicated involvement of parasitic antigens, in causing the marked proliferative response. However, this response might also be attributable to the increase in bacteria in the abomasum which follows the elevation of the pH of the gastric fluid in severe cases of ostertagiasis (Jennings et al, 1966).

To my knowledge, this is the first time that the quantified simultaneous administration of different species of a nematode (as distinct from a genus) has resulted in an obvious enhancement of establishment of this species. The precise reason for such a phenomenon is unknown and further studies are necessary to ascertain what happens if the proportion and timing of administration of the different species inoculated is varied. From the practical point of view the enhanced establishment and accompanying severe pathology of

the mixed inocula are important considerations for control methods involving mixed or alternate grazing of sheep and cattle; particularly as Bisset (1980b) has demonstrated that O. ostertagi can more readily establish in sheep in the presence of O. leptospicularis although I have not observed this interaction.

CHAPTER 5

FIELD STUDIES ON THE EPIDEMIOLOGY AND PATHOGENICITY OF DIFFERENT ISOLATES OF BOVINE Ostertagia SPP.

1) 1980 Experiment

2) 1981 Experiment

1. 1980 Experiment

INTRODUCTION

Field studies on the epidemiology and pathogenesis of bovine ostertagiasis has received much attention over a number of years (reviewed by Armour, 1970 and Michel, 1976), but the possible occurrence of different "strains" of the parasite with variation in their epidemiology and pathogenicity has received little attention except in relation to possible drug resistance. In the previous chapter it was shown that three separate groups of calves showed quite different pathogenic effects after receiving comparable experimental larval challenges, with different isolates of Ostertagia spp. and that none of the "strains" consisted purely of O. ostertagi. The Weybridge "strain", widely used in experiments throughout Britain and a recent field isolate consisted primarily of O. ostertagi and a low proportion of S. lyrata. In contrast, the other isolate which was markedly more infective than the others, also contained a proportion of O. leptospicularis.

The experiment reported here was a field study on the epidemiology and pathogenicity of the "strains" in which calves were set-stocked on different pastures which carried infection with the three isolates.

EXPERIMENTAL DESIGN

Twelve helminth-naive, 4-month old, male Friesian X calves were allocated on a weight basis to 3 equal groups. Each group was grazed on a 0.33 hectare plot in the grounds of Glasgow University Veterinary School from May to September, each plot having been contaminated with one of the three isolates in the preceding year. To supplement the infection which was already present as overwintered larvae, each calf was infected orally with 5,000 L_3 of the appropriate isolate one week prior to being put to graze.

Larval Isolates

The isolates used were:

GB3 - a field infection consisting mainly of O. ostertagi with a small component of S. lyrata, which had been passaged three times in culture calves.

GC3 - a field infection consisting mainly of O. ostertagi and O. leptospicularis with a small component of S. lyrata, which had also been passaged three times in culture calves.

W - the "Weybridge strain", consisting mainly of O. ostertagi and a small component of S. lyrata, which had been passaged an unknown number of times in culture calves.

OBSERVATIONS

Each week the calves were examined clinically and faecal samples were taken, and the animals were weighed each fortnight. Herbage samples were collected each week from the three plots, and the mean weekly temperature and rainfall were recorded.

Faecal samples were examined by a modified McMaster technique (MAFF Technical Bulletin No.19), the results being expressed as eggs per gram of faeces (epg).

Pasture larval counts were determined by the method of Parfitt (1955) and the larval numbers expressed as L_3/kg dried herbage.

Pepsinogen levels were estimated on the plasma samples by the method of Edwards et al (1960) and expressed in international units (i.u.) of tyrosine.

At post-mortem the gastro-intestinal tract was removed and examined for nematodes by the method of Ritchie et al (1966). In addition, when the abomasum was opened two portions of tissue were taken for histological examination, one being fixed in 10% formol saline and the other in Carnoy's medium.

The nematodes were counted, identified and classified as

adults (i.e. mature male and female worms), 5th larval stages (L_5) and arrested (EL_4) and developing (L_4) fourth larval stages. The arrested fourth larval stages were identifiable since, prior to slaughter, the calves were housed for one week to allow normally developing larvae to progress beyond this stage.

RESULTS

Clinical Findings

Diarrhoea was first observed in the GC3 calves at the beginning of August, after 13 weeks grazing, and this became severe by the end of August, necessitating the slaughter of two calves in early September and the remaining calves of the group in mid-September. In the other two groups only calf 16 (GB3) showed diarrhoea. For comparison with the severely ill animals of the GC3 group, two animals from each of the other groups were necropsied in early and mid-September. None of the survivors showed any severe diarrhoea.

The mean fortnightly liveweight gains are given in Fig. 3 and individual figures shown in Appendix G. For the first seven weeks at grass the increases were similar in all three groups, but thereafter the GC3 group calves ceased to gain weight, and by the end of August there was a difference of 50 Kg. in weight between this group and the others.

Climatic Data

The climatic records are given in Fig. 4. Temperatures were within the normal range for the West of Scotland , and the rainfall pattern was generally typical except for August and September when it was abnormally heavy.

Parasitological Findings

Faecal egg counts

Appendix H. details individual faecal eggs counts and the mean figures are shown in Fig. 5. The GC3 calves were positive by the third week and the other two groups of calves by the fourth week of grazing.

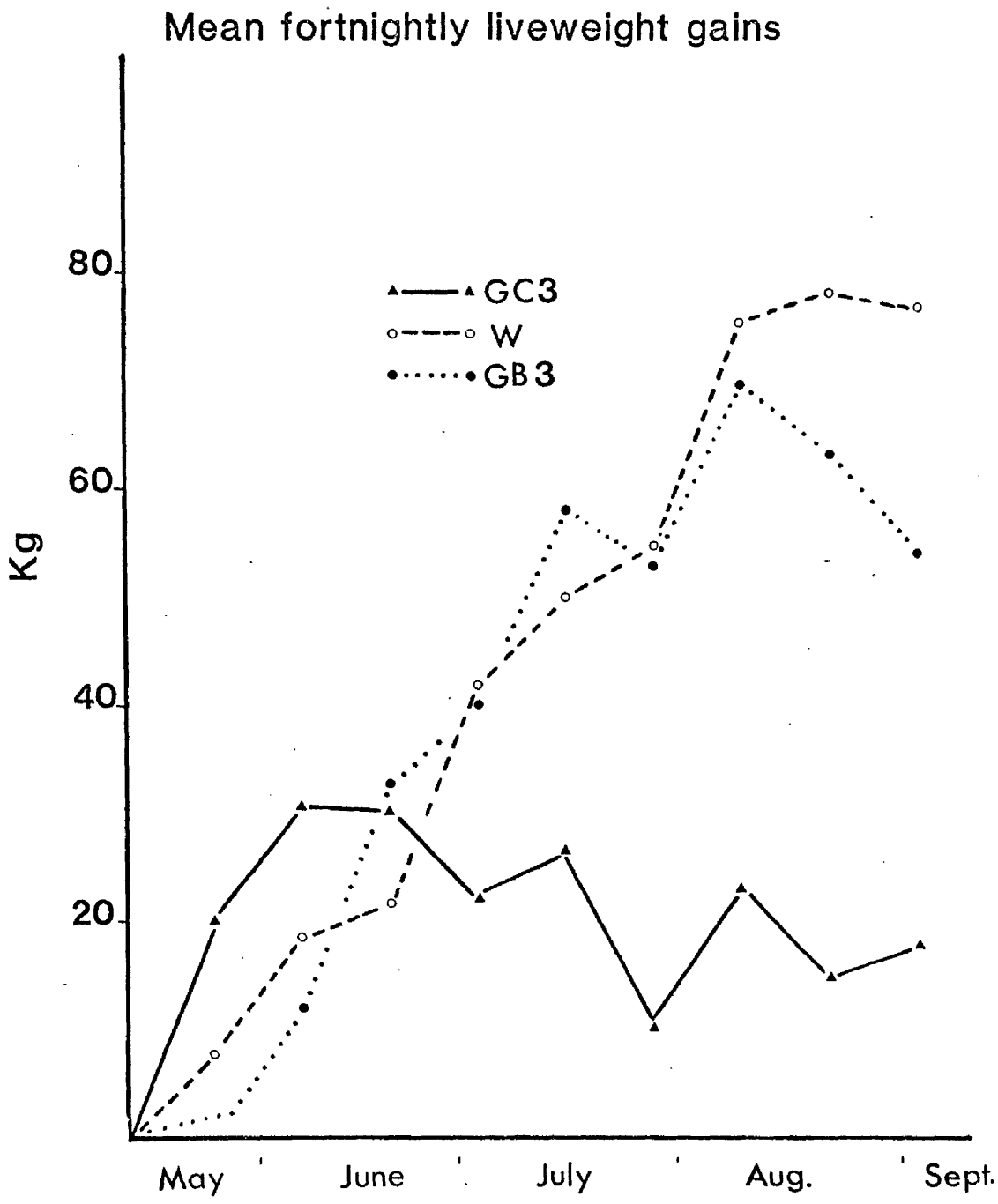


Figure 3. Mean fortnightly liveweight gains of calves infected with different isolates of Ostertagia spp.

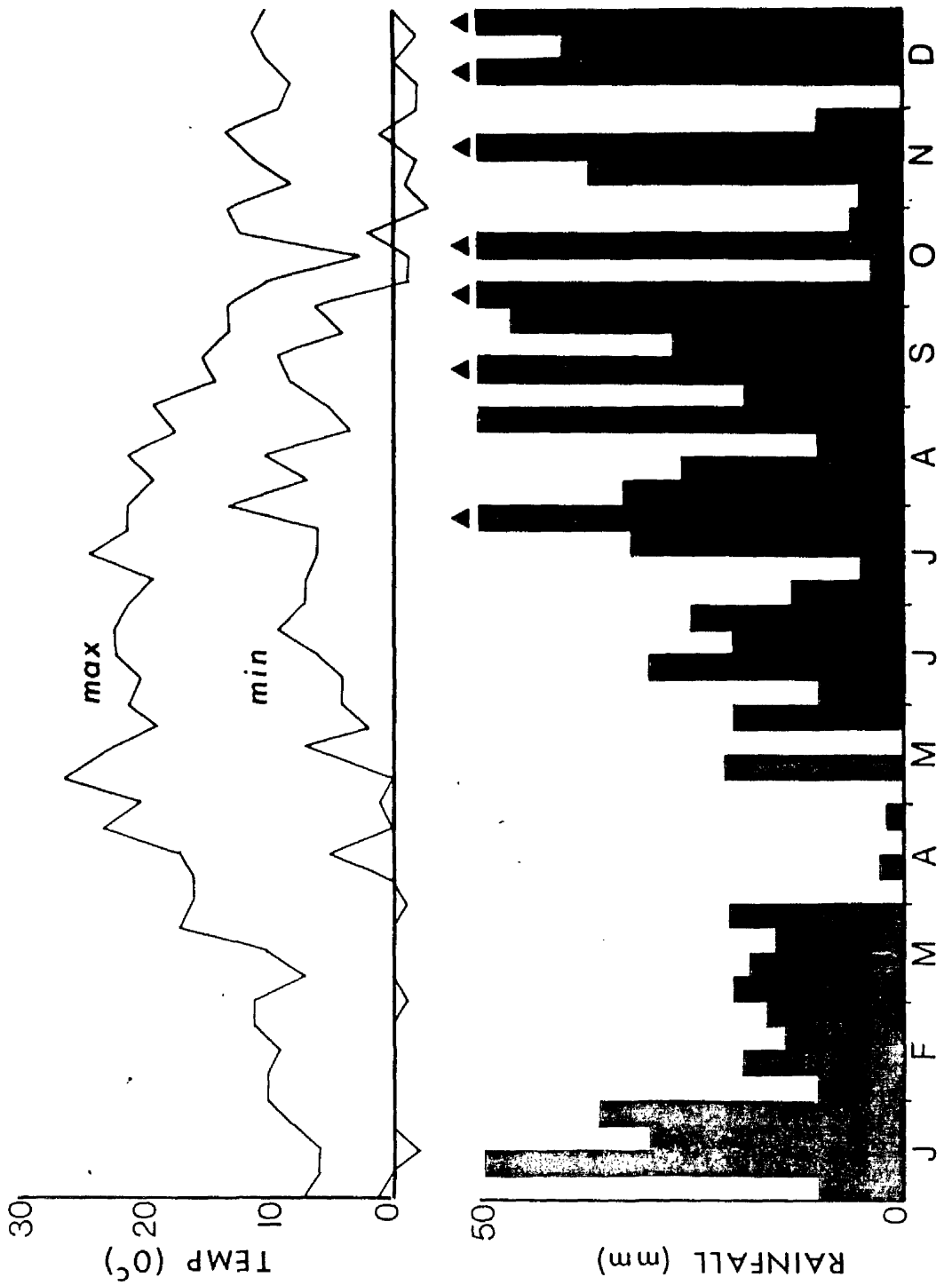


Figure 4. Climatic data 1980

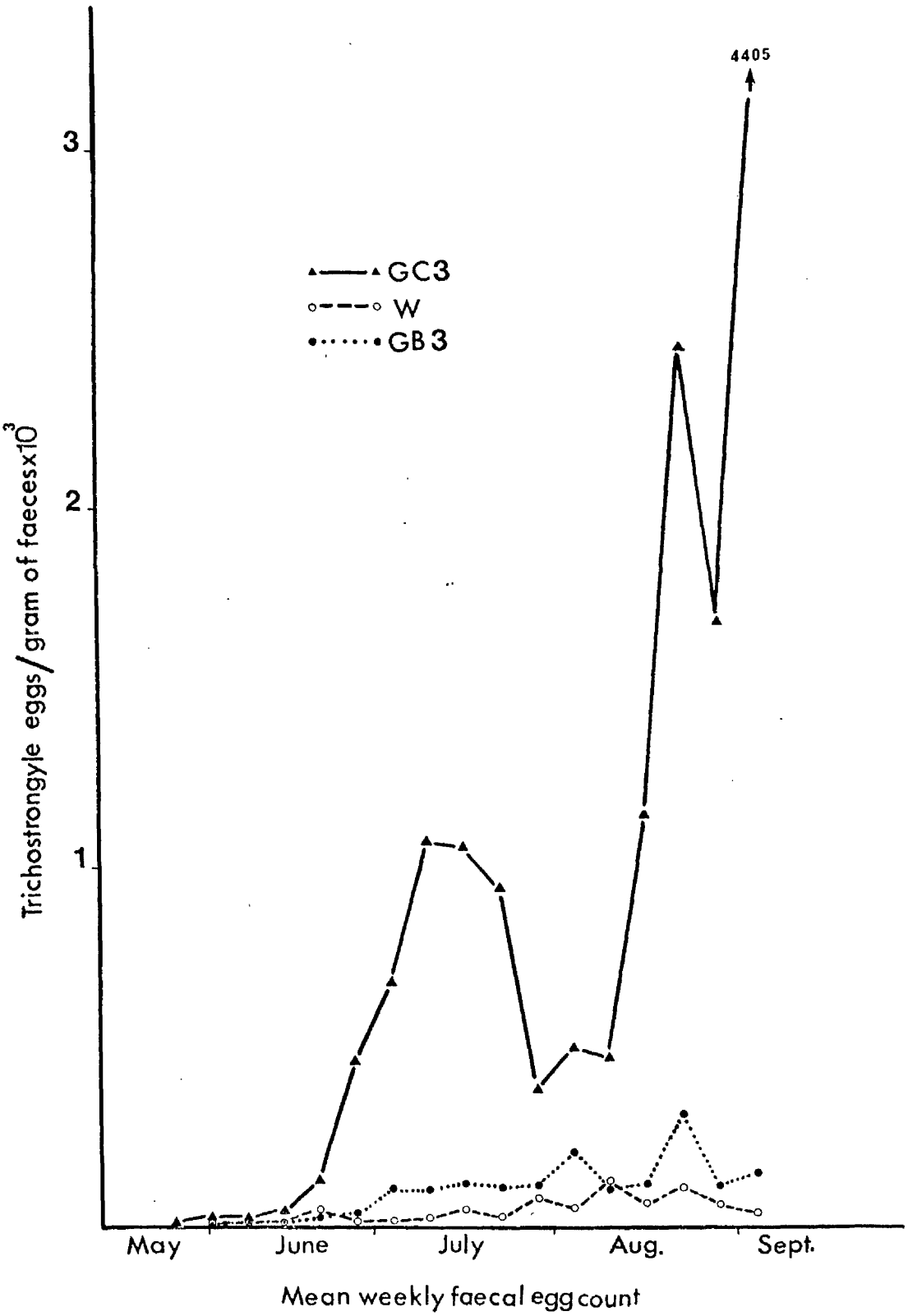


Figure 5. Faecal egg counts of grazing calves infected with different isolates of Ostertagia spp.

Though all three groups had positive egg counts these were markedly higher in the GC3 calves which, in mid-July, after ten weeks grazing, had a mean count of 1007. Thereafter, while the egg counts of the other groups showed a slight and variable rise, that of the GC3 calves fell to 380 epg at 13 weeks (late July) remained at this level for three weeks and then rose to a second peak of 4405 epg at week 19 (mid-September).

Pasture larval counts

Ostertagia spp. pasture larval counts (OL_3) are shown in Fig.6.and detailed in Appendix I. Low numbers of larvae had overwinteredbut by mid-June these were at negligible levels, and in the GC3 group were undetectable until early July when a rapid and continuous increase occurred reaching a maximum of 62700 L_3 /Kg in week 16 (end of August) followed by a rapid fall to under 10,000 L_3/Kg in early September and remained low

The pasture larval counts of the GB3 group began to rise in mid-July reaching a peak 6,700 L_3/Kg between mid-July to early August and then a gradual reduction in numbers to low levels in early September.

