

**((EPIDEMIOLOGY AND CONTROL OF GASTROINTESTINAL PARASITE
INFECTIONS OF DAIRY CATTLE IN KIAMBU DISTRICT, KENYA AND IN
DENMARK WITH EMPHASIS ON PARASITIC GASTROENTERITIS //**

BY

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A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy in Veterinary Parasitology

of the University of Nairobi

Department of Veterinary Pathology and Microbiology,

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DECLARATION

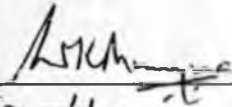
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DEDICATION

This work is dedicated to my son, **Fred Waruiru Maina**.

ABSTRACT

a) *Field survey*

The present study was designed to establish a profile of gastrointestinal (GI) parasites and to determine the influence of season, farm, age and sex on their prevalence, burden and distribution in dairy cattle. A survey of GI parasite infections of young (< 6 months old), yearlings (6-12 months old) and adult (> 12 months old) cattle on 16 farms in Kiambu District was conducted during an exceptionally dry season (September 1991 to January 1992) and during a wet season (March to July 1992). The survey was based on monthly coproparasitological examination of cohorts and worm counts in tracer calves. The effects of age, sex, farm and season on the prevalence and intensity of helminth and coccidia infections were determined. Faecal egg and oocyst counts revealed that the overall prevalences were: strongylids (85.5%), liver flukes (34.0%), coccidia (30.9%) and tapeworms (9.6%). Eight species of the protozoan *Eimeria* were identified and the most prevalent species were *E. bovis* and *E. zuernii*. The most prevalent nematode genera were *Haemonchus*, *Cooperia*, *Oesophagostomum* and *Trichostrongylus*. Season, farm and age of the animals had a significant influence on the intensity ($P < 0.05$) of infection with strongylids, liver flukes and coccidia, whereas the sex of the animals had no significant ($P > 0.05$) effect on the prevalence or intensity of infections. A higher intensity of infection with strongylids and coccidia was found in the wet season compared to the dry season ($P < 0.05$). The age specific intensity was in the following order: for strongylids, yearlings had the highest egg counts, followed by calves and adults. Calves had significantly ($P < 0.05$) higher oocyst counts compared to yearlings and adults. Liver fluke egg counts did not differ significantly ($P > 0.05$) between yearlings and adult cattle.

b) *Case study*

The aim of this study was to investigate the cause of poor condition and death among weaner calves at Iganjo farm. Parasitic gastritis due to *H. placei* was found at necropsy of a nine month weaner calf while, clinical signs and history of other sick animals examined included occasional diarrhoea, unthrifty coats, submandibular oedema, anaemia, weakness and progressive emaciation. The animals, however, had continued to eat until shortly before death. At necropsy, lesions were predominantly haemorrhagic gastritis, generalized oedema, pale mucus membranes and fat degeneration. Adult and immature *Haemonchus* worms were recovered in large numbers from the lumen of the abomasum. Relatively small numbers of other worm species, including *T. axei*, *Cooperia* spp. *Nematodirus helvetianus*, *O. radiatum* and *Trichuris globulosa* were recovered. On treatment of other cases with albendazole and supportive drugs there was gradual recovery. The cause of the outbreak was attributed to the release of highly susceptible calves onto a highly contaminated pasture during the wet season.

c) *Abattoir survey*

The objective of this survey conducted from August 1992 to July 1993 was to provide information on the spectrum and prevalence of GI nematodes of cattle and to assess the occurrence of hypobiosis in *H. placei*. Gastrointestinal (GI) tracts of 672 crossbred cattle were examined for the presence of GI nematodes. Eight nematode species were found in 583 (86.8%) of the animals. The nematodes were, in order of prevalence: *H. placei* (67.0%), *C. pectinata* (53.0%), *C. punctata* (41.7%), *O. radiatum* (38.4%), *T. axei* (24.3%), *N. helvetianus* (19.6%), *T. globulosa* (9.7%) and *Strongyloides papillosus* (3.6%). The intensity of the nematode infections was moderate: the mean burden being less than 7,000 worms. *H. placei* accounting, on average, for 52.3% of the total nematode burden. The total burden was

least during dry seasons and increased gradually during the rainy seasons. Adult *H. placei* persisted in the host throughout the year and there was no indication of hypobiosis. The heaviest GI worm burdens were detected in 1.5 to 3 year-old animals.

d) *One-year epidemiological observations*

The purpose of the present work was to more precisely define seasonal prevalence, abundance and importance of GI nematodes of weaner-yearling cattle over a 13-month period. The epidemiology of *H. placei* and other GI nematodes in yearling dairy cattle was examined monthly in two farms during the period running from April 1993 to April 1994. In each farm, 32 head of newly weaned dairy calves were given a single dose of albendazole and then placed on experimental pastures. Twelve of the animals were designated for bi-monthly slaughter ($n=2$) and analysis of worm population characteristics and 20 were designated for blood and faecal collection and for weighing. Parasite-free tracer calves were grazed alongside the weaner calves each month ($n=2$) throughout the study period and were also slaughtered for analysis of worm populations. Faecal egg counts, haematological and serum pepsinogen determinations, herbage larval counts, and animal liveweight changes were recorded monthly. The study revealed that *H. placei*, *T. axei*, *Cooperia* spp. and *O. radiatum* were responsible for parasitic gastroenteritis (PGE) and *H. placei* was the predominant nematode present in young cattle of both farms. Faecal egg counts from resident cattle and necropsy worm counts revealed that pasture larval levels were directly related to the level of rainfall. Total worm burdens present in the animals were highest during the rainy seasons (March/June and October/December) and lowest during the dry seasons (July/September and January/February). The very low recovery of *H. placei* immature larvae in tracer calves indicated that arrested development is not a feature of the life cycle of this parasite in central

Kenya. The maintenance of the parasite population depended on the continuous cycle of infection between the host and the pasture. The agro-climatic conditions of the study area revealed that, in general, favourable weather conditions for the development and survival of the free-living stages of GI nematodes existed all the year round.

e) *Plot studies*

The objective of this work was to determine the annual pattern of development of strongylid eggs to infective larvae (L_3) on pasture, and relates environmental factors on their abundance. On a series of pasture plots, 2 kg pats of bovine faeces containing known numbers of strongylid (*Haemonchus*, *Cooperia*, *Oesophagostomum* and *Trichostrongylus*) eggs were deposited at intervals of 4 weeks from July 1995 to June 1996. The plots were sampled every two weeks after contamination and infective larvae identified and counted. Larvae of all genera developed throughout the year but the pats exposed during the rainy seasons yielded abundant larvae on to herbage. Irrespective of season of deposition of the pats, larval counts were found in larger numbers from 2 to 6 weeks after deposition and generally declined to below detectable levels within 12 to 16 weeks of contamination. The comparatively short survival times noted in this experiment may present opportunities for manipulation of GI nematode population dynamics in the tropical environment of Kenya.

f) *Control studies using morantel sustained release trilaminar bolus*

The aim of this 10-month study was to determine the comparative efficacy of morantel sustained release trilaminar (MSRT) bolus and conventional anthelmintic treatments against GI nematodes of cattle under field conditions. Forty weaner calves were randomly divided into 4 groups of 10 calves each and grazed from March to December 1993 on adjacent,

similarly contaminated paddocks. Group 1 calves (T-1) served as untreated controls while group 2 calves (T-2) were dosed at turnout with MSRT boluses designed to release morantel tartrate continuously for 90 days. Group 3 calves (T-3) were drenched with albendazole on day zero, and group 4 calves (T-4) on day zero and day 14, respectively. The efficacy of these dosings was assessed by comparison of weight gains, clinical status of the animals and parasitological data (faecal worm egg counts, herbage larval counts, worm counts from tracers and set-stocked trial calves, determination of haematological parameters and pepsinogen levels). Faecal egg counts from the treated groups (T-2, T-3 and T-4) remained significantly ($P < 0.05$) lower than counts from control T-1 calves for the first three months post-treatment; notably, egg counts were reduced by 100% 28 days after treatment in T-2 and T-4 groups and by 97% in T-3 treated calves. Egg counts in T-2 calves remained significantly ($P < 0.05$) lower than counts from T-1, T-3 and T-4 calves up to trial termination. The use of MSRT boluses resulted in a reduction of 92% ($P < 0.001$) in the number of GI nematodes in set-stocked calves at the end of the study and a 55 to 85.7% reduction in herbage larval infectivity as reflected in lowered parasite burdens in tracer calves. At the trial termination, the control (T-1) calves had gained on average ($\pm$ S.D) 59.4 ± 4.8 kg (200 ± 7.4 g day⁻¹) and the T-2 ones on average 128.6 ± 10.5 kg (530 ± 13.1 g day⁻¹). T-3 calves gained on average 52.5 ± 5.7 kg (170 ± 6.9 g day⁻¹) and T-4 ones on average 82.6 ± 6.3 kg (270 ± 9.1 g day⁻¹).