The other group (W) did not show an appreciable increase in number until the begininning of August when they rose rapidly to a peak of 34,900 L_3/Kg at the end of August. Thereafter, like the GC3 group there was a very rapid loss of larvae form the pasture.

Worm Burdens

Individual worm burdens are shown in Table 11. Because of the severity of the clinical ostertagiasis in the GC3 group, calves were secrificed at two points.

There was a reduction in the total worm burdens of the W and GB3 calves sacrificed on September 15th compared with those on September 1st; in contrast, the worm burdens in the GC3 calves were generally similar on both dates.

The overall mean burdens of the four calves were higher in the GC3 group (135,200) than in either the GB3 (45,100) or the W(38,000).

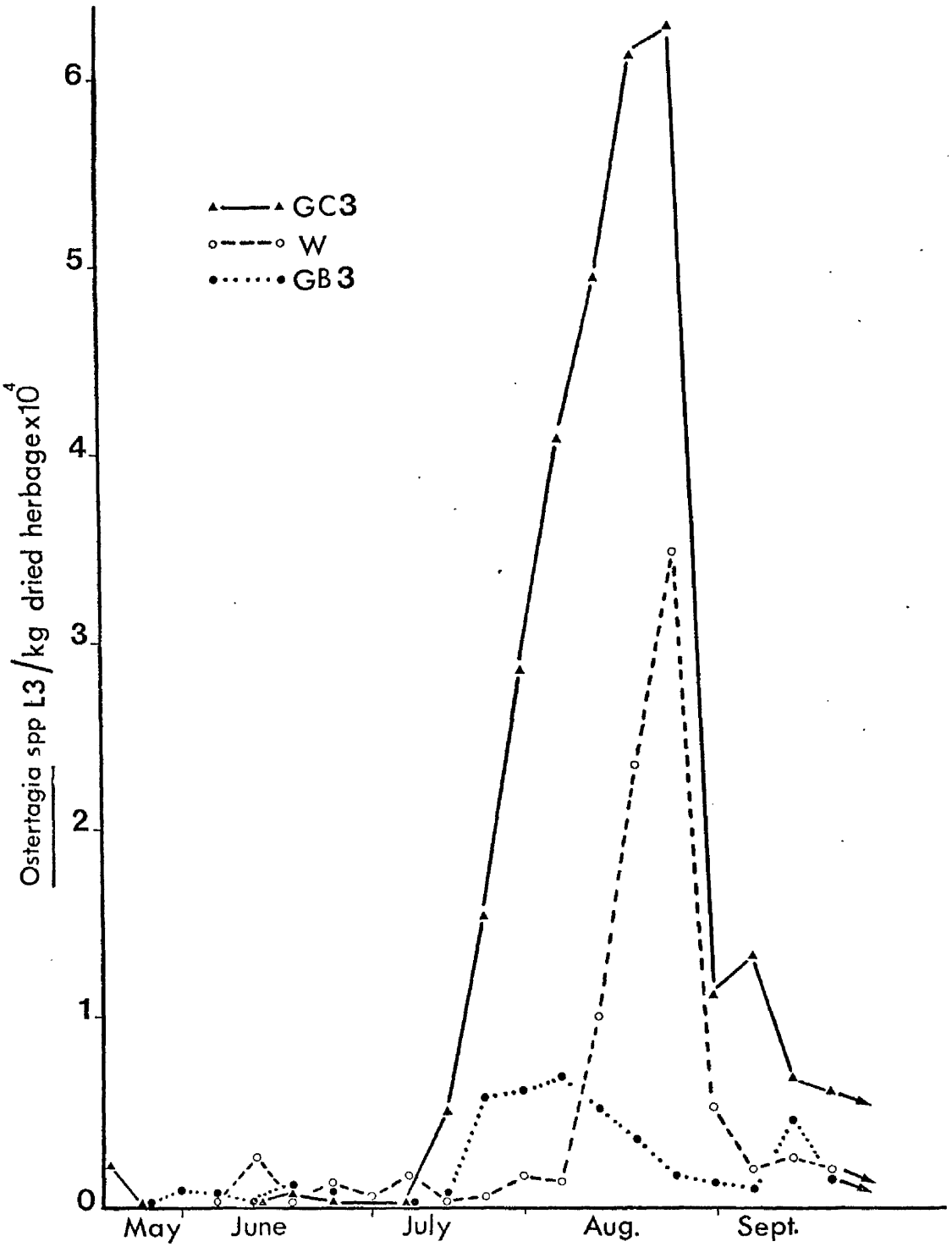


Figure 6. Numbers of Ostertagia L_3 recovered form pastures infected with different isolates of Ostertagia spp.

Table 11. Post mortem worm burdens of grazing calves infected with different isolates of Ostertagia spp.

Isolate	Grazing Period	Calf No.	Abomasal pH	Total Worm Burden	Abomasal Worm Burdens			L_4	EL_4
					Adults of Individual Species (% of total adult worm burden)				
					O. oster.	S. lyrata	O. lepto.		
W	May 12 to Sept 1	17	5.1	64500	58657(97.6)	1443(2.4)	0	0	4400
		53	5.7	64700	63294(97.8)	1406(2.2)	0	0	0
	May 12 to Sept 15	3	3.6	8100	6844(93.8)	456(6.2)	0	0	800
		41	4.8	14550	11158(96.2)	442(3.8)	0	0	2950
Mean			4.8	37963	34988(96.4)	937(3.6)			2038
GB3	May 12 to Sept 1	16	7.4	111300	100011(90.1)	10989(9.9)	0	0	300
		57	5.1	31600	25967(85.7)	4333(14.3)	0	0	1300
	May 12 to Sept 15	11	4.8	15500	9379(97.7)	221(2.3)	0	4500	1400
		50	4.1	22200	5056(95.4)	244(4.6)	0	200	16700
Mean			5.4	45150	35103(92.2)	3947(7.8)		1175	4925
GC3	May 12 to Sept 1	4	6.2	83000	80458(98.0)	1642(2.0)	0	0	900
		55	6.7	188400	164280(88.8)	8510(4.6)	12210(6.6)	0	3400
	May 12 to Sept 15	21	6.8	125600	108973(86.9)	7900(6.3)	8527(6.8)	100	100
		58	6.9	143800	132316(92.4)	5442(3.8)	5440(3.8)	0	600
Mean			6.7	135200	121507(91.5)	5874(4.2)	6544(4.3)	25	1250

The male:female ratios were GC3 - 0.8:1; GB3 and W -0.7:1. Nearly all the worms were mature adults or 5th larval stages with one exception, Calf No. 50 in the GB3 group, where 75% of the total worm burden were arrested EL_4.

On detailed examination of the female worms a mean and standard deviation of counts of eggs in utero per gravid Ostertagia female (it is not possible to identify these to species level) were: GC3 group - 26.0 $\pm$ 7.1; GB3 group - 19.6 $\pm$ 7.2; W group - 17.0 $\pm$ 7.2. On statistical analysis, using a two sample t-test, the count for GC3 was significantly greater ($p > 0.001$) than those in each of the other groups.

With few exceptions the intestinal worm populations, composed solely of Cooperia and Nematodirus spp., were low and the individual figures are detailed in Appendix J .

Biochemical Data

While increased plasma pepsinogen levels were observed in all calves at the middle of the grazing season (July) this was more pronounced in the GC3 group (to 3.4 i.u.). Maximum pepsinogen values were reached by the end of the grazing period as can be seen in Fig 7. which illustrates these results individual figures being given in Appendix K .

Pathology

Pathological change was especially severe in the calves of group GC3 with hyperaemia, oedema and diphtheresis which, as is characteristic in extreme ostertagiasis, in many cases obscured the discrete nodule formation. On histological examination many mature adult parasites were found in the abomasal glands of the GC3 group calves, a feature of the pathogenesis of this isolate previously noted. The calves receiving the other isolates had mild lesions,

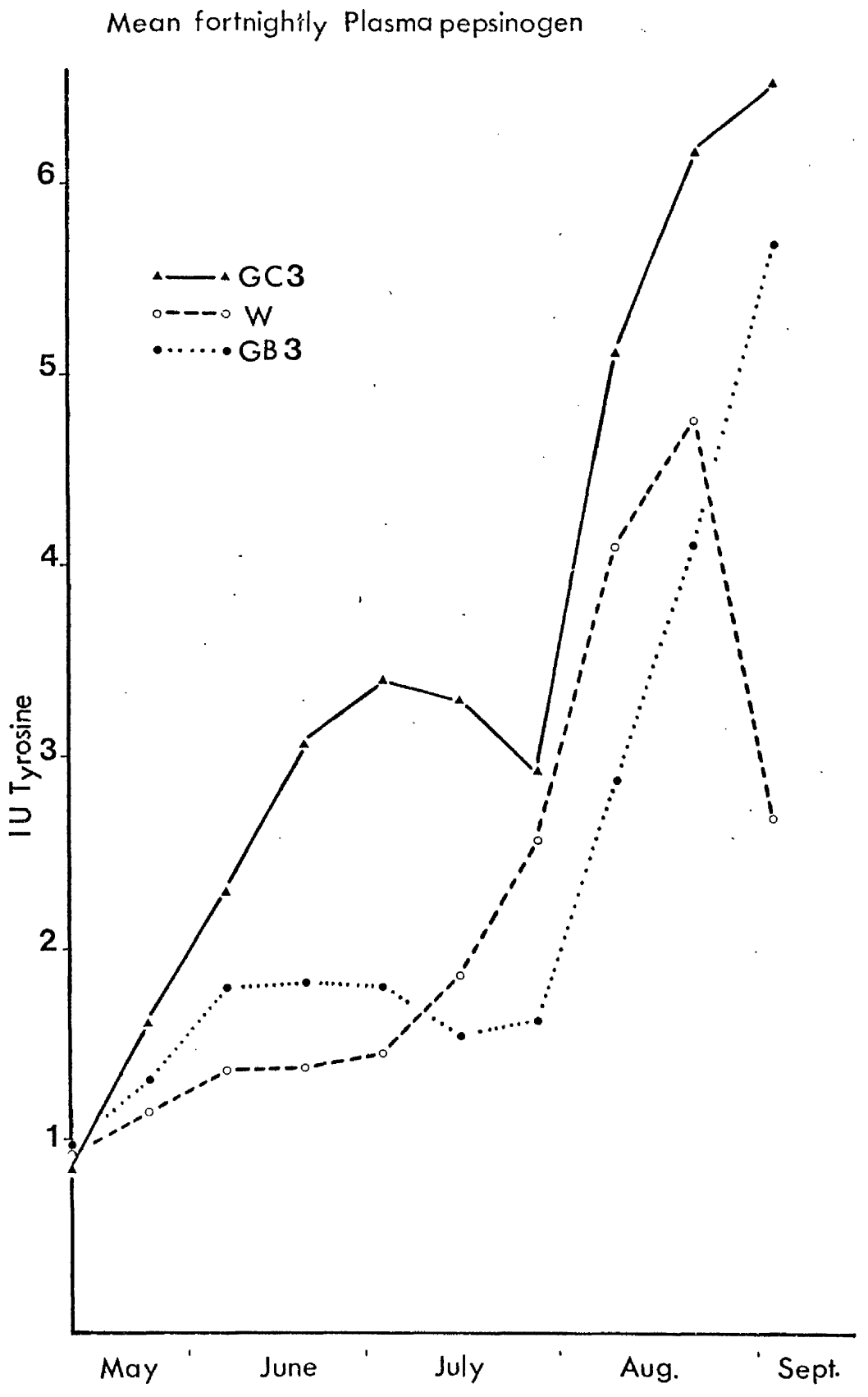


Figure 7. Mean fortnightly plasma pepsinogen levels from calves grazing pastures infected with different isolates of Ostertagia spp.

with the exception of a single animal in the GB3 group (No.16), which had severe lesions characteristic of Type I ostertagiasis as described by Anderson et al (1966).

DISCUSSION

The OL_3 ingested by the calves at the commencement of grazing had two origins - the experimental inoculum of 5,000 OL_3 of the appropriate isolate, and surviving overwintered OL_3; since the numbers of overwintered OL_3 recovered were very low in all plots (less than 1,000 L_3/kg dried herbage) it is reasonable to assume that all the calves were exposed to comparable levels of infection. However, despite this similarity in original larval intake, marked differences were shown in the subsequent patterns of faecal egg count, herbage larval count, post-mortem worm burden and degree of pathological change.

In all groups the egg counts, first detected during the third and fourth weeks, were consistently low until week 7, when a clear difference in the pattern of the GC3 group began to appear. Though the mean egg counts of the GB3 and W groups remained at, or below, 300 epg throughout the season, that of the GC3 group rose to more than three times this figure by week 10 (mid-July), though very few OL_3 had been available on the pasture. There was a brief period of diminished egg output between weeks 10 and 13, the reason for which cannot be known, but it may indicate the loss of the initial infection which, by this time, would be approaching senility. Thereafter, the rise continued, reaching a maximum of 4,405 epg at week 19 in mid-September, a figure 15 times greater than those of the others.

This remarkable difference in faecal egg count following comparable initial larval exposure to the different isolates may be attributable to an increased ability of the ingested larvae to become established and/or to a greater fecundity of the female worms

present. Since in the experimental studies with these isolates it was observed that a greater proportion of the GC3 infective larvae became established and in the present study a higher number of eggs per female worm occurred in the GC3 group, it would appear that both factors may be involved. Of the total Ostertagia population derived from the GC3 infection, 91.5% were O. ostertagi and the male:female ratio was 1:1, so that the increased egg production must be attributable to this species.

The pattern of pasture larval counts followed in general the seasonal sequence described for south west Scotland by Armour (1978a), with increases occurring on all three plots in the second half of the grazing period. Here again, however, as happened with the egg counts, the maximum larval counts showed diversity in timing and dimension. The plot with the lowest maximum count (GB3 with 6,700 Ostertagia spp. L_3/kg dried herbage) reached this figure in week 13, in early August, three weeks before the others had arrived at their maxima of 34,900 (W) and 62,700 (GC3). Though the high count of GC3 was predictable considering the very high faecal epg of the calves grazing there, the figure of 37,000 L_3/kg on plot W was quite unexpected, deriving as it did from an initial egg output less than that on GB3, but reaching a larval count four times as great. There is no immediately apparent reason for this discrepancy, but it might be attributable to differences between the isolates in translation potential from egg to L_3 on the herbage.

A second deviation from the expected pattern was the disappearance of 90 per cent of larvae from the herbage of all three plots by early September. It is difficult to explain this atypical behaviour, but a contributory factor may have been excessively heavy rainfall during August (Fig. 4), which could have caused premature distintegration of the faecal pats and downward lavage of the free larvae into the soil.

The worm burdens at post-mortem examination reflected the different levels of available herbage L_3 and the faecal egg output. Thus, the worm numbers in the calves grazing the GC3 plot had a range of 83,000 to 188,400, with all the animals showing the extreme clinical signs of terminal ostertagiasis, and characteristically severe abomasal lesions at post-mortem; in the other groups the counts were at, or below, 65,000 and the calves had shown only mild diarrhoea with the single exception of calf No.16 in the GB3 group, which had a burden of 111,300 worms and alone showed clinical signs and lesions comparable in severity to those in the GC3 calves. Arrested larvae were present in only low numbers, with the exception of calf No.50 in group GB3, and this was to be expected as the calves were necropsied just prior to the period when such larvae usually appear.

On detailed examination it was found that the worm burdens on the W and GB3 plots consisted primarily of O. ostertagi with a few S. lyrata, whereas on the GC3 plot O. leptospicularis was present in three of the calves in proportions between 4% and 7% of the total count. O. leptospicularis were not detected in the other calf No.4. This level of O. leptospicularis was much lower than expected, since the experimental infections with the same larval isolate had yielded burdens of this species representing 28% of the infective larval intake. Two explanations are possible. First, there may have been a preferential loss of O. leptospicularis from the calves prior to slaughter and this could explain the absence of this species from calf No.4 which had been severely diarrhoeic. Second, the presence of O. leptospicularis could have interfered with the turnover or loss of O. ostertagi which is clearly evident in the other groups but is apparently absent from the GC3 calves. Thus in the face of a decreasing larval challenge during late August and September (Fig. 6) the worm burdens of the two calves each of plots W and

GB3 killed on September 15th were lower than the burdens of those killed in early September; in contrast, the burdens of the GC3 calves remained constant despite a markedly reduced larval intake, an indication, perhaps, that a large part of the O. ostertagi burden, which would normally have been shed as in the other groups, had been able - or even assisted - to retain its station in the abomasum. The pathogenic consequences of such an alteration in behaviour are clear.

Though an enhanced establishment of worm species has been recorded when larvae of two different genera are administered at the same time e.g. Trichostrongylus colubriformis and Nematodirus spathiger (Herlich, 1959) the results of the previous experiments and of the one reported here appear to show the only instance in which an interaction of two species of the same genus has resulted in such enhancement. Indeed, in a recent experiment with Oesophagostomum columbianum and Oe. venulosum, Dash (1981) recorded the opposite effect, with a 50 per cent reduction in the establishment of Oe. venulosum. These results confirm the fact that the GC3 isolate has a greater pathogenicity than is normally encountered in bovine ostertagiasis, and this appears to be at least partly attributable to a faculty of its infective larvae to become established in the abomasum in greater proportion than those of other isolates. The role of O. leptospicularis in the pathogenesis of this isolate, and its possible influence on the dynamics of the relationship between the host and O. ostertagi, require further investigation. The apparent loss of infectivity and pathogenicity by the Webridge isolate in these experiments when compared with previous results (Anderson et al, 1966; Ritchie et al, 1966) indicates the need for further comparison between the characteristics of isolates in laboratory passage and in the field.