g) *Duddingtonia flagrans: a study on herbage infectivity*

The present investigation, conducted in 1994 was designed to determine the overwintering residual grass infectivity in a pasture previously grazed by *D. flagrans* treated and untreated calves. Herbage infectivity was monitored by use of tracer calves over a period

of 7 weeks. The experimental pasture had previously been divided into two comparable plots, 1 and 2 which were grazed by two sets of calves, groups 1 and 2 respectively. Group 1 calves had been fed fungal material (*D. flagrans* in barley grains) once daily over a three month period from turnout. The controls, group 2 calves received barley grains as a placebo. In the present experiment, 10 parasite naive male Jersey calves aged 6 months were randomly allocated to either an experimental group (B) or a control group (A). Each group was grazed on adjacent plots, group B on the previous group 1 plot and group A on the previous group 2 plot for 4 weeks before being housed for three weeks prior to slaughter upon termination of the experiment. Body weights were recorded and individual faecal samples taken at regular intervals throughout the experimental period. Pasture nematode contamination was monitored by larval counts on herbage and worm counts of tracer calves grazed on each plot. The results demonstrated that fungal treatment did not significantly lower the overwintering larval population in plot B compared to plot A as tracer calves grazing Plot A acquired worm burdens roughly comparable, or perhaps slightly lower, than those of the plot B tracers.

h) *Duddingtonia flagrans*: fungal dose-nematode larvae interactions

The present experiment was designed to quantify the influence of egg counts and fungal dose levels on the nematode-trapping capability of *D. flagrans* against free-living stages of GI nematodes of cattle. In an *in-vitro* experiment, the interactions between free-living stages of four bovine GI parasites and the nematode-trapping fungus *D. flagrans* were evaluated using a faecal culture assay. Faeces were collected from donor calves infected with monocultures of *H. placei*, *T. axei*, *C. oncophora* and *O. radiatum*, and the number of parasitic eggs per gram of faeces (epg) were determined. The assay consisted of four faecal worm egg levels: low (40-50 epg), medium (200-280 epg), high (600-680 epg) and very high

(1288-4800) and four fungal concentrations: 0 (controls), 1000, 5000 and 25000 chlamydospores g⁻¹ faeces. The number of infective third stage larvae (L₃) which developed in faecal cultures were determined after cultures had been incubated for two weeks in darkness at 25°C and 95 % relative humidity. Results showed that the nematode-trapping capability of *D. flagrans* was dependent on the fungal concentration and number of eggs in the faecal cultures. Thus, percent reductions increased with corresponding increase in fungal concentration and epg levels in all the four species of parasitic nematodes examined. The average trapping efficacy of *D. flagrans* at the medium epg level exceeded 80% for *H. placei*, *T. axei* and *C. oncophora* compared to 53% for *O. radiatum* at the 1000 and 5000 fungal spore concentrations.

In conclusion, the results of this study suggest that GI nematode infections, especially haemonchosis, are major constraints to the health of young dairy cattle of the study area. To increase the productivity of cattle, helminthosis control should be based on epidemiological observations and should not rely on anthelmintics only. Alternative ways of control, such as biological control of free-living stages of strongylid nematodes of cattle by using nematophagous fungi merit serious consideration.

GENERAL INTRODUCTION

There is a growing recognition of the contribution of livestock and their products to the national economies as well as the socio-economic development and welfare of rural communities in developing countries. This is evident from the research and development programmes of a number of international agencies and organizations such as European Union, the International Livestock Research Institute (ILRI), the Food and Agriculture Organisation (FAO) of the United Nations and the United Nations Environment Programme (UNEP), which are aimed at the improvement of the health and productivity of livestock in Kenya. However, in the animal health sector, these programmes, over the years, have been concerned largely with the major epidemics caused by viral and bacterial pathogens such as rinderpest, foot and mouth disease and contagious bovine pleuropneumonia. The only parasitic diseases which have received worthy attention are trypanosomosis and East Coast Fever.

Until recently, there appeared to be no comparable nationally or internationally sponsored or coordinated programmes on helminth diseases and infections of farm animals in Kenya, probably because, under field conditions, helminth diseases are characteristically insidious and subclinical or chronic in nature and therefore attract relatively little attention. This ignores the fact that they are probably the most prevalent endemic parasitoses of all classes of livestock and that the hidden production losses which they cause can be very considerable, even in subclinically infected animals (Chiejina, 1991). These losses arise primarily through loss of draught power, poor meat and milk yield, carcass and offal condemnation and impaired reproductive efficiency (Fabiya, 1987; Chiejina, 1991). In Kenya recent observations in situations of subclinical infections, have shown that growth can be depressed by 25-50%, fecundity by 30% and milk yields by up to 30% (Carles, 1992).

Gastrointestinal (GI) nematode parasitism is one of the major animal health problems facing the ruminant livestock enterprises in Kenya (Mango *et al.*, 1974; Allonby and Urquhart, 1973). These enterprises are based predominantly on year-round grazing of pastures on which infective larvae are always potentially available, albeit with seasonal fluctuations dependent mainly on rainfall. It is widely believed by farmers that nematode parasites are more important to the sheep and goat industry than to the cattle industry (Barger, 1993a). This is probably a consequence of the ability of pathogenic species such as *Haemonchus contortus* and, to a lesser extent, *Trichostrongylus colubriformis* to cause the death of large numbers of small ruminants, whereas death of cattle attributable to nematode infection are less common. However, nematode infections in both cattle and small ruminants cause similar reduction in weight gains (Barger, 1982; Holmes, 1994).

It is generally believed that of all the internal parasites of cattle, the GI nematodes are of the most serious economic consequence. This is based on the overall numbers of worms, numbers of genera and species present, general levels of pathogenicity and widespread distribution (Gibbs and Herd, 1986; Nansen, 1987). The most common nematodes present in cattle on pasture in the tropics include *H. placei*, *T. axei*, *Cooperia* spp., (*C. pectinata*, *C. punctata* and *C. onchophora*) and *Oesophagostomum radiatum* (Round, 1962; Winks *et al.*, 1983; Chiejna, 1994). Of these, *H. placei* and *O. radiatum* are recognized as being the most pathogenic and economically important parasites of cattle in the tropics (Roberts *et al.*, 1951; Hutchinson *et al.*, 1980; Waruiru *et al.*, 1993a).

The epidemiology of GI parasitism in grazing cattle has been well documented in several countries, especially in Europe (Armour, 1980; Nansen *et al.*, 1990), North America (Williams *et al.*, 1987; Rickard and Zimmerman, 1992), South America (Benitez-Usher *et al.*, 1984; Bianchin and Honer, 1987) and Australia (Henderson and Kelly, 1978; Winks *et al.*,

1983). This has led to the improvement in control measures and a decrease in production losses (Michel, 1985). In contrast, little knowledge is available on the epidemiology and severity of bovine strongylosis in Kenya (Straat, 1979). The literature, especially on the ecology of the free-living stages of the nematodes concerned, is scarce and concentrates mainly on sheep (Dinnik and Dinnik, 1958; 1961; Allonby and Urquhart, 1973) and no systematic epidemiological studies have been undertaken on bovine GI nematodes in Kenya. Thus, extensive applied research on the epidemiology of GI nematode infections is absolutely necessary as this information is important in the formulation of parasite control strategies.

As opposed to other infections, helminthosis is closely associated with the local soil, climate and management systems. Thus, external larval development and survival are dependent on specific ecological conditions and/or existence of vectors and intermediate hosts which are dependent on characteristic geoclimatographic factors. This implies that the impact and character of the infections vary widely from locality to locality. Although very little work has been done on the ecology and bionomics of nematodes in Kenya, Round (1962) stated that there is such a wide diversity of environmental conditions in Kenya that studies carried out in one area would almost undoubtedly not be applicable to other areas. Therefore, to develop strategic preventive measures against nematodosis, it is necessary to have a fairly precise knowledge of the seasonal dynamics of nematode infections of an area (Arambulo and Moran, 1981), and one should ideally analyze the representative local herd situation (Nansen, 1991).