2. 1981 Experiment

INTRODUCTION

The severe clinical effects and the pathological changes observed in the calves given larvae of the GC isolate in the field experiment in 1980 prompted a further investigation designed to clarify the epidemiology and pathogenicity of this strain. Having postulated a positive role for O. leptospicularis in infections with the GC strain an assessment of the seasonal variation in the herbage larval population of this species seemed desirable. However, as the larval stages of O. leptospicularis cannot as yet be readily distinguished from those of the other bovine Ostertagia species, the worm burdens of tracer calves were used to assess the seasonal fluctuation of O. leptospicularis L_3 on herbage.

EXPERIMENTAL DESIGN

Five 4 month old, helminth-naive, male Friesian X calves were set-stocked (permanent calves) from May to September, 1981 on the same 0.33 hectare plot as was contaminated in the 1980 field experiment by the GC isolate. To overcome the problem of over-stocking and overgrazing which occurred in 1980, an adjacent area, previously separated by a double fence, of 0.33 hectare was included; this area had not been grazed by livestock for 3 years and L_3 could not be recovered by the pasture sampling technique.

Information on the level of Ostertagia larvae of the different species ie O. ostertagi, O. leptospicularis and S. lyrata was obtained by the use of helminth-naive tracer calves; single tracers were introduced in May, July, August and September, allowed to graze for 3 weeks and then housed for 1 week prior to slaughter and analysis of their worm burdens. The experimental design is summarised in Table 12 .

Table 12. Experimental design.

Permanents

Calf No.	Management	Slaughter
14	Set stocked May - October	14th July 1981
4	"	15th Sept 1981
52	"	"
53	"	"
57	"	"

Tracers

Calf No.	Management	Slaughter
42	Grazed 1st - 21st May	28th May 1981
39	" 7th - 28th July	4th Aug 1981
38	" 5th - 26th August	2nd Sept 1981
37	" 10th - 31st August	7th Sept 1981
522	" 15th Sept - 6th Oct.	13th Oct 1981
526	" 22nd Sept - 13th Oct.	20th Oct 1981

Table 13. Biochemical and Parasitological findings at post-mortem.

Calf No.	Abomasal pH	Plasma pepsinogen (iu)	Epg.
14	3.9	3.0	750
4	6.3	9.7	1300
52	6.1	6.7	2250
53	7.0	7.7	1300
57	7.1	4.8	1100

OBSERVATIONS

Permanent calves

Each week the calves were examined clinically and faecal samples were taken. Each fortnight, blood was collected from the jugular vein into tubes containing heparin, after which the plasma was removed for the determination of the plasma pepsinogen level by the method of Edwards et al (1960). Calf weight gains were obtained each fortnight.

Weekly herbage samples were collected for estimation of the larval populations. These samples were obtained from the total area and therefore included the area which was thought to be free of L_3.

Tracer calves

The calves were examined weekly when particular attention was paid to appetite and faecal consistency. One week prior to slaughter, the calves were housed in order to facilitate the recognition of any arrested larvae.

RESULTS

One animal, No.14, had to be slaughtered due to severe non parasitic disease before the end of the experiment.

Diarrhoea was first observed in two of the other calves (53 and 57) at the end of August 1981 i.e. after 18 weeks grazing and at the beginning of September in the remaining two permanent calves, and this became severe by mid-September necessitating their slaughter. The tracer calves grazed in August and September showed similar clinical symptoms at the end of their 3 week grazing period.

The mean fortnightly liveweight gains are given in Fig. 8. The permanents gained a mean of 32.5 kg. liveweight up to the beginning of August (i.e. 0.31 kg/day) thereafter the body weight declined

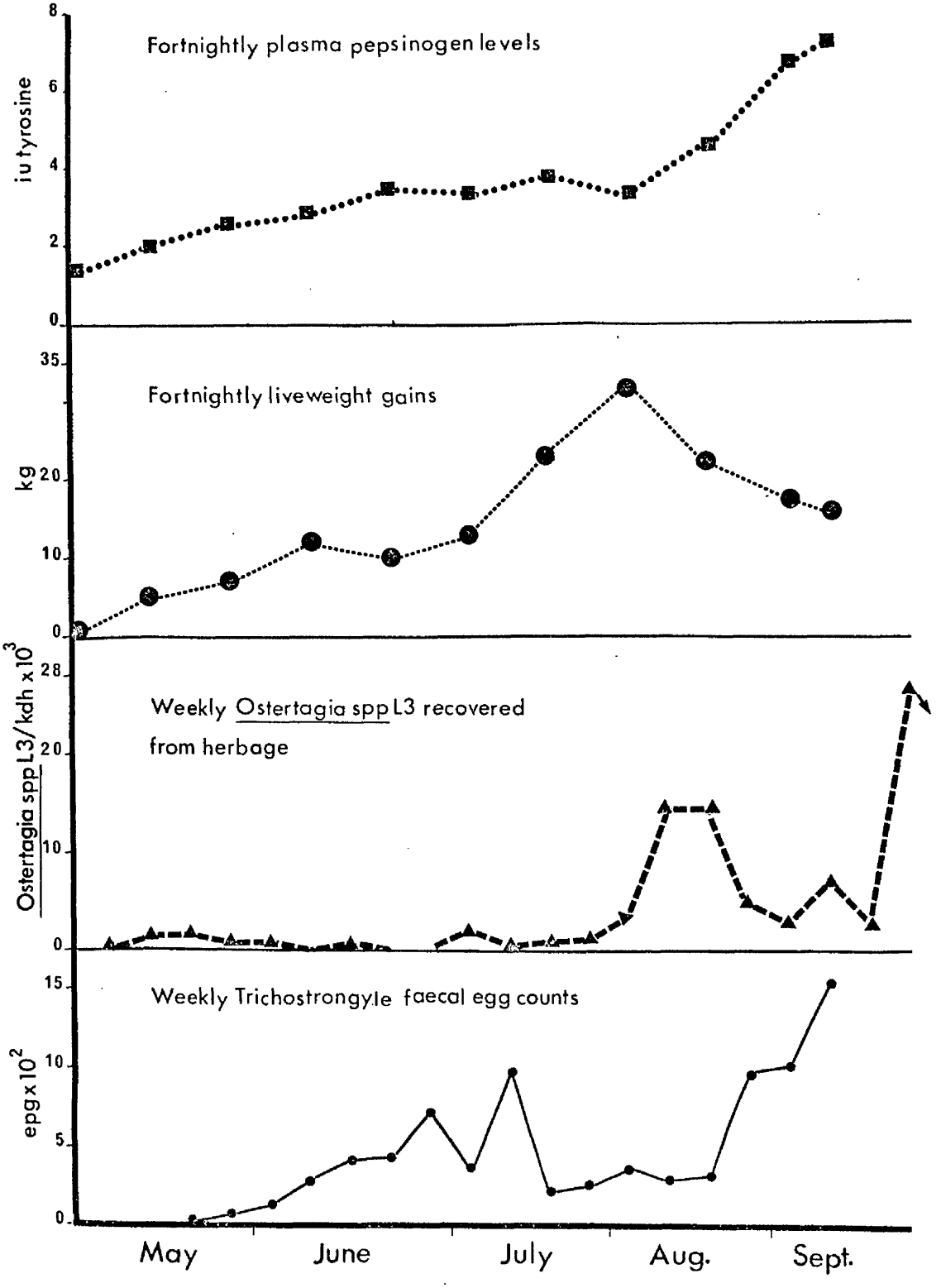


Figure 8. Mean fortnightly plasma pepsinogen levels, liveweight gains and weekly herbage Ostertagia L_3 levels plus faecal egg counts from the 1981 experiment.

and by mid-September the mean liveweight level had decreased by 16 kg.

Climatic data

The climatic records are given in Fig.9. Temperatures were within the normal range for the West of Scotland and the rainfall pattern was generally similar until July; throughout this month and during August the rainfall was much lower than in the previous year.

Parasitological findings

Faecal egg counts: positive faecal counts from individual permanent calves were first detected on 27th May i.e. after 4 weeks grazing and ranged from 0 to 150 epg. In mid-July they increased to a mean of 990 epg only to decline again in mid-August to a mean of 250 epg. In September a further increase occurred reaching a mean maximum of 1550 epg. Fig. 8 illustrates the pattern of faecal egg output, pasture samples, plasma pepsinogen and body weight gains, and individual results are given in Appendices L,M,N and O respectively.

Herbage larval counts

At the commencement of grazing on May 1st, Ostertagia spp. L_3 could not be detected on the pasture. However, larvae were observed during the remainder of the May samplings; a maximum of 1700 L_3 being recorded on the 18th. During June L_3 were detected on only two occasions. Thereafter they were consistently present although the number fluctuated; peaks of 13200 in mid-August and 27000 on 21st September were observed (Fig.8).

Biochemical data

As expected the pH values at post-mortem were highest in the calves harbouring the greatest worm burdens and ranged in the permanent calves from 3.9 to 7.1 and from 3.2 to 7.0 in the tracer calves.

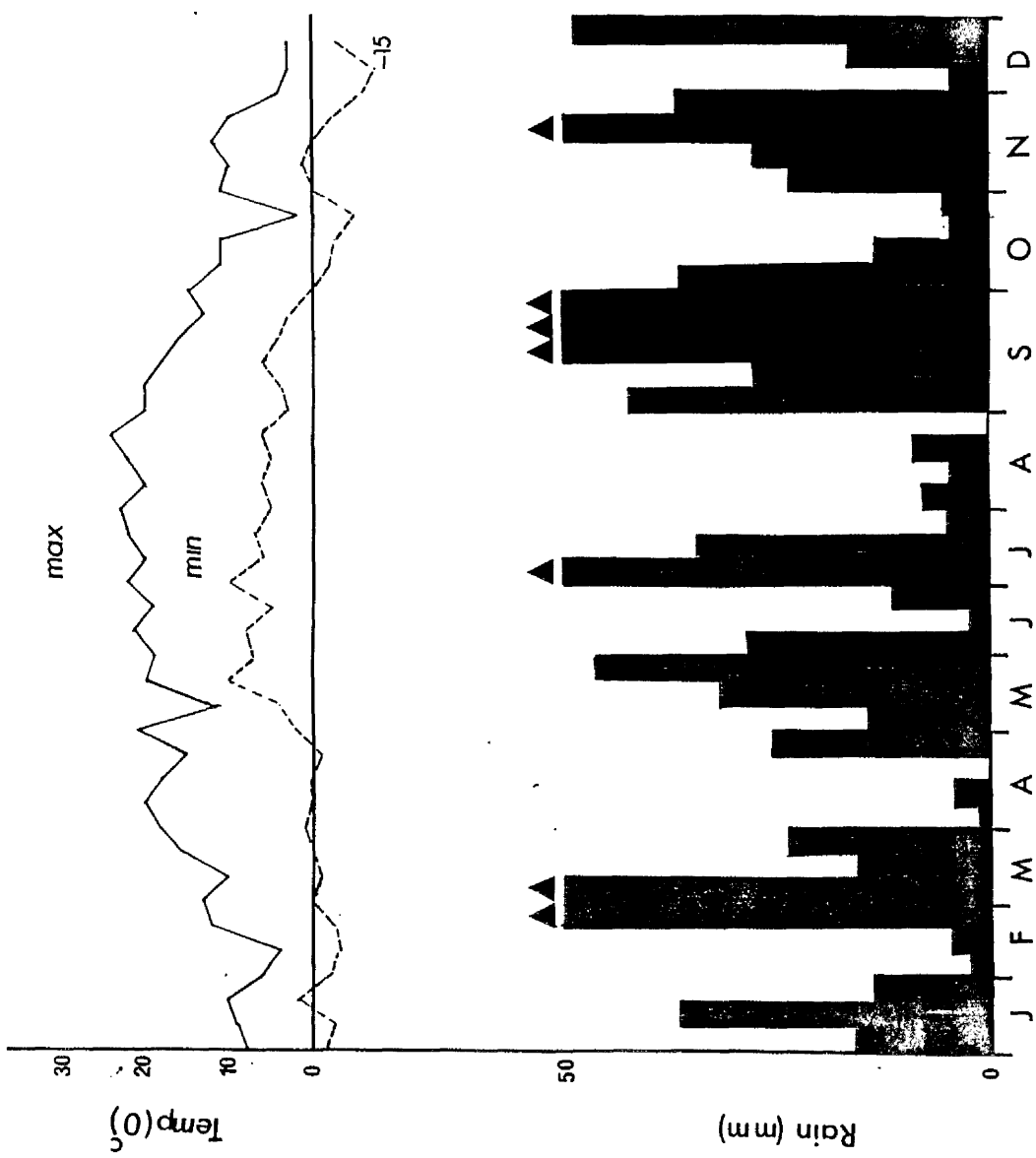


Figure 9. Climatic data 1981

Similarly the pepsinogen values were also elevated to the greatest extent in the calves with most worms. At post-mortem, pepsinogen levels in the permanent calves ranged from 3.0 to 9.7 i.u. Biochemical and parasitological findings are shown in Table 13.

Worm burdens

Parasites recovered from individual permanent and tracer animals are tabulated in Tables 14 and 15 respectively. The Ostertagia spp. worm burdens in the set-stocked permanents ranged from 13,150 to 231,800; the lowest figure being recorded from the animal slaughtered in the middle of the grazing period (i.e. 16th July).

Of the Ostertagia spp. present, the highest percentage were O. ostertagi (range 70.7 - 92.1%) followed by O. leptospicularis (range 4.4 - 26.0%) and S. lyrata 2.0 - 5.7%.

Most of the parasites present were mature stages; relatively low numbers of fourth stage larvae being recorded (50 - 25600).

Tracer worm burdens ranged from 1450 to a maximum of 268800 the highest recoveries being observed in the calf slaughtered at the beginning of September (No. 38 T3). Species distribution was generally similar to that observed in the permanent calves in that the percentage range of O. ostertagi present was 64.9 - 94%, that of O. leptospicularis being 2.6 - 14.8% while S. lyrata was present in slightly higher proportions (range 3.0 - 14.5%). In addition, another species, similar to O. antipini (Plate 27) was found in the two tracer calves slaughtered during October.

Figure 10 illustrates the pattern of worm recoveries from these animals.

Pathology

The increasingly severe pathological changes observed in the material taken from early, mid and late season tracer calves (Plates 28, 29 and 30) reflected the upward trend in the pasture

Table 14. Post-mortem worm burdens of permanent calves infected with Ostertagia spp.

Calf No.	Total worm burden	Individual spp. Adults (% total adult population) O. lepto.	O.oster.	S.lyr.	EL_4	L_4
14*	13150	764(5.8)	12109(92.1)	277(2.1)	0	0
4	105600	27107(26.0)	73836(70.7)	3507(3.3)	50	1100
52	72400	8014(11.3)	61352(86.7)	1434(2.0)	0	1600
53	231800	20465 (9.9)	178393(86.5)	7342(3.6)	0	25600
57	205700	8678 (4.4)	177652(89.9)	11270(5.7)	0	8100

* Animal slaughtered early due to severe clinical disease.

Table 15. Post-mortem worm burdens of tracer calves infected with Ostertagia spp.

Calf No.	Total worm burden	Individual spp. Adults (% total adult population) O.lepto	O.oster.	S.lyr.	Other Oster.*	EL_4	L_4
42 T1	9000	614(6.8)	8056(89.5)	330(3.7)	0	0	0
39 T2	1450	88(6.0)	1318(91.0)	44(3.0)	0	0	0
38 T3	268800	6818(2.6)	246776(94.0)	8906(3.4)	0	0	6300
37 T4	171500	11928(7.0)	151860(88.5)	7712(4.5)	0	0	0
522 T5	58150	8180(14.8)	42346(76.4)	2910(5.2)	2014 (3.6)	0	2700
526 T6	26600	1951(9.0)	14025(64.9)	3111(14.5)	2513(11.6)	0	5000

*Male spicule similar to that of the deer parasite O. antipini.

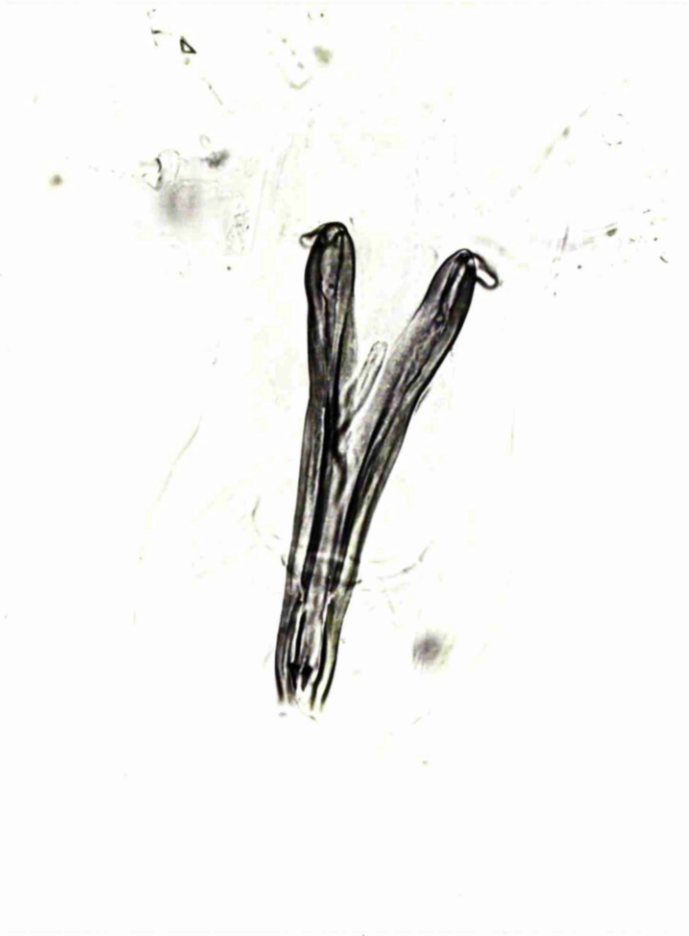


Plate 27. Ostertagia spp. spicules similar to O. antipini x 150.

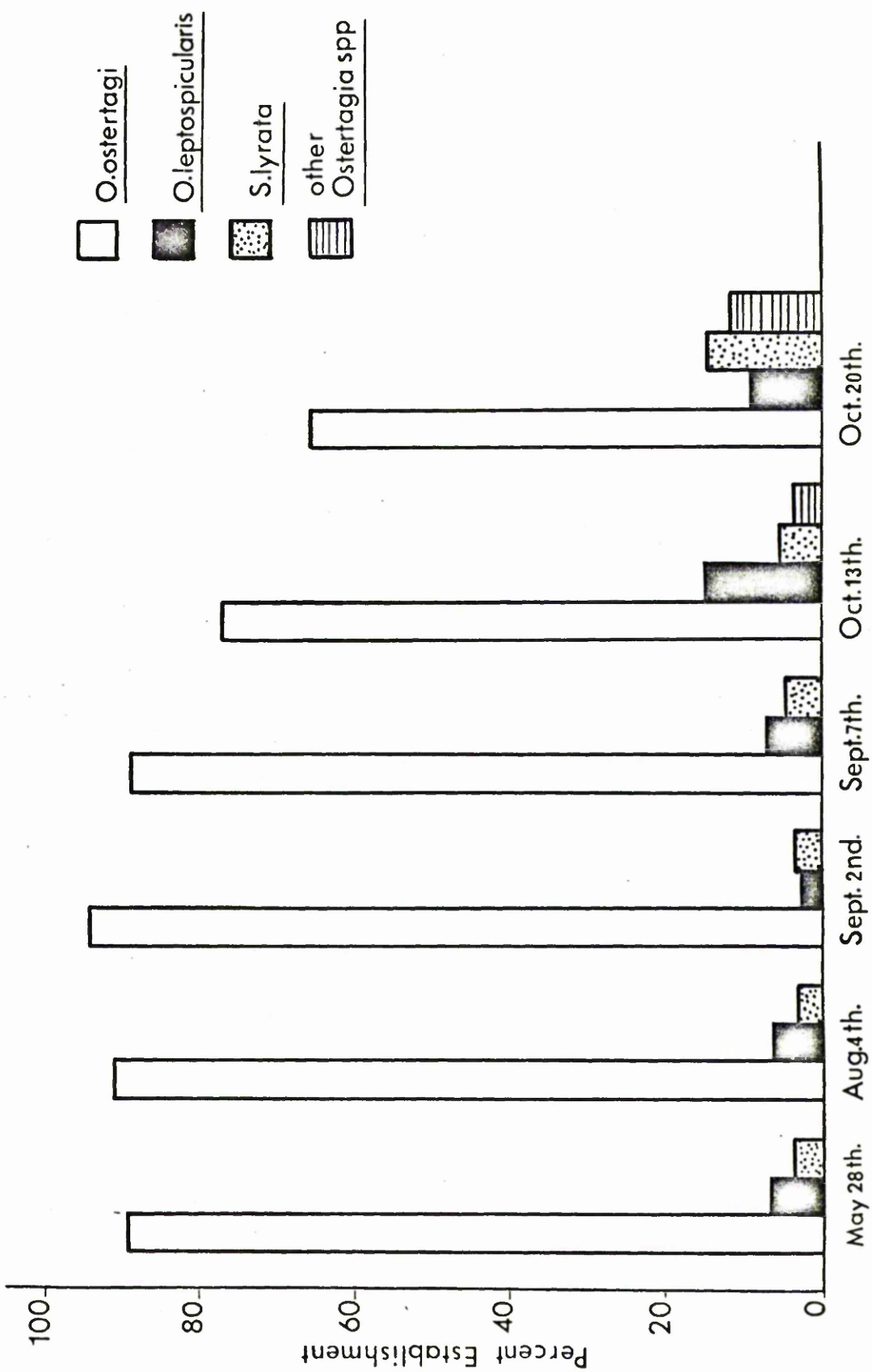


Figure 10. Percentage establishment of different Ostertagia spp in tracer calves.

Plate 28. Abomasum of tracer calf grazed in May 1981.

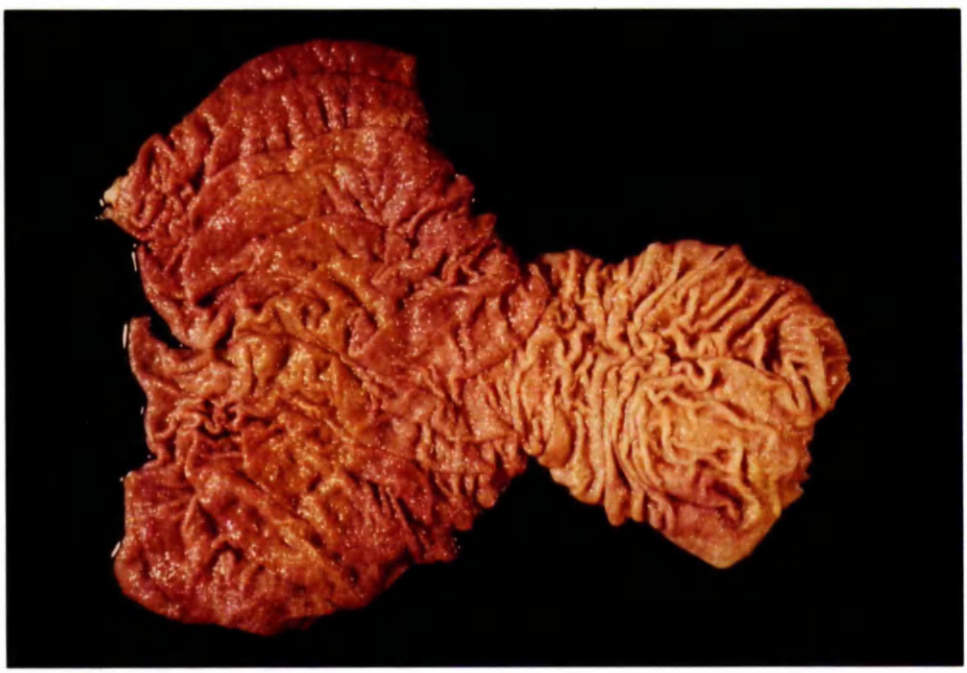


Plate 29. Abomasum of tracer calf grazed in August 1981.

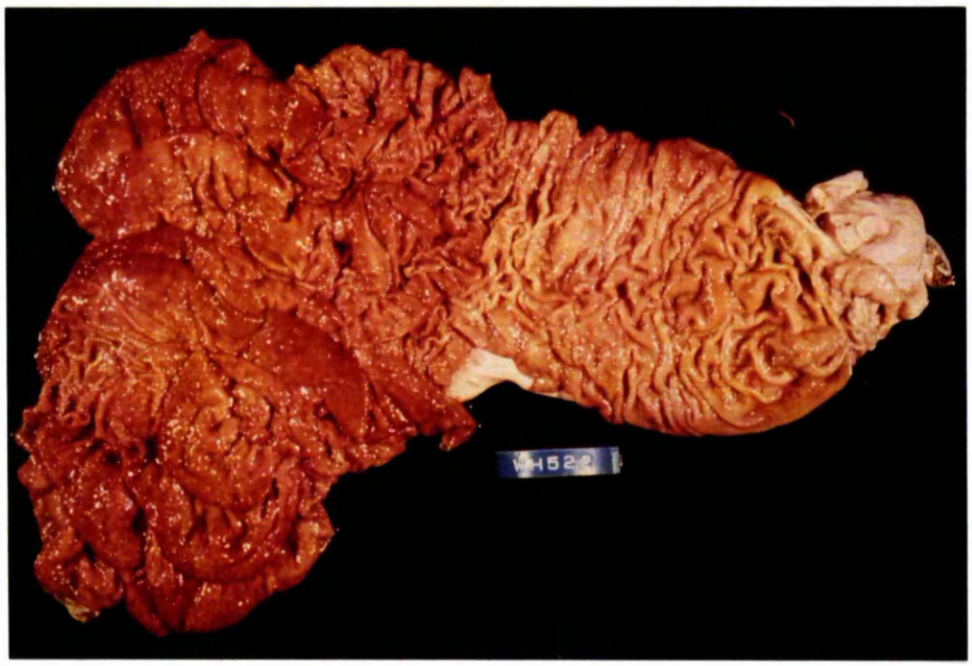


Plate 30. Abomasum of tracer calf grazed in October 1981.

larval counts while at slaughter the lesions observed in the permanent animals were very similar.

DISCUSSION

A fundamental difference between this study and that of 1980 was the lower stocking rate used, due to the incorporation of an adjacent paddock which was free of Ostertagia L_3. This factor must obviously be taken into account when interpreting the findings.

Thus the pasture levels of Ostertagia spp. L_3 were much lower than those observed during the previous study in 1980, and while this was most probably a reflection of the dilution effect of the "clean" part of the extended grazing area, larval translation, so dependent on moisture, may also have been limited by the dry conditions in August.

However, despite the climatic conditions being less suitable for larval migration and the pasture number of L_3 being lower, the number of Ostertagia spp. established in both the permanent and tracer calves (Tables 14 and 15) in August and September were very high, thus confirming the extremely high infectivity of the GC strain of Ostertagia spp. containing both O. ostertagi and O. leptospicularis.

On examination of the tracer worm burdens there were seasonal trends in the distribution of different Ostertagia spp.; thus O. leptospicularis accounted for 9.0 - 14.8% of the total population in the late autumn tracers (October), whereas only 2.6 to 7.0% were found in spring through early summer (May - September), suggesting that O. leptospicularis develops preferentially in the latter half of the summer as has recently been observed in The Netherlands (Eysker and Jansen, 1982) where it was found that the parasite is able to build up from low pasture infectivity levels in spring to fairly high populations in September.

The same trend occurred in the worm burdens of the permanent,

thus the calf which had to be slaughtered in July had only 5.8% O. leptospicularis where those slaughtered in September had 4.4, 9.9, 11.3 and 26.0% O. leptospicularis. Since the two calves 4.4 and 9.9% also had the highest total worm burden and most severe diarrhoea (Nos. 53 and 57) these figures may be an underestimate since many worms could have been lost during the diarrhoeic phase.

The presence at the end of the grazing period, of low numbers of another Ostertagia spp., similar to the deer parasite O. antipini is interesting. Its role in the epidemiology of bovine ostertagiasis is however, obscure and may merit further investigation.

It is interesting that the percentage of arrested larvae present in the permanent calves and those tracers sacrificed in September and October were rather lower than might be expected from previous studies (Anderson et al, 1965a,b). Whether this is simply a reflection of the weather pattern in 1981 or indicates some other interaction between Ostertagia species is not known but the former seems the most likely reason.

Finally, when the availability of larvae on pasture was gauged by the use of both herbage larval counting techniques and tracer calves, the overall pattern of contamination and infection obtained by both methods was similar and this is clearly shown when larval populations are plotted against a histogram of the actual worm burdens (Fig.11).

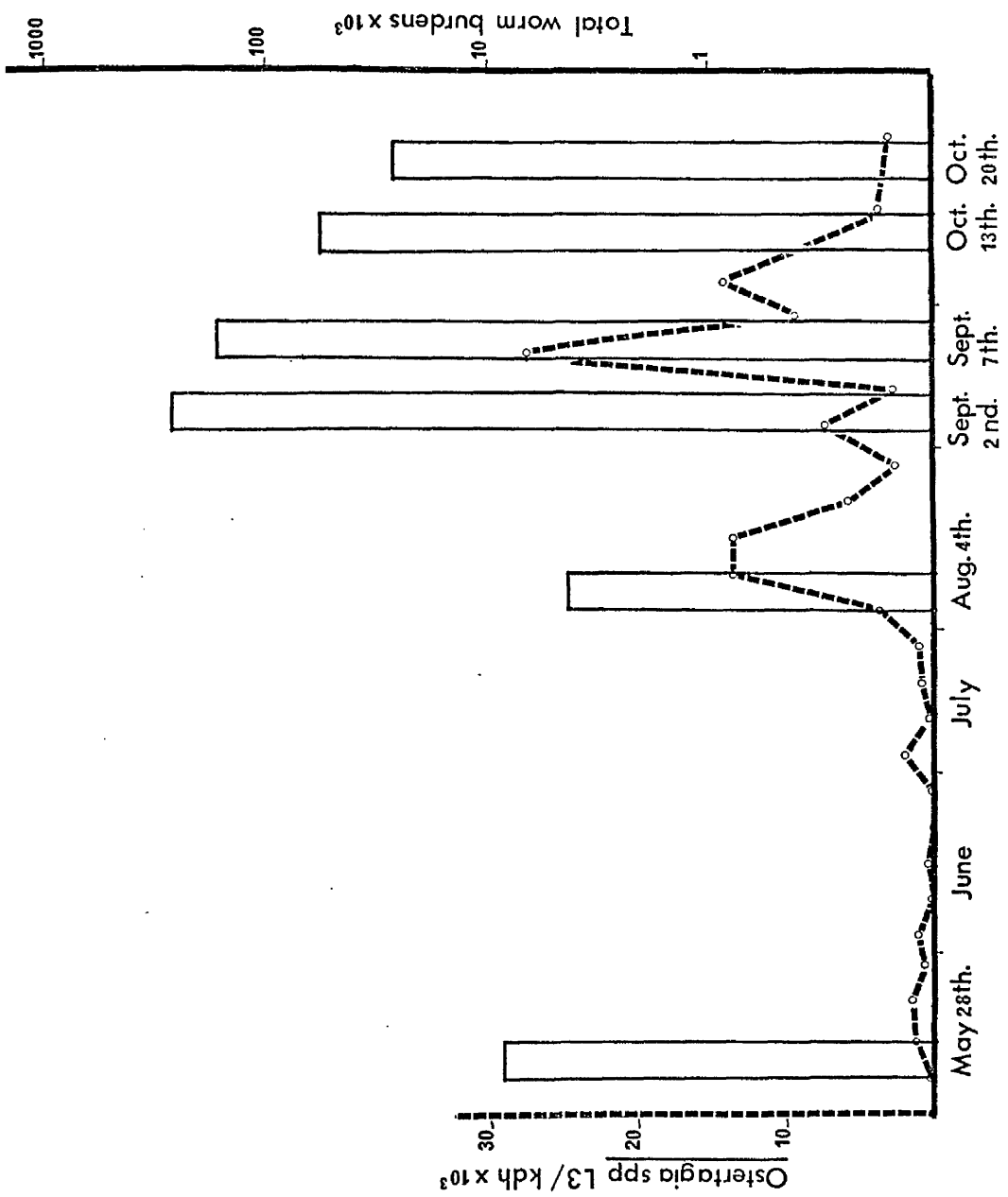


Figure 11. Histogram of tracer calf worm burdens with superimposed graph showing levels of Ostertagia L_3 on pasture.

GENERAL DISCUSSION

GENERAL DISCUSSION

The two fundamental objectives of this thesis were, first to investigate the existence or otherwise of significant populations of bovine Ostertagia spp. infective larval migration in the soil and second, to probe the possible presence of strains of bovine Ostertagia spp. with an especially high infectivity and/or pathogenicity.

The results of the studies in Chapter 3 clearly demonstrate that significant numbers of Ostertagia larvae are present in the soil to a depth of at least 15 cm. for 12 months and possibly up to 20 months. Some fluctuations in the larval numbers present in the herbage and soil layers occurred during the 12 month period which cannot be completely explained but may be related to a vertical migration movement of larvae from beneath the lower soil layers sampled, possibly aided by migrating earthworm or terrestrial beetle populations. It may be that the variation in larval numbers as measured by the recovery technique employed could also be related to the physical state of the larvae. A prolonged period of drought could so dehydrate the larvae that they are unable to physically respond to the stimuli used in their recovery, yet they were able to recover motility and so respond after a period of persistent heavy rain and provided rehydration. These areas require further study.

The results in Chapters 4 and 5 show that isolates of Ostertagia spp. also occur which possess a high infectivity which in some instances can result in a more severe pathology. Such isolates also appear to produce a higher egg production by the worms established and in the field very high pasture counts of infective larvae. However, in the isolates studied any enhanced infectivity was related to the presence of O. leptospicularis in combination with O. ostertagi (and some S. lyrata) rather than to a very highly infective strain

of O. ostertagi. O. leptospicularis is a parasite of deer but also occurs on sheep and cattle usually in low numbers and the process whereby it came to form a significant part of the bovine isolate investigated is not known. One may speculate that it could have occurred following mixed grazing of sheep and cattle, the presence of deer in the vicinity of the grazing or it may reflect either the inherent fecundity of O. leptospicularis or selection by anthelmintics which are more effective against this species than O. ostertagi. These areas also require further investigation.

The practical implication of these findings should be considered in relation to the recommendations for the control of bovine ostertagiasis in Britain. The measures advocated in the most recent publication in this subject (Agricultural Development and Advisory Service i.e. ADAS Publication No. 2154, 1981) are based primarily on the studies of Michel (1969a). Working in Southern England he showed that the vast majority of bovine Ostertagia spp. L_3 have a maximum life span of approximately 9 months and that pasture contaminated in one grazing season and ungrazed thereafter was virtually clean of OL_3, by the following spring and could be labelled "clean" grazing. The results of the soil studies described in Chapter 3 show that in the grazing areas under experiment and in the climate present in West Scotland, these measures could have serious consequences. Since clinical problems of parasitic bronchitis and ostertagiasis have already been described (Duncan et al, 1979; Bairden et al, 1979) in cattle on so called "clean" grazing, these recommendations may have to be revised or at least include a cautionary note.

In the same publication i.e. ADAS 2154 an alternative method of producing "clean" pastures is to alternate the grazing of sheep and cattle on an annual basis based on the premise that in most parts of Britain the species of the important gastro-intestinal

nematodes of cattle do not readily establish in sheep and vice-versa. The authors of the booklet have rather ignored possible cross-infection with Trichostrongylus axei and dismiss Haemonchus contortus another nematode common to cattle and sheep as being of only local importance. The result of experiments described in Chapters 4 and 5 clearly illustrate that O. leptospicularis must also be seriously considered as a pathogen, at least in cattle, and that its presence appears to enhance the infectivity of O. ostertagi. Since Bissett (1980b) has reported similar findings with O. leptospicularis and O. circumcincta in sheep, the presence of O. leptospicularis within ruminant livestock in which parasitism is controlled by an alternate host grazing system constitutes a major hazard to its successful functioning.

Other interesting findings in the work described in this thesis were the rather low proportion of arrested larvae in the calves involved in the field experiment; the severe fibrosis in the lamina propria adjacent to gastric glands occupied by O. leptospicularis and the marked response in the draining lymph nodes; the occurrance of O. antipini in cattle, and the influence of stocking rate on the level of Ostertagia infective larvae which accumulate on pasture.

The relatively low proportion of EL_4 stages was surprising since some of the calves were not sacrificed until October, by which month the number of arrested larval stages usually exceeded 80% of the total Ostertagia population in calves in West Scotland (Anderson et al, 1965a). It may be that the weather patterns in 1980 and 1981 were not conducive to the stimuli which trigger arrested development to occur in Ostertagia larvae ingested during autumn or perhaps the isolate studied had a low propensity to arrest. It is interesting that the recent work of Smeal and Donald (1981) in Australia has shown that a considerable variation exists in

the inherent ability of different "strains" of O. ostertagi to become arrested. The possible effect of concomitant infection with O. leptospicularis in the host immune system and the interaction of this with the induction of arrested development must also be considered.

The severe and extensive fibrosis of the lamina propria of the gastric mucosa was quite unexpected and in contrast to anything previously seen in O. ostertagi. It may be a response to the deposition of eggs within the mucosa and presumably in heavy infections could interefere with the restoration of normal abomasal gastric function. The lymph node response was also interesting and the theory that O. leptospicularis could be more immunogenic requires further study particularly as a possible method of immunisation using attenuated larvae.

O. antipini is a parasite of deer and its presence in tracer calves in 1981 is most interesting and perhaps adds support to the theory that O. leptospicularis originated from casual deer grazing on the cattle pasture where the GC strain originated. It does not of course explain the increased presence of the latter parasite in the calves.

Some authors, namely Michel (1969a) and Southcott (1979) have suggested that the stocking rate of calves on permanent pasture has little influence on the level of trichostrongyle larvae which accumulates on pasture. The validity of this suggestion has been questioned by Armour (1980) and also shown to be incorrect by Nansen et al (1978). The results of the field studies in the present thesis confirm the view of Armour and Nansen and his co-workers in that the pasture levels of Ostertagia L_3 were consistently lower where the stocking rate was low as in Section 2 of Chapter 5 when compared to the level of pasture larvae attained in Section 1,

Chapter 5 when the stocking rates were higher.

Although these extra observations provide interesting matter for discussion, their importance is minimal compared to the main findings of the work discussed in the thesis i.e. that a reservoir of bovine Ostertagia L_3 can occur in soil and that an Ostertagia spp. namely O. leptospicularis which is common to deer, sheep and cattle can select out and accumulate in cattle. Furthermore, the presence of O. leptospicularis seems to promote a higher establishment of other Ostertagia spp. worms and more severe pathological changes.

GENERAL SUMMARY

SUMMARY

This thesis is divided into 5 Chapters. In the first, following a general introduction, there is a comprehensive review of the literature pertaining to the pathogenesis, epidemiology, treatment and control of bovine ostertagiasis. Chapter 2 described the materials and methods. In Chapter 3, for the first time a sequential study is made on the population of bovine Ostertagia spp. infective larvae in the soil. Herbage and stratified soil samples from a previously grazed calf pasture were collected regularly over a 12 month period in 1979 and 1980 and infective larvae were present on each sampling occasion to a depth of at least 15 cm. Analysis of the vertical distribution of the larvae suggested a possible downward migration in the winter followed by an upward trend in the spring and summer. This interpretation was based on a statistical anlysis of both the actual larval numbers present and the positivity of each sample collected. Most important was the finding that the numbers of Ostertagia L_3 present in August 1980 at the interface between soil and pasture was estimated at 6.4×10^6 per hectare as similar figures to that estimated for the previous November (6.6×10^6) despite the absence of contamination during this period. The infectivity of these larvae was confirmed by inoculation to calves. Using parasite-naive tracer calves it was also shown that infection persisted for at least another 8 months and were capable of establishing in calves.

In Chapter 4 experimental infections were carried out in calves with three different isolates of bovine Ostertagia spp. The most highly infective and pathogenic contained approximately 30 per cent of O. leptospicularis in addition to O. ostertagi (70 per cent) and a negligible number of S. lyrata. The other isolates consisted solely of O. ostertagi (approximately 90 per cent) and

S. lyrata (approximately 10 per cent) and these showed comparable infectivity and pathogenicity to that commonly recorded. The greater pathogenicity of the isolate containing O. leptospicularis was attributed to its higher infectivity and the greater damage caused to the gastric mucosa by individual parasites when compared with O. ostertagi.

The role of O.leptospicularis in enhancing the infectivity of Ostertagia spp. isolates was confirmed by administration of experimental infections of O. ostertagi or O. leptospicularis or a mixed isolate of equal mixture of both these species. The percentage of worms established using the mixed inoculum was approximately double that of the O. ostertagi isolate and two thirds greater than that of pure O. leptospicularis. This is the first time that an enhanced establishment has been recorded when two parasitic nematode species from the same genus have been administered.

In Chapter 5 the epidemiological features of the three isolates used in the experimental infections in Chapter 4 were studied under field conditions. Two of the isolates, which contained namely O. ostertagi and a low proportion of S. lyrata behaved in the conventional epdiemiological way although the worm burdens established did not give rise to the expected clinical signs. The third isolate which also contained O. leptospicularis behaved in a different way, yielding very high faecal egg counts which in turn were followed by very high pasture larval counts, heavy worm burdens and clinical disease, thus confirming the interaction between the different species noted in Chapter 4 using experimental infections.

Finally, the implications which the findings have for the control of ostertagiasis are discussed. Most recommended strategies are based on the premise that pastures are clear of Ostertagia

infective larvae after resting for 9-12 months. The results of the study on the soil and herbage populations of infective larvae (Chapter 3) clearly shows that in the West of Scotland this is not so and that it would be dangerous to base control measures solely on this assumption.

An optional strategy to resting pasture for the control of ostertagiasis is to alternate the grazing of sheep and cattle. The finding that O. leptospicularis, which is usually present in low numbers in cattle and sheep, can establish in large numbers in cattle and can also enhance the infectivity and pathogenicity of concomitant O. ostertagi infections could severely compromise such control strategies.

REFERENCES

Ackert, J.E. and Muldoon, W.E. (1920). Strongylosis (Ostertagia) in cattle. J.Am.vet.med.Ass., 58, 138-146.

Agricultural Development and Advisory Service (1981). Control of worm in grazing ruminants. ADAS Publication No. 2154, U.K..

Andersen, F.L., Wang, G.T. and Levine, N.D. (1966). Effects of temperature on survival of the free-living stages of Trichostrongylus colubriformis. J.Parasitol. 52, 713-721.

Anderson, N. (1971). Ostertagiasis in beef cattle. Vict. Vet.Proc., 30, 36-38.

Anderson, N. (1977). The efficiency of levamisole, thiabendazole and fenbendazole against naturally acquired infections of Ostertagia ostertagi in cattle. Res.Vet.Sci., 23, 298-302.

Anderson, N., Armour, J., Jennings, F.W., Ritchie, J.S.D. and Urquhart, G.M. (1965a). Inhibited development of Ostertagia ostertagi. Vet.Rec., 77, 146-147.

Anderson, N., Armour, J., Jarrett, W.F.H., Jennings, F.W., Ritchie, J.S.D. and Urquhart, G.M. (1965b) A field study of parasitic gastritis in cattle. Vet.Rec., 77, 1196-1204.

Anderson, N., Armour, J., Eadie, R.M., Jarrett, W.F.H., Jennings, F.W., Ritchie, J.S.D. and Urquhart, G.M. (1966). Experimental Ostertagia ostertagi infections in calves: results of single infections with five graded dose levels of larvae. Am.J.vet.Res., 27, 1259-1265.

Anderson, N., Armour, J., Jennings, F.W., Ritchie, J.S.D. and Urquhart, G.M. (1969). The sequential development of naturally occurring ostertagiasis in calves. Res.Vet.Sci., 10, 18-28.

Anderson, N. and Lord, V. (1979). Anthelmintic efficiency of oxfendazole, fenbendazole and levamisole against naturally acquired infections of Ostertagia ostertagi and Trichostrongylus axei in cattle. Aust.Vet.J., 55, 158-162.

Armour, J. (1967). Field and experimental studies on Ostertagi ostertagi infections in the bovine. Ph.D. Thesis, University of Glasgow, 226 pp.

Armour, J. (1970). Bovine ostertagiasis: a review. Vet. Rec., 86, 184-190.

Armour, J. (1974). Parasitic gastroenteritis in cattle. Vet.Rec., 95, 391-395.

Armour, J. (1978a). Intensive grassland use and livestock health. Proceedings of Joint British Grassland Society and British Veterinary Association Conference, 16th-17th February, 1978, pp. 81-88.

Armour, J. (1978b). Facts and Reflections III. Arrested development of nematodes in sheep and cattle. Editors Borgsteede, F.H.M., Armour, J. and Jansen, J. Central Veterinary Institute, Lelystad, The Netherlands pp. 77-88.

Armour, J. (1980). The epidemiology of helminth disease in farm animals. Vet.Parasitol., 6, 7-46.

Armour, J., Jarrett, W.F.H. and Jennings, F.W. (1966). Experimental Ostertagia circumcincta infections in sheep, development and pathogenesis of a single infection. Am.J.Vet.Res., 27, 1267-1278.

Armour, J., Jennings, F.W. and Urquhart (1967a). The possible existence of two strains of Ostertagia ostertagi. Vet.Rec., 80, 208-209.

Armour, J., Jennings, F.W., Kirkpatrick, K.S., Malczewski, A., Murray, M. and Urquhart, G.M. (1967c). The use of thiabendazole in bovine ostertagiasis. Treatment of experimental Type I disease. Vet.Rec., 80, 510-514.

Armour, J., Jennings, F.W. and Urquhart, G.M. (1969a). Inhibition of Ostertagia ostertagi at the early fourth larval stage. II. The influence of environment on host or parasite. Res.Vet.Sci., 10, 238-244.

Armour, J. and Bruce, R.G. (1974). Inhibited development in Ostertagia ostertagi infections - a diapause phenomenon in a nematode. Parasitol., 69, 161-174.

Armour, J., Jennings, F.W., Reid, J.F.S. and Selman, I.E. (1975). Treatment of bovine ostertagiasis. Vet. Rec., 97, 188.

Armour, J., Duncan, J.L. and Reid, J.F.S. (1978). Activity of oxfendazole against inhibited larvae of Ostertagia ostertagi and Cooperia oncophora. Vet.Rec., 102, 263-264.

Armour, J., Bairden, K., Duncan, J.L., Jennings, F.W. and Parkins, J.J. (1979). Observations on ostertagiasis in young cattle over two grazing seasons with special reference to plasma pepsinogen levels. Vet.Rec., 105, 500-503.

Armour, J., Alsaqur, I.M., Bairden, K., Duncan, J.L. and Urquhart, G.M. (1980a). Parasitic bronchitis and ostergasiasis on aftermath grazing. Vet.Rec., 106, 184-185.

Armour, J., Bairden, K. and Preston, J.M. (1980b). Anthelminthic efficacy of ivermectin against naturally acquired bovine gastro-intestinal nematodes. Vet.Rec., 107, 226-227.

Arundel, J.H. and Hamilton, D. (1975). The effect of mixed grazing of sheep and cattle on worm burdens in lambs. Aust.Vet.J., 51, 436-440.

Bailey, W.S. and Herlich, H. (1953). An epizootic of parasitic gastritis in mature cattle. Auburn Vet., 9, 105-110.

Bairden, K. (1980). Factors modifying the prevalence of bovine ostertagiasis with special reference to infective larvae in soil. M.Sc. Thesis, University of Glasgow, 149 pp..

Bairden, K., Parkins, J.J. and Armour, J. (1979). Bovine ostertagiasis: a changing epidemiological pattern? Vet.Rec., 105, 33-35.

Bissett, S.A. (1980a). Species involved in ostertagiasis in calves. New Zealand Vet.J., 28, 54.

Bissett, S.A. (1980b). Goats and sheep as hosts for some common cattle Trichostrongylids. Vet.Parasitol., 7, 363-368.

Borgsteede, F.H.M. (1977). The epidemiology of gastro-intestinal helminth infections in young cattle in the Netherlands. Ph.D. Thesis, University of Utrecht.

Borgsteede, F.H.M. (1981). Experimental cross-infections with gastrointestinal nematodes of sheep and cattle. Z. Parasitenkd., 65, 1-10.

Bruford, J.W. and Fincham, I.H. (1945). A phenothiazine trial in calves. Vet.Rec., 57, 421-424.

Brunsdon, R.V. (1972). The potential role of pasture management in the control of Trichostrongyle worm infection in calves with observations on the diagnostic value of plasma pepsinogen determinations. New Zealand Vet.J., 20, 214-220.

Bryan, R.P. (1973). The effects of dung-bettle activity on the numbers of parasitic gastro-intestinal helminth larvae recovered from pasture samples. Aust.J.Agricul.Res., 24, 161-168.

Burg, R.W., Miller, B.M., Baker, E.E., Birnbaum, J., Currie, S.A., Hartman, R., Kong, Y.L., Monaghan, R.L., Olson, G., Putter, I., Tunac, J.B., Wallick, H., Stapley, E.O., Oiwa, R. and Omura, S. (1979). Avermectins, new family of potent anthelmintic agents: Producing organism and fermentation. Antimicrobial Agents and Chemotherapy, 15, 361-367.

Burger, H.J., Eckert, J., Wetzel, H. and Michael, S.A. (1966). Zur epizootologie des Trichostrongyliden-Belfalles des Rindes in Nordwestdeutshchland. Dtsch. Tierartzl.Wochenschr., 73, 503-508.

Burger, H.J., Eckert, J., Chevalier, H.J., Rahman, M.S.A. and Konigsmann, G. (1968). Parasitic gastro-enteritis in cattle. II. Immunization of calves with X-irradiated Ostertagia and cooperia larvae. Vet.med. Nachr., 4, 312-332.

Callinan, A.P.L. (1978). The ecology of the free-living stages of Trichostrongylus axei. Int. J. Parasitol., 8, 453-456.

Callinan, A.P.L.(1979). The ecology of the free-living stages of Trichostrongylus vitrinus. Int. J. Parasitol., 9, 133-136.

Cawley, G.D. and Lewis, C.J. (1975). Some observations on the treatment of ostertagiasis. Vet.Rec., 97, 107-108.

Christie, M.G. (1963). The disintegration of sheep dung and the pre-parasitic stages of trichostrongylids. J.comp.Path., 73, 416-423.

Ciordia, H. and Bizzell, W.E. (1963). The effect of various constant temperatures on the development of the free-living stages of some nematode parasites of cattle. J.Parasitol., 49, 60-63.

Ciordia, H., Ernst, J.V., Stuedemann, J.A., Wilkinson, S.R. and McCampbell, H.C. (1977). Gastrointestinal parasitism of cattle on fescue pastures fertilised with broiler litter. Am.J.Vet.Res., 38, 1335-1339.

Ciordia, H., Stuedemann, J.A., Ernst, J.V., McCampbell, H.C. and Wilkinson, S.R. (1980). Effects of level of nitrogen fertilization of fescue pastures on gastrointestinal parasitism of beef cattle. Am.J. Vet.Res., 41, 893-898.

Clunies Ross, I.C. (1936). The passage of fluids through the ruminant stomach. III. The effects of volume of fluid and the site of stimulation on the reflex closure of the oesophageal groove. With a note on the influence of size of dose on anthelmintic efficiency. Aust.Vet.J., 12, 4-8.

Cobbold, T.S. (1864). Entozoa: an introduction to the study of helminthology with reference, more particularly, to the internal parasites of Man. Groombridge and Sons, London.

Cobbold, T.S. (1873). The internal parasites of our domesticated animals; a manual of the entozoa of the ox, sheep, dig, horse, pig and cat, First Edition. "The Field" Office, London.

Coop, R.L. and Angus, K.W. (1981). How helminths affect sheep. In Practice, 3, 4-11.

Cornwell, R.L., Jones, R.M. and Pott, J.M. (1973b). Autumn treatment with morantel/diethylcarbamazine in yarded cattle. Vet.Rec., 92, 668-671.

Craig, T.M. (1979). Seasonal transmission of bovine gastrointestinal nematodes in the Texas Gulf Coast. J.Am.vet.med.Ass., 174, 844-847.

Craig, T.M. (1980). Epidemiologic aspects of transmission of ruminant helminths in Texas. Proceed. 25th Meeting of the Am.Ass.Vet.Parasitologists, pp.36.

Craig, T.M. and Bell, R.R. (1978). Evaluation of fenbendazole as an anthelmintic for gastrointestinal nematodes of cattle. Am.J.Vet.Res., 39, 1037-1038.

Crofton, H.D. (1954). The vertical migration of infective larvae of strongyloid nematodes. J.Helminthol., 28, 35-52.

Crofton, H.D. (1958b). Nematode parasite populations in sheep on lowland farms. VI. Sheep behaviour and nematode infections. Parasitol., 48, 251-260.

Crofton, H.D. (1963). Nematode parasite populations in sheep and on pasture. Technical Communication No.35. Commonwealth Agricultural Bureaux, Farnham Royal.

Cummins, L.J. and Callinan, A.P.L. (1979). Effects of levamisole and human chorionic gonadotrophin on ostertagiasis in western Victoria, Australia. Vet. Rec., 104, 77-78.

Dargie, J.D. (1980). The pathophysiological effects of gastro-intestinal and liver parasites in sheep. In: Ruckesbusch, Y.: Thrivend, P.(editors), Digestive Physiology and Metabolism in Ruminants, Lancaster, U.K., MTP Press, 341-363.

Dash, K.M. (1981). Interaction between Oesophagostomum columbianum and Oesophagostomum venulosum in sheep. Int.J.Parasitol., 11, 201-207.

Dinaburg, A.G. (1944). Development and survival under outdoor conditions of eggs and larvae of the common ruminant stomach worm, Haemonchus contortus. J. Agricul.Res., 69, 421-433.

Donald, A.D. (1968). Ecology of the free-living stages of nematode parasites of sheep. Aust.Vet.J., 44, 139-144.

Douvres, F.W. (1956). Morphogenesis of the parasitic stages of Ostertagia ostertagi, a nematode parasite of cattle. J.Parasitol., 42, 626-635.

Downey, N.E. (1978). Action of albendazole on gastrointestinal nematodes in naturally infected calves. Vet.Rec., 103, 427-428.

Downey, N.E. and Fallon, R. (1973). The control of nematode infections in replacement dairy calves. Farm and Food Research, 4, 102-106.

Drudge, J.H., Leland, S.E., Wyant, Z. and Elam, G.W. (1954). Observations on the effectiveness of phenothiazine in the control of gastro-intestinal nematodes of sheep. Report of the Kentucky Agricultural Experimental Station of the University of Kentucky, p.56.

Drudge, J.H., Leland, S.E. and Wyant, Z.N. (1957). Strain variation in the response of sheep nematodes to the action of phenothiazine. II. Studies on pure infections of Haemonchus contortus. Am.J.Vet.Res., 18, 317-325.

Duncan, J.L., Armour, J., Bairden, K., Jennings, F.W. and Urquhart, G.M. (1976). The successful removel of inhibited fourth stage Ostertagia ostertagi larvae by fenbendazole. Vet.Rec., 98, 342.

Duncan, J.L., Armour, J., Bairden, K., Jennings, F.W. and Urquhart, G.M. (1977). The activity of fenbendazole against inhibited 4th stage larvae of Ostertagia ostertagi. Vet.Rec., 101, 249.

Duncan, J.L., Armour, J. and Bairden, K. (1978). Autumn and winter fenbendazole treatment against inhibited 4th stage Ostertagia ostertagi larvae in cattle. Vet. Rec., 103, 211-212.

Duncan, J.L., Armour, J., Bairden, K., Urquhart, G.M. and Jørgensen, R.J. (1979). Studies on the epidemiology of bovine parasitic bronchitis. Vet.Rec., 104, 274-278.

Durie, P.H. (1961). Parasitic gastroenteritis of cattle: the distribution and survival of infective strongyle larvae on pasture. Aust.J.Agricult.Res., 12, 1200-1211.

Durie, P.H. (1962). Parasitic gastro-enteritis of cattle: seasonal fluctuation in populations of strongyle larvae on a calf pasture and their significance in infection of the grazing animal. Aust.J.Agricult.Res., 13, 767-777.

Edwards, K., Jepson, R.P. and Wood, K.F. (1960). Value of plasma pepsinogen estimation. Br.Med.J., 1, 30-32.

Eissenegger, H. and Eckert, J. (1975). The epizootiology and prophylaxis of dictyocauliasis and trichostrongylosis of Swiss cattle. Schweizer Archiv für Tierheilkunde, 117, 255-286.

Ellenby, C. (1968). Desiccation survival of the infective larva of Haemonchus contortus. J.Exp.Biol., 49, 469-475.

Elliot, D.C. (1977). The effect of fenbendazole in removing inhibited early-fourth-stage Ostertagia ostertagi from yearling cattle. New Zealand Vet.J., 25, 145-147.

Eysker, M. and Jansen, J. (1982). Population build up of gastrointestinal nematode infections in ewes and lambs on pasture grazed by calves in the previous year. Res.Vet.Sci., 32, 203-205.

Fincher, G.T. (1973). Dung beetles as biological control agents for gastrointestinal parasites of livestock. J.Parasitol., 59, 399.

Fincher, G.T. (1975). Effects of dung beetle activity on the number of nematode parasites acquired by grazing cattle. J.Parasitol., 61, 759-762.

Fincher, G.T. and Stewart, T.B. (1979). Vertical migration by nematode larvae of cattle parasites through soil. Proceed.Helminth.Soc.Wash., 46, 43-46.

Foster, W.D. (1965). A history of parasitology, First Edition. E. and S. Livingstone, Edinburgh and London.

Furman, D.P. (1944a). Effects of environment on the freeliving stages of Ostertagia circumcincta (Stadelmann) Trichostrongylidae: I. Laboratory experiments. Am.J.Vet.Res., 5, 79-86.

Furman, D.P. (1944b). Effects of environment on the freeliving stages of Ostertagia circumcincta (Stadelmann) Trichostrongylidae: II. Field experiments. Am.J.Vet.Res., 5, 147-153.

Gardener, R.C.B. (1911). Parasitic gastritis in sheep and cattle. J.South-Eastern Agricul.Coll., Wye, Kent, 20, 482-493.

Gibson, T.E. and Everett, G. (1968). A comparison of set stocking and rotational grazing for the control of trichostrongylosis in sheep. Br.vet.J., 124, 287-298.

Gordon, H.McL. (1940). The anthelmintic efficiency of Lentin (Merck) against Oesophagostomum columbianum. J.Council for Scientific and Industrial Research, Australia, 13, 87-90.

Gordon, H.McL. (1956). The influence of particle size on the anthelmintic efficiency of phenothiazine in sheep. Aust.Vet.J., 32, 258-268.

Gordon, H.McL. (1962). Recent advances in anthelmintics for use in sheep. Aust.Vet.J., 38, 170-176.

Gordon, H.McL. (1963). The evaluation of anthelmintics in sheep by field experiments. Proceed. of the first International Conference, World Association for the Advancement of Veterinary Parasitology, pp. 90-104.

Gordon, H.McL. (1967b). Some aspects of the control of helminthosis in sheep. Vet.Inspector, 31, 88-99.

Gordon, H.McL. and Whitlock, H.V. (1939). A new technique for counting nematode eggs in sheep faeces. J.Council for Scientific and Industrial Research, Australia, 12-50.

Gracey, J.P. (1960). Survey of livestock diseases in Northern Ireland. HMSO, Belfast.

Grønvold, J. (1979). On the possible role of earthworms in the transmission of Ostertagia ostertagi third-stage larvae from faeces to soil. J.Parasitol., 65, 831-832.

Halliday, G.J., Dalton, R.G., Anderson, N. and Mulligan, W. (1965). Total body water and total body solids in cattle affected with ostertagiasis. Br.vet.J., 121, 547-551.

Health and Safety at Work Act 1980, Department of Health and Social Security, Gt.Britain.

Heath, D.D., Southcott, W.H. and May, P.F. (1970). The use of sheep fistulated at the oesophagus for the recovery of strongyloid larvae from pasture. Parasitol. 60, 281-289.

Henriksen, S.A., Jørgensen, R.J., Nansen, P., Sejrsen, S.B.L. and Klausen, S. (1975). Gastro-intestinal strongyles in cattle. I. Investigations on the infection in grazing calves. Dan.Vet.Tidestir., 58, 441-444.

Herd, R.P. (1980). Animal health and public health aspects of bovine parasitism. J.Am.vet.med.Ass., 176, 737-743.

Herlich, H. (1959). Experimental infection of cattle with the stomach worms, Ostertagia ostertagi and Trichostrongylus axei. Proceed.Helminth.Soc.Wash., 26, 97-102.

Herlich, H. (1960). Age resistance of cattle to nematodes of the gastro-intestinal tract. J.Parasitol., 46, 392-397.

Hotson, I.K. (1967). Ostertagiosis in cattle. Aust.Vet. J., 43, 383-387.

Inderbitzen F. (1976). Experimentall erzeugte entwicklungshemmung von Dictyocaulus viviparus des Rindes. Thesis. Institut fur Parasitologie der Universitat Zürich (Switzerland).

Jacobs, D.E., Tod, M.E., Dunn, A.M. and Walker, J. (1968). Farm-to-farm transmission of porcine oesophagostomiasis. Vet.Rec., 82, 57.

Jennings, F.W., Armour, J., Lawson, D.D. and Roberts, R. (1966). Experimental Ostertagia ostertagi infections in calves: studies with abomasal cannulas. Am.J. Vet.Res., 27, 1249-1257.

Jørgensen, R.J. (1975a). Isolation of infective Dictyocaulus larvae from herbage. Vet.Parasitol., 1, 61-67.

Jørgensen, R.J. (1981). Studies on the lungworm Dictyocaulus viviparus (Bloch, 1782) and its epidemiology in young cattle. Ph.D. Thesis, Royal Vet. and Agricul. University, Copenhagen, 77pp..

Kates, K.C. and Turner, J.H. (1953). A comparison of the pathogenicity and course of infection of two nematodes of sheep, Nematodirus spathiger and Trichostrongylus colubriformis, in pure and mixed infections. Proceed. Helminth.Soc.Wash., 20, 117-124.

Keith, R.K. (1953). The differentiation of the infective larvae of some common nematode parasites of cattle. Aust.J.Zool., 1, 223-235.

Kelly, J.D., Chevis, R.A.F. and Goodman, H.T. (1975). Effect of particle size on the anthelmintic efficacy of mebendazole against Nippostrongylus brasiliensis in the rat. Int.J.Parasitol., 5, 275-280.

Kelly, J.D., Gordon, H.McL. and Whitlock, H.V. (1976). Anthelmintics for sheep: historical perspectives, classification/usage, problem areas and future prospects. New South Wales Vet.Proceed., 12, 18-31.

Kelly, J.D., Whitlock, H.V., Thompson, H.G., Hall, C.A., Martin, I.C.A. and LeJambre, L.F. (1978). Physiological characteristics of free-living and parasitic stages of Haemonchus contortus susceptible or resistant to benzimidazole anthelmintics. Res. Vet.Sci., 25, 376-385.

Kelly, J.D. and Hall, C.A. (1979a). Resistance of animal helminths to anthelmintics. Adv.Pharmacol.Chemother., 16, 89-128.

Kelly, J.D. and Hall, C.A. (1979b). Anthelmintic resistance in nematodes. I. History, present status in Australia, genetic background and methods for field diagnosis. New South Wales Vet.Proceed., 15, 19-31.

Kingsbury, P.A. (1958). Effect of particle size on the anthelmintic properties of phenothiazine in sheep. Vet.Rec., 70, 523-528.

Kistner, T.P., Wyse, D. and Averkin, E. (1979). Efficacy of oxfendazole against inhibited Ostertagia ostertagi larvae in naturally infected cattle. Aust.Vet.J., 55, 232-235.

Lancaster, M.B. and Hong, C. (1971). The nematode fauna of the Chinllingham wild white cattle. Br.vet.J., 127, 113-117.

Lancaster, M.B. and Hong, C. (1977). Action of fenbendazole on arrested fourth stage larvae of Ostertagia ostertagi. Vet.Rec., 101, 81-82.

Lancaster, M.B., Hong, C. and Michel, J.F. (1981). Further observations on the action of fenbendazole against inhibited fourth stage larvae of Ostertagia ostertagi. Vet.Rec., 108, 473-475.

Leaver, J.D. (1970). A comparison of grazing systems for dairy herd replacements. J.Agricul.Sci., Cambridge, 75, 265-272.

LeJambre, L.F., Southcott, W.H. and Dash, K.M. (1978). Development of simultaneous resistance in Ostertagia circumcincta to thiabendazole, morantel tartrate and levamisole. Int.J.Parasitol., 8, 443-447.

Levine, N.D. (1978). The influence of weather on the bionomics of the free-living stages of nematodes. In: Weather and Parasitic Animal Disease (Ed. T.E. Gibson). Technical Note No. 159, World Meteorological Organisation.

Levine, N.D. and Clark, D.T. (1961). The relation of weekly pasture rotation to acquisition of gastrointestinal nematodes of sheep. Illno.Vet., 4, 80-97.

Levine, N.D. and Todd, K.S. (1975). Micrometeorological factors involved in development and survival of free-living stages of the sheep nematodes Haemonchus contortus and Trichostrongylus colubriformis. A review. Int.J.Biometrol., 19, 174-183.

Malezewski, A. (1970). Gastro-intestinal helminths of ruminants in Poland. III. Seasonal incidence of the stomach worms in calves, with consideration of the effect of the inhibition phenomenon on the spring rise phenomenon. Acta Parasitologica Polonica, 18, 417-434.

Malezewski, A., Westcott, R.B., Spratling, B.M. and Gorham, J.R. (1975). Internal Parasites of Washington cattle. Am.J.Vet.Res., 36, 1671-1675.

Martin, W.B., Thomas, B.A.C. and Urquhart, G.M. (1957). Chronic diarrhoea in housed cattle due to atypical parasitic gastritis. Vet.Rec., 69, 736-739.

Michel, J.F. (1963). The phenomena of host resistance and the course of infection of Ostertagia ostertagi in calves. Parasitol., 53, 63-84.

Michel, J.F. (1966). The epidemiology and control of parasitic gastroenteritis in calves. Fourth Int. Meeting of the World Ass. Buiatrics, Zurich, pp. 272.

Michel, J.F. (1969a). The epidemiology and control of some nematode infections of grazing animals. In: Advances in Parasitology (Ed. B. Dawes). Academic Press, London and New York, 7, 211-282.

Michel, J.F. (1969b). Some observations on the worm burdens of calves infected daily with Ostertagia ostertagi. Parasitol., 59, 575-595.

Michel, J.F. (1974). Arrested development of nematodes and some related phenomena. In: Advances in Parasitology (Ed. B. Dawes). Academic Press, London and New York, 12, 280-311.

Michel, J.F. (1976). Nematode infections in grazing animals. In: Advances in Parasitology (Ed. B. Dawes). Academic Press, London and New York, 14, 355-387.

Michel, J.F. and Rose, J.H. (1954). Some observations on the free-living stages of the cattle lungworm in relation to their natural environment. J.Comp.Path., 64, 195-205.

Michel, J.F. and Parfitt, J.W. (1956). An experimental study of the epidemiology of parasitic bronchitis in calves. Vet.Rec., 68, 706-710.

Michel, J.F., Lancaster, M.B. and Hong, C. (1972a). The epidemiology of gastro-intestinal nematode infection in the single-suckled calf. Vet.Rec., 91, 301-306.

Michel, J.F., Lancaster, M.B. and Hong, C. (1976a). Observation on the resumed development of arrested Ostertagia ostertagi in naturally infected yearling cattle. J.Comp.Path., 86, 73-80.

Michel, J.F., Lancaster, M.B. and Hong, C. (1976b). The resumed development of arrested Ostertagia ostertagi in experimentally infected calves. J.Comp.Path., 86, 615-619.

Michel, J.F., Lancaster, M.B. and Hong, C. (1979). The effect of age, acquired resistance, pregnancy and lactation on some reactions of cattle to infection with Ostertagia ostertagi. Parasitol., 69, 157-168.

Ministry of Agriculture, Fisheries and Food (1971). Manual of veterinary parasitological technique. Technical Bulletin 18, U.K.

Murray, M., Jennings, F.W. and Armour, J. (1970). Bovine ostertagiasis: structure, function and mode of differentiation of the bovine gastric mucosa and kinetics of the worm loss. Res.Vet.Sci., 11, 417-427.

Nagle, E.J., Brophy, P.O., Caffrey, P.J. and ÔNuallain, T. (1980). Control of ostertagiasis in young cattle under intensive grazing. Vet.Parasitol., 1, 143-152.

Nansen, P. and Jørgensen, M. (1977). Increased problems with gastro-intestinal trichostrongylosis following slurry application in cattle herds. Dansk Veterinaer Tidsskrift, 60, 249-254.

Nansen, P., Jørgensen, R.J., Hansen, J.W. and Sejresen, K.R. (1978). Some factors influencing the exposure of grazing cattle to trichostrongyle infection. Vet.Sci. Commun., 2, 193-205.

Nilsson, O. and Sorelius, L. (1973). Trichostrongyle infections of cattle in Sweden. Nordisk Veterinaer-medicin, 25, 65-78.

Oakley, G.A. (1981). Survival of Dictyocaulus viviparus infection in earthworms. Res.Vet.Sci., 30, 255-256.

Osborne, J.C., Batte, E.G. and Bell, R.R. (1960). The pathology following single infections of Ostertagia ostertagi in calves. Cornell Vet., 50, 223-235.

Parfitt, J.W. (1955). Two techniques used for the detection and enumeration of the larvae of Dictyocaulus viviparus in faeces and herbage. Lab. Prac., 4, 15-16.

Parkins, J.J., Bairden, K. and Armour, J. (1982). Ostertagia ostertagi in calves: growth, nitrogen balance and digestibility studies conducted during winter feeding following different fenbendazole therapy programmes. Res.Vet.Sci., 32, 74-78.

Persson, L. (1974a). Studies on the bionomics of eggs and infective larvae of Ostertagia ostertagi in soil. Zentralblatt fur Veterinarmedizin, B.21, 318-328.

Pirie, H.M., Doyle, J., McIntyre, W.I.M. and Armour, J. (1971). The relationship between pulmonary lymphoid nodules and vaccination against Dictyocaulus viviparus. Proc. IV. Inter.Conf.W.A.A.V.P.Pathology of Parasitic Disease. Ed. S.M. Gaafar, Purdue University Studies.

Porter, D.A. and Cauthen, G.E. (1946). Experimental infection of calves with the small stomach worm, Ostertagia ostertagi. J.Parasitol., 32, Suppl.39.

Pott, J.M., Jones, R.M. and Cornwell, R.L. (1974). Control of bovine parasitic gastroenteritis by reduction of pasture larval levels. Proceed. III Inter.Cong. Parasitol., Munich Proceed., Vol.II, pp. 747-748.

Pouplard, L. (1968). A control programme for bovine dictyocaulosis. Vet.Med.Nachr., 68, 3-18.

Prichard, R.K. (1970). Mode of action of the anthelmintic thiabendazole in Haemonchus contortus. Nature, London, 228, 684.

Prichard, R.K. (1973). The fumarate reductase reaction of Haemonchus contortus and the mode of action of some anthelmintics. Int.J.Parasitol., 3, 409-417.

Prichard, R.K., Donald, A.D., Dash, K.M. and Hennessy, D.R. (1978a). Factors involved in the relative anthelmintic tolerance of arrested 4th stage larvae of Ostertagia ostertagi. Vet.Rec., 102, 382.

Prichard, R.K., Kelly, J.D. and Thompson, H.G. (1978b). The effects of benzimidazole resistance and rate of administration on the uptake of fenbendazole and thiabendazole by Haemonchus contortus and Trichostrongylus colubriformis in sheep. Vet. Parasitol., 4, 243-255.

Randall, R.W. and Gibbs, H.C. (1981). Effects of clinical and subclinical gastrointestinal helminthiasis on digestion and energy metabolism in calves. Am.J. vet.Res., 42, 1730-1734.

Raynaud, J.P., Laudren, G. and Jolivet, G. (1971). Epidemiology of gastrointestinal nematodes of cattle as interpreted by tracer calves. Ann.Rech.Vet., 5, 115-145.

Raynaud, J.P. and Bouchet, A. (1976). Bovine ostertagiasis, a review. Analysis of types and syndromes found in France by post mortem examinations and total worm counts. Ann.Rech.Vet., 7, 253-280.

Reinecke, R.K. (1960). A field study of some nematode parasites of bovines in a semi-arid area, with special reference to their biology and possible methods of prophylaxis. Onderstepoort J.Vet.Res., 28, 365-464.

Ritchie, J.D.S., Anderson, N., Armour, J., Jarrett, W.F.H., Jennings, F.W. and Urquhart, G.M. (1966). Experimental Ostertagia ostertagi infections in calves: parasitology and pathogenesis of a single infection. Am.J.vet.Res., 27, 659-667.

Roberts, F.H.S., O'Sullivan, P.J. and Riek, R.F. (1952). The epidemiology of parasitic gastro-enteritis of cattle. Aust.J.Agricul.Res., 3, 187-226.

Robinson, J. (1962). Pilobolus spp. and the translation of the infective larvae of Dictyocaulus viviparus from faeces to pasture. Nature, London, 193, 353-354.

Rose, J.H. (1956). The bionomics of the free-living larvae of Dictyocaulus viviparus. J.Comp.Path., 66, 228-240.

Rose, J.H. (1961). Some observations on the free-living stages of Ostertagia ostertagi, a stomach worm of cattle. Parasitol., 51, 295-307.

Rose, J.H. (1962). Further observations of the free-living stages of Ostertagia ostertagi in cattle. J.Comp. Path., 72, 11-18.

Rose, J.H. (1963). Ecological observations and laboratory experiments on the free-living stages of Cooperia oncophora. J.Comp.Path., 73, 285-296.

Rose, J.H. (1965). The rested pasture as a source of lungworm and gastro-intestinal worm infection for lambs. Vet.Rec., 77, 749-752.

Rose, J.H. (1966). Investigations into the free-living phase of the life-cycle of Nematodirus helvetianus. Parasitol., 56, 679-691.

Rose, J.H. (1970). Parasitic gastro-interitis in cattle. Factors affecting the time of the increase in the worm population of pastures. Res.Vet.Sci., 11, 199-208.

Ross, J.G. and Dow, C. (1965). Field and experimental infections of calves with the nematode parasite, Ostertagia ostertagi. Br.Vet.J., 121, 18-27.

Ross, J.G., Purcell, A., Todd, J.R. and Dow, C. (1968). Combined infections of calves with the nematodes parasites Trichostrongolus axei and Ostertagia ostertagi. Br.Vet.J., 124, 229-304.

Rutter, W. (1975). Sheep from grass. Bulletin No.13. East of Scotland College of Agriculture.

Selman, I.E., Reid, J.F.S., Armour, J. and Jennings, F.W. (1976). Type II ostertagiasis in adult cattle. Vet.Rec., 99, 141-143.

Silangwa, S.M. and Todd, A.C. (1964). Vertical migration of trichostrongylid larvae on grasses. J.Parasitol., 50, 278-285.

Silverman, P.H. and Campbell, J.A. (1958). Studies on parasitic worms of sheep in Scotland. I. Embryonic and larval development of Haemonchus contortus at constant conditions. Parasitol., 49, 23-37.

Skinner, W.D. and Todd, K.S. (1980). Lateral migration of Haemonchus contortus larvae on pasture. Am.J.vet. Res., 41, 395-398.

Smeal, M.G., Fraser, G.C. and Robinson, G.G. (1980). Seasonal changes in the structure of nematode populations of cattle in New South Wales in relation to inhibited larval development. Aust.Vet.J., 56, 80-86.

Smeal, M.G. and Donald, A.D. (1981). Effects on inhibition of development of the transfer of Ostertagia ostertagi between geographical regions of Australia. Parasitol., 82, 389-399.

Smith, H.J. (1973). Inhibited development of Ostertagia ostertagi, Cooperia oncophora and Nematodirus helvetianus in parasite-free calves grazing fall pastures. Am.J.vet.Res., 35, 935-937.

Smith, H.J. (1974). On the natural seeding of marshland pastures with bovine gastro-intestinal parasites. Can.J.comp.Med., 38, 139-144.

Southcott, W.H. (1979). Management control for nematode parasites. New South Wales Vet.Proceed. 17-18.

Southcott, W.H. and Barger, I.A. (1975). Control of nematode parasites by grazing management. II. Decontamination of sheep and cattle pastures by varying periods of grazing with the alternate host. Int.J.Parasitol., 5, 45-48.

Spedding, C.R.W. (1969). The eradication of parasitic disease. Vet.Rec., 84, 625.

Stadelmann, H. (1891). Ueber den anatomischen Bau des Strongylus convolutus Ostertag, nebst einigen Bemerkungen zu seiner Biologie. Archiv fur Naturgeschichte, 1, 149-176.

Steel, R.G.D. and Torrie, G.A. (1980). Principles and procedures of statistic. A biometrical approach. 2nd. Edition. Published by McGraw Hill, New York.

Stewart, W.L. and Crofton, H.D. (1941). Parasitic gastritis in cattle. Vet.Rec., 53, 619-621.

Stödter, W. (1901). Die Strongyliden in dem Labmagen der gezähmten Wiederkäuer und die Magenwurmseuche. Inaugural dissertation, Hamburg.

Stoodly, K.D.C., Lewis, T. and Stainton (1980). Applied Statistical Techniques. Publication by Ellis Horwood, England.

Sturrock, R.F. (1965). The control of trichostrongyle larvae (Nematoda) by fumigation in relation to their bionomics. I. Bionomics results. Parasitol., 55, 29-44.

Tarshis, I.B. (1958). A preliminary study of lateral migration by infective larvae of some cattle nematodes on experimentally contaminated forage plots. Proceed.Helminth.Soc.Wash., 25, 99-106.

Taylor, E.L. (1930). Some factors concerning the occurrence of disease due to strongyloid worms in grazing animals - verminous gastritis, hoose and equine strongyloidosis. Vet.Rec., 10, 602-606.

Taylor, E.L. (1938). Grazing hygiene with respect to parasitic diseases. Report of the 13th International Veterinary Congress, Zurich-Interlaken, 1, 672-680.

Tharaldsen, J. (1970). Nematodes in young cattle on permanent and new pastures. Nytt magasin for zoologi, 18, 106.

Thomas, P.L. and Elliot, D.C. (1957). The use of fine-particle phenothiazine against Trichostrongylus colubriformis in sheep with observations on its use against other species of worms. New Zealand Vet.J., 5, 66-69.

Threlkeld, W.L. (1946). The life history of Ostertagia ostertagi. Virginia Agricultural Experimental Station, Technical Bulletin No. 100, pp. 1-14.

Tripathi, J.C. (1974). Longevity and migration of infective larvae of some common nematodes of goats in different types of soil. Indian J.An.Sci., 44, 104-108.

Wallace, H.R. (1961). The bionomics of the freeliving stages of zoo-parasitic and phyto-parasitic nematodes - a critical survey. Helminthol.Abstracts, 30, 1-22.

Wedderburn, J.F. (1970). Ostertagiasis in adult cattle: a clinical report of an outbreak in the field. New Zealand Vet.J., 18, 168-170.

Whitten, L.K. (1956). The efficiency of phenothiazine against Trichostrongylus colubriformis in sheep. New Zealand Vet.J., 4, 63-68.

Williams, J.C. (1980). Epidemiology of ostertagiasis in cattle in Louisiana. Proceed.25th Meeting Am.Ass.Vet. Parasitol., p.37.

Williams, J.C. and Bilkovitch, F.R. (1971). Development and survival of infective larvae of the cattle nematode, Ostertagia ostertagi. J.Parasitol., 57, 327-338.

Williams, J.C. and Knox, J.W. (1976). Effect of nematode parasite infection on the performance of stocker cattle at high stocking rates on coastal Bermuda grass pastures. Am.J.Vet.Res., 37, 453-463.

Williams, J.C., Knox, J.W., Sheehan, D. and Fuselier, R.H. (1977). Efficacy of albendazole against inhibited early fourth larvae of Ostertagia ostertagi. Vet. Rec., 101, 484-486.

Williams, J.C., Knox, J.W., Baumann, B.A., Snider, T.G. and Hoerner, T.J. (1981a). Further studies on the efficacy of fenbendazole against inhibited larvae of Ostertagia ostertagi. Vet.Rec., 108, 228-230.

Williams, J.C., Knox, J.W., Baumann, B.A., Snider, T.G. and Hoerner, T.J. (1981b). Anthelmintic efficacy of albendazole against inhibited larvae of Ostertagia ostertagi. Am.J.Vet.Res., 42, 318-321.

Worley, D.E. and Sharman, G.A.M. (1966). Gastritis associated with Ostertagia bisonis in Montana Range Cattle. J.Am.Vet.Med.Ass., 149, 1291-1294.

Yazwinski, T.A. and Gibbs, H.C. (1975). Survey of helminth infections in Maine dairy cattle. Am.J.Vet.Res., 36, 1677-1682.

Yazwinski, T.A., Williams, M., Greenway, T. and Tilley, W. (1981). Anthelmintic activities of ivermectin against gastro-intestinal nematodes of cattle. Am. J.Vet.Res., 42, 481-482.

Young, R.R. and Trojstman, A.C. (1980). A rapid technique for the recovery of strongyloid infective larvae from pasture and soil samples. Parasitol., 80, 425-431.

APPENDICES

Appendix A. Technique for plasma pepsinogen estimation.

Reaction

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

Reagents

2% Bovine Serum Albumin (BSA)

2N HCl

4% Trichloracetic Acid (TCA)

N/4 Caustic Soda

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

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

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

Procedure

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

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

1. 6.0 ml aliquots of tests were pipetted into universals and incubated at $37^{o}C$ for 24 hours.
2. 6.0 ml aliquots of BSA blanks were pipetted into another set of universals and the protein precipitated with 10 ml of 4% TCA.

3. Precipitated blanks were allowed to stand for ten minutes and then filtered through a No.44 Whatman filter paper.
4. Tests were precipitated after incubation with 10 ml 4% TCA then processed as in (3) above.
5. 2 ml of all filtrates were pipetted into suitably labelled flasks containing 20 ml N/4 NaOH.
6. Flasks containing 2 ml of each working standard were set up with 20 ml N/4 NaOH ie 2 u mols and 4 u mols tyrosine.
7. A reagent blank containing 2 ml H_2O with 20 ml N/10 NaOH was set up .
8. 3.0 ml diluted Folin and Ciocalteau's reagent was added to all flasks.
9. After standing for 30 minutes the blue colour was read in a spectrophotometer at a wavelength of 680 mu.

Calculation of Results

1. The reagent blank was subtracted from all readings.
2. From tyrosine standards the factor for conversion of all spectrophotometer readings to u mols tyrosine was calculated and all readings converted to u mols tyrosine.
3. If incubated BSA and plasma = A
   and non-incubated BSA and plasma = B
   then A-B = total release of tyrosine on incubation.
4. If incubated BSA alone = C
   and non-incubated BSA alone = D
   then C-D = release of tyrosine from BSA substrate due to incubation alone, ie NO PEPSINOGEN.
5. Therefore (A-B) - (C-D) = tyrosine in u mols released on incubation of the equivalent of 0.125 ml serum for 24 hours with substrate.
6. The amount of tyrosine in u mols released per 1000 ml plasma per minute = International Units or x 1000 = milli Units tyrosine. (u mol (5)x 5.56).

Appendix B Individual numbers of Ostertagia spp. L_3 recovered from the herbage component of soil cores.

	Replicate No.																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Oct.	58	43	9	73	49	38	131	37	31	20															
Nov.	0	0	0	11	0	0	9	0	10	12	8	21	0	0	9	11									
Jan.	0	10	0	0	11	19	22	18	0	0	8	12	10	0	11	0	0	0	0	10	0	0	0	12	0
Mar.	0	0	0	0	0	0	0	2	0	0	2	2	0	0	0	0	0	1	0	0	0	1	0	0	1
Apr.	42	5	0	91	0	0	4	4	0	4	0	36	1	1	1	0	1	0	5	0	0	2	0	3	0
May	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
Jun.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	6	11	0
Jul.	0	1	0	0	18	0	0	1	0	0	1	0	0	0	3	28	0	3	0	1	1	10	3	0	0
Aug.	0	0	1	0	0	0	0	0	2	3	0	0	0	1	0	0	0	0	0	0	3	0	0	0	0
Sept.	0	0	0	0	1	0	1	0	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0

Appendix B (contd.) Individual numbers of Ostertagia spp. L_3 recovered from the B (Root-mat) component of soil cores.

	Replicate No.																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Oct.	0	40	8	31	19	10	11	30	22	28															
Nov.	0	0	0	0	0	0	12	0	48	10	51	0	9	22	0	0									
Jan.	0	0	0	11	0	0	0	0	8	0	0	12	0	0	10	0	29	0	0	11	10	0	0	0	0
Mar.	0	0	0	1	1	0	0	0	0	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0
Apr.	9	1	0	0	0	0	0	1	0	3	0	0	2	1	4	0	0	0	0	0	0	0	1	0	0
May	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jun.	2	0	0	2	0	0	0	0	1	0	0	2	1	3	0	0	1	1	0	0	0	0	0	0	0
Jul.	1	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	1	2	0	0
Aug.	1	15	0	0	0	0	1	2	3	2	3	0	2	6	3	7	0	0	0	0	169	14	0	0	2
Sept.	0	1	0	0	0	10	0	8	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Appendix B (contd.) Individual numbers of Ostertagia spp. L_3 recovered from the C (upper soil, 0.5 - 7 cm) composed of soil cores.

	Replicate No.																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Oct.	0	9	23	68	41	37	14	21	2	7															
Nov.	0	10	9	52	11	9	20	0	46	14	61	39	10	39	12	9									
Jan.	8	0	0	22	12	9	21	0	0	0	0	0	0	18	32	38	10	0	9	11	0	10	76	0	0
Mar.	0	2	0	0	2	0	0	2	7	0	0	0	2	0	0	0	0	1	1	0	0	0	0	0	0
Apr.	146	157	0	0	0	0	92	1	84	3	3	0	1	1	8	0	0	0	2	0	0	0	2	0	1
May	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Jun.	0	0	0	0	0	8	0	0	0	1	0	1	1	0	2	0	1	1	1	0	1	0	1	0	6
Jul.	0	0	1	1	0	8	161	0	0	281	1	2	2	2	0	0	0	19	1	41	0	0	0	0	6
Aug.	0	0	2	1	0	2	1	0	3	0	27	1	0	1	0	0	0	3	0	1	2	0	0	0	0
Sept.	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	2	0	0	0	0	0	21	0	1

Appendix B (contd.) Individual numbers of Ostertagia spp. L_3 recovered from the D (lower soil, 7-15 cm) composed of soil cores.

	Replicate No.																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Oct.	0	30	0	38	12	0	0	0	0	21															
Nov.	0	0	0	0	0	0	12	0	18	10	19	11	0	0	0	0									
Jan.	0	0	0	9	21	18	12	10	29	0	0	81	0	0	0	0	0	11	0	10	9	0	0	0	0
Mar.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	1	0	0
Apr.	0	0	0	0	0	0	0	0	0	174	0	0	0	0	0	0	1	0	1	6	2	0	1	3	0
May	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jun.	2	0	50	1	0	0	2	1	0	1	0	2	2	0	0	1	1	0	0	0	1	0	0	0	0
Jul.	0	0	0	0	0	0	20	0	2	1	0	0	1	1	0	0	0	0	0	0	0	1	20	0	0
Aug.	5	0	0	0	0	0	7	0	0	1	0	0	5	2	4	0	0	0	0	0	1	0	2	0	1
Sept.	0	0	0	1	0	0	3	0	1	0	0	2	0	0	1	0	0	0	0	0	2	0	0	0	0

Appendix C Weekly pasture larval nubmers (L_3/kg dried herbage) recovered from paddock ungrazed by calves from May to September 1980.

	Week	Ostertagia spp.	C. oncophora	N. helvetianus
May 12th	1	0	290	0
	2	0	222	0
	3	449	449	0
June 2nd	4	727	727	0
	5	0	0	0
	6	495	0	0
	7	0	0	0
July 1st	8	0	0	0
	9	519	0	130
	10	0	0	0
	11	500	0	0
	12	1110	0	1110
Aug. 4th	13	1400	350	0
	14	256	0	0
	15	0	0	0
	16	468	468	234
Sept. 1st	17	0	0	0
	18	260	0	0
	19	520	260	260
	20	243	0	0

Appendix D Individual daily trichostrongyle egg counts (eggs/gram) of indoor calves.

(W isolate)

Day	Calf number 16	21	22	31	33
0	0	0	0	0	0
17	100	10	30	110	90
18	160	20	140	260	230
19	690	380	390	320	2640
20	180	410	1240	330	4460
21	120	420	1210	300	2320

(GB3 isolate)

Day	Calf number 14	24	32	35	37
0	0	0	0	0	0
17	0	20	300	30	50
18	6	30	540	60	300
19	50	680	550	320	130
20	660	720	170	330	340
21	1090	1470	180	460	190

(GC3 isolate)

Day	Calf number 52	37	46	22	44
0	0	0	0	0	0
17	40	350	370	280	510
18	50	610	550	270	870
19	30	410	630	390	960
20	660	260	380	760	1070
21	640	1460	2020	1590	3500

Appendix E. Individual plasma pepsinogen levels (i.u.) of the indoor calves

(W isolate)

	Calf number				
Day	16	21	22	31	33
0	0.8	0.7	0.7	0.7	0.7
3	1.1	1.1	0.8	1.0	0.8
6	1.1	0.9	1.0	1.1	1.1
10	0.9	0.9	1.1	1.0	1.3
13	1.4	1.1	1.7	1.5	1.7
17	1.9	1.8	3.1	2.6	5.3
20	1.8	2.3	3.8	5.2	7.5
21	2.0	3.0	4.0	4.3	6.9

(GB3 isolate)

	Calf number				
Day	14	24	32	35	37
0	0.6	0.7	0.5	0.9	0.7
3	0.6	0.9	0.8	1.1	0.9
6	0.9	0.9	0.8	1.1	1.0
10	0.7	1.1	0.9	1.1	1.1
13	0.9	1.1	0.9	1.6	1.4
17	1.0	1.9	1.7	2.5	2.9
20	1.5	3.6	7.5	4.8	4.9
21	1.6	6.6	9.7	4.3	5.7

(GC3 isolate)

	Calf number				
Day	52	37	46	22	44
0	0.7	0.6	0.7	0.8	0.6
3	0.6	1.0	0.7	1.0	0.9
6	0.9	1.2	0.9	1.3	1.3
10	0.8	1.5	1.0	1.7	1.7
13	0.8	1.3	1.0	1.7	1.7
17	1.1	2.3	1.6	2.8	3.2
20	1.8	5.8	3.2	7.8	7.4
21	1.8	5.7	3.9	8.8	8.2

Appendix F. Individual daily faecal egg counts (eggs/gram) of the indoor calves.

(Group A) (100,000 *O. ostertagia* L_3)

Day	Calf number 34	22	21	39
0	0	0	0	0
17	50	50	50	0
18	-	150	350	0
19	-	600	800	500
20	-	2450	550	750
21	-	650	650	750
22	-	-	350	750
23	-	-	150	1050
24	-	-	250	1750
25	-	-	150	500
26	-	-	150	400
27	-	-	200	600
28	-	-	100	700
29	-	-	-	150
30	-	-	-	200
31	-	-	-	1900
32	-	-	-	1050
33	-	-	-	750
34	-	-	-	950
35	-	-	-	650

Appendix F. (contd.)

(Group B) (100,000 *O. leptospicularis* L_3)

Day	Calf number 49	56	32	18
0	0	0	0	0
17	50	0	0	200
18	-	50	150	150
19	-	250	150	850
20	-	1500	550	1150
21	-	2050	450	1250
22	-	-	350	1450
23	-	-	750	1000
24	-	-	250	750
25	-	-	50	700
26	-	-	50	1050
27	-	-	100	850
28	-	-	100	1200
29	-	-	-	950
30	-	-	-	400
31	-	-	-	100
32	-	-	-	100
33	-	-	-	700
34	-	-	-	550
35	-	-	-	850

Appendix F (contd.)

(Group C) (50,000 *O. ostertagi* L_3 + 50,000 *O. leptospicularis* L_3)

Day	Calf number 50	11	45	31
0	0	0	0	0
17	0	50	50	0
18	-	50	300	150
19	-	850	2100	1950
20	-	2450	800	2500
21	-	3600	1900	3400
22	-	-	1600	3450
23	-	-	2950	2200
24	-	-	1900	2600
25	-	-	1400	2150
26	-	-	1750	1550
27	-	-	950	1100
28	-	-	1250	550
29	-	-	-	1600
30	-	-	-	650
31	-	-	-	750
32	-	-	-	600
33	-	-	-	950
34	-	-	-	850
35	-	-	-	700

Appendix G. Individual fortnightly body weight (kg) of set-stocked calves grazed from May to September 1980.

(W isolate)

	Week	Calf number 17	53	3	41
May 12th	1	155	137	106	165
	3	162	154	100	174
June 9th	5	190	160	115	170
	7	190	190	115	174
July 7th	9	214	190	121	206
	11	230	198	140	194
Aug. 4th	13	226	198	150	218
	15	255	230	158	222
	17	260	235	158	222
Sept. 15th	19	-	-	178	257

(GB3 isolate)

	Week	Calf number 16	57	11	50
May 12th	1	130	175	155	170
	3	140	180	137	158
June 9th	5	166	196	162	154
	7	178	222	182	180
July 7th	9	158	248	190	194
	11	190	256	206	210
Aug. 4th	13	190	248	198	206
	15	194	288	206	218
	17	186	292	194	210
Sept. 15th	19	-	-	198	224

Appendix G (contd.)

Week		(GC3 isolate) Calf number 4	55	21	58
May 12th	1	130	155	170	190
	3	154	166	194	210
June 9th	5	182	166	186	235
	7	178	174	186	230
July 7th	9	184	158	186	206
	11	174	170	186	218
Aug. 4th	13	162	146	174	202
	15	172	158	186	222
	17	166	154	174	206
Sept. 15th	19	-	-	170	190

Appendix H. Individual weekly faecal egg counts (eggs/gram) of set-stocked calves grazed from May to September 1980.

(W isolate)

	Week	Calf number 17	53	3	41
May 12th	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
June 2nd	4	0	4	0	0
	5	0	0	2	0
	6	0	2	0	0
	7	30	30	30	40
July 1st	8	30	40	20	30
	9	3	20	4	7
	10	10	20	10	5
	11	40	80	10	40
	12	30	20	20	20
Aug. 4th	13	30	130	20	110
	14	70	4	30	100
	15	20	370	30	20
	16	50	110	20	50
	17	70	220	50	110
Sept. 8th	18	-	-	140	180
	19	-	-	220	240

Appendix H (contd.)

(GB3 isolate)

	Week	Calf number 16	57	11	50
May 12th	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
June 2nd	4	2	0	0	0
	5	2	0	0	0
	6	20	0	0	0
	7	20	30	5	50
July 1st	8	30	20	50	50
	9	200	30	100	90
	10	130	60	150	70
	11	180	40	170	100
	12	270	50	80	40
Aug. 4th	13	30	130	120	190
	14	460	40	220	120
	15	170	50	20	180
	16	250	30	140	60
	17	1020	30	100	100
Sept. 8th	18	-	-	180	50
	19	-	-	210	90

Appendix H (contd.)

(GC3 isolate)

	Week	Calf number 4	55	21	58
May 12th	1	0	0	0	0
	2	0	0	0	0
	3	0	10	0	10
June 2nd	4	0	20	0	10
	5	0	20	0	20
	6	20	70	30	30
	7	110	90	210	130
July 1st	8	820	870	100	60
	9	970	1170	470	110
	10	1230	1520	1040	490
	11	1740	1170	700	620
	12	1300	1620	490	370
Aug. 4th	13	380	N.S.	640	120
	14	160	1630	180	30
	15	650	590	630	30
	16	1920	1220	1180	260
	17	5600	3140	690	350
Sept. 8th	18	-	-	2730	660
	19	-	-	3760	5250

Appendix I. Weekly pasture larval numbers (L_3/kg dried herbage) recovered from paddocks grazed by set-stocked calves from May to September 1980.

(W isolate)

	Week	Ostertagia spp.	C. oncophora	N. helvetianus
May 12th	1	0	0	0
	2	0	0	0
	3	900	900	0
June 2nd	4	259	0	0
	5	125	93	0
	6	156	260	0
	7	1411	0	0
July 1st	8	193	0	0
	9	1120	40	40
	10	168	0	24
	11	252	0	28
	12	1727	0	22
Aug. 14th	13	1135	27	27
	14	10000	2500	0
	15	23370	898	0
	16	34857	1452	726
	17	5106	3404	0
Sept. 8th	18	2000	0	0
	19	2660	0	0

Appendix I (contd.)

(GB3 isolate)

	Week	Ostertagia spp.	C. oncophora	N. helvetianus
May 12th	1	0	0	0
	2	0	0	0
	3	0	0	0
June 2nd	4	120	0	0
	5	2640	940	40
	6	1012	506	0
	7	583	0	0
July 1st	8	0	0	0
	9	0	0	0
	10	283	850	0
	11	5853	487	487
	12	5700	1425	285
Aug. 4th	13	6612	1102	551
	14	5063	506	506
	15	3928	491	981
	16	1557	519	1557
	17	1176	1176	2352
Sept. 8th	18	833	417	0
	19	4500	500	0

Appendix I (contd.)

(GC3 isolate)

	Week	Ostertagia spp.	C. oncophora	N. helvetianus
May 12th	1	620	0	310
	2	159	0	0
	3	0	0	0
June 2nd	4	0	0	0
	5	0	0	0
	6	0	0	0
	7	0	0	0
July 1st	8	0	0	0
	9	0	0	0
	10	5000	1666	0
	11	15357	3071	0
	12	28120	2009	0
Aug. 4th	13	40000	6666	3333
	14	49086	8181	1023
	15	61215	6801	3401
	16	62666	8952	8952
	17	11000	1100	0
Sept. 8th	18	13103	6552	0
	19	6779	6779	0

Appendix J. Individual C. oncophora and N. helvetianus worm burdens of the set-stocked calves grazed from May to September 1980.

		(W isolate)			
		Calf number			
		17	53	3	41
C. oncophora					
"	Male	400	100	600	1400
"	Female	400	500	200	800
"	EL_4	200	0	400	400
"	L_4	400	0	200	400
	Total	1400	600	1400	3000
N. helvetianus					
"	Male	600	200	800	600
"	Female	200	100	400	400
"	EL_4	0	100	0	0
"	L_4	0	100	0	0
	Total	800	500	1200	1000

		(GB3 isolate)			
		Calf number			
		16	57	11	50
C. oncophora					
"	Male	2400	1200	600	200
"	Female	11200	800	1000	200
"	EL_4	600	0	0	0
"	L_4	400	0	0	0
	Total	14600	2000	1600	400
N. helvetianus					
"	Male	200	600	600	800
"	Female	100	600	200	200
"	EL_4	0	200	400	0
"	L_4	0	0	0	0
	Total	300	1400	1200	800

Contd.

Appendix J (contd.)

		(GC3 isolate) Calf number 4	55	21	58
C. oncophora					
"	Male	8600	31100	3000	1000
"	Female	28300	32200	13800	100
"	EL4	400	4000	300	300
"	L_4	300	6200	450	200
	Total	37600	73500	17550	1600
N. helvetianus					
"	Male	1900	900	750	300
"	Female	1800	1300	750	200
"	EL_4	0	0	0	0
"	L_4	0	0	0	0
	Total	3700	2200	1500	500

Appendix K. Individual fortnightly plasma pepsinogen value (i.u. tyrosine) of the set-stocked calves grazed from May to September 1980.

(W isolate)

	WEEK	Calf number 17	53	3	41
May 12th	1	1.1	1.3	0.7	0.7
	3	1.3	1.3	1.1	0.9
June 9th	5	1.5	1.4	1.1	1.4
	7	1.9	1.9	1.0	1.1
July 7th	9	1.4	2.0	1.1	1.1
	11	1.5	3.1	1.5	1.4
Aug. 4th	13	2.2	4.0	2.3	1.8
	15	3.0	8.2	2.4	2.8
	17	4.0	9.6	1.8	3.6
Sept. 15th	19	-	-	1.5	3.9

(GB3 isolate)

		Calf number 16	57	11	50
May 12th	1	1.1	0.5	1.1	1.2
	3	1.6	1.2	0.9	1.5
June 9th	5	2.0	1.6	1.3	2.3
	7	2.3	1.4	1.6	2.0
July 7th	9	2.2	1.3	1.8	2.0
	11	1.8	1.3	1.4	1.7
Aug. 4th	13	2.2	1.2	1.5	1.6
	15	4.4	2.1	2.8	2.2
	17	3.1	4.4	5.8	3.1
Sept. 15th	19	-	-	7.6	3.5

Appendix K (contd.)

(GC 3 isolate)

		Calf number			
		4	55	21	58
	Week				
May 12th	1	1.0	0.7	0.8	0.8
	3	2.1	1.4	1.1	1.9
June 9th	5	2.9	1.8	2.0	2.6
	7	3.0	2.7	2.7	4.0
July 7th	9	3.5	3.2	3.0	4.0
	11	3.9	2.8	2.5	4.0
Aug 4th	13	4.1	2.2	2.3	3.1
	15	7.3	4.4	4.0	4.8
	17	11.0	2.9	4.6	6.8
Sep 15th	19	-	-	6.3	6.8

Appendix L. Individual weekly faecal egg counts (eggs/gram) of set-stocked calves grazed from May to September 1981.

		Calf number				
	Week	14	4	52	53	57
May 1st	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	150	100	0	0	0
June 1st	6	150	300	50	50	0
	7	50	700	250	300	0
	8	350	1350	50	150	250
	9	600	1150	200	50	200
	10	1300	1050	250	250	N.S.
July 8th	11	1200	150	50	N.S.	0
	12	3750	550	200	100	350
	13	-	450	100	150	100
	14	-	450	100	200	250
Aug. 3rd	15	-	500	350	250	N.S.
	16	-	400	300	200	200
	17	-	700	250	0	250
	18	-	1400	150	1100	1250
Sept. 1st	19	-	850	450	1200	1550
	20	-	1300	2550	1300	1100

Appendix M. Weekly pasture larval numbers (L_3/kg dried herbage) recovered from paddock grazed by calves from May to September 1981.

	Week	Ostertagia spp.	C. oncophora	N. helvetianus
May 1st	1	0	0	0
	2	0	0	0
	3	1212	606	1212
	4	1666	3333	3333
	5	690	690	0
June 1st	6	800	400	400
	7	0	0	328
	8	333	666	666
	9	0	426	0
	10	0	0	0
July 8th	11	2000	0	666
	12	210	0	0
	13	734	247	367
	14	1034	345	345
Aug. 3rd	15	3600	800	400
	16	13170	6585	6585
	17	13142	6571	1095
	18	5714	2857	2857
Sept. 1st	19	2609	870	870
	20	7428	3714	3714
	21	2903	2252	2581
	22	27333	16000	16666
	23	8421	10000	9474

Appendix N. Individual fortnightly plasma pepsinogen levels (i.u. tyrosine) of set-stocked calves grazed from May to September 1981.

	Week	Calf number 14	4	52	53	57
May 1st	1	0.9	2.5*	0.7	1.0	1.0
	3	1.1	3.6	1.5	2.0	1.8
	5	1.6	4.9	1.6	2.2	2.0
June 6th	7	1.8	4.7	1.9	3.0	2.5
	9	2.9	4.7	2.2	3.7	3.5
July 7th	11	3.0	3.8	2.2	4.2	3.2
	13	2.9	3.3	3.7	5.4	2.6
Aug. 3rd	15	-	3.4	2.4	3.9	3.6
	17	-	5.2	3.0	5.2	5.0
Sept. 1st	19	-	9.9	5.7	6.7	4.4
	20	-	9.7	6.7	7.7	4.8

* In the absence of any other signs of parasitism the unusually high p.p. level of this animal was attributed to some other non-parasite condition.

Appendix O. Individual fortnightly liveweight gains (kg) of set-stocked calves grazed from May to September 1981.

	Week	Calf number 14	4	52	53	57
May 1st	1	109	121	146	124	124
	3	118	127	146	134	124
	5	112	130	146	140	134
June 6th	7	121	140	150	146	130
	9	112	140	137	140	146
July 7th	11	92	158	134	140	166
	13	-	154	140	143	154
Aug. 3rd	15	-	178	150	151	150
	17	-	174	134	134	146
Sept. 1st	19	-	174	132	130	134
	20	-	170	130	134	134