Further information is needed on the effect of the environment on the ecology of the free-living stages of cattle parasitic nematodes, as a better understanding of the characteristics of pasture stages of these parasites may lead to the development of alternative strategies of control aimed at improved managerial practices, or directly at free-living stages on pasture

(Krecek *et al.*, 1991; Grønvold *et al.*, 1993a).

The effects of environmental conditions on the development of cattle nematodes infective larvae (L₃) have been studied under wet tropical conditions in Australia (Fabiya *et al.*, 1988), in wet and dry seasons in Nigeria (Fakae and Chiejina, 1988), and in South Africa both under semi-arid conditions (Reinecke, 1960) and on irrigated pasture (Horak and Louw, 1978). Gatongi *et al.* (1988) reported on the influence of weather on the bionomics of L₃ of cattle in Kenya. While many of these investigators used tracer calves to assess larval abundance on pasture, those with a focus on the microhabitat and pasture larval counts are few in number (Krecek *et al.*, 1991).

The main constraint on larval development and survival of ruminant nematodes, is availability of moisture inside the faeces (Berbigier *et al.*, 1990) and herbage (Gruner *et al.*, 1989; Besier and Dunsmore, 1993). Since weather conditions vary from place to place, studies of the bionomics of the larvae under local conditions are needed in planning locally applicable control strategies (Okon and Enyenihi, 1977).

Presently the primary factor for the control of parasitic strongylids is by curative, strategic or tactical treatment of the animals with anthelmintics (Van Wyk, 1990a). The implementation of different pasture management practices is another way of reducing the effects of the parasite infection (Van Wyk, 1990b). However, in the tropics, these methods are limited by the high costs of anthelmintics, their uncertain availability, increasing frequency of drug resistance (particularly in small ruminants) and limited scope in many communal pastoral systems for controlled grazing.

In Kenya, anthelmintics are used on a considerable scale in the more important livestock producing areas like dairy farming (Kinoti *et al.*, 1994). Outside these areas, they are utilized in intensive livestock operations such as government farms and large commercial

enterprises. Use of anthelmintics in small and nomadic production systems is limited and is based on rare haphazard anthelmintic treatments with no account of epidemiological principles (Mbaria *et al.*, 1995).

The benefits of the strategic use of anthelmintics to control parasitic nematode infections in ruminants are well known (McKeller, 1988) and in cattle especially these regimes have been refined into a variety of systems of intraruminal boluses based on either continuous or intermittent release of the anthelmintic drug. The intermittent release system has been designed to release therapeutic doses of the drug at intervals close to the prepatent period of the parasites. However, the continuous release devices releases a subtherapeutic dose and relies on the persistence of the drug in the environment of the parasites to deter their establishment in the host.

With respect to continuous release devices, the release profile of the drug is particularly important in relation to the likelihood that the device will increase selection for resistant parasites (Donald, 1985). The preferable release profile for a continuous release device is a constant level of anthelmintic release followed by a rapid decline once the device is exhausted, avoiding the potential for underdosing with its associated implications for the development of resistance (Anderson, 1985). In the light of these possibilities, it is important that there is detailed study of those features of controlled release technology that have a direct bearing upon the development of resistance, i.e., efficacy, release rate characteristics and duration of release (Bell *et al.*, 1996). The FAO recommended high priority be given to maintaining the efficacy and availability of low-cost parasiticides, and more research on livestock production systems in developing countries that apply strategic drug management be undertaken (FAO, 1991).

Anthelmintics will continue to constitute a major control measure, but it is unlikely

that there will be any acceleration in the rate of commercial release of new compounds. However, ongoing modifications and new formulations of existing anthelmintics will continue to be produced, and implementation at the farm level of the proper use of anthelmintics and other control measures will be one of the important tasks of the coming century. Until now, the development of anthelmintic resistance in cattle has been negligible, but it may possibly pose a potential risk over the coming decades. With regard to some new anthelmintics that have environmental concerns related to their faecal excretion (Herd *et al.*, 1993; Strong *et al.*, 1996), this should be carefully examined in the future.

A general review of chemical control of livestock parasites (Strong and Wall, 1990) suggests chemical and non-chemical control be integrated and more specific reviews of anthelmintic resistance reach similar conclusions (Waller, 1994). Control in the form of vaccination or biological control by microfungi or others would be attractive alternatives that should be given a high research priority (Nansen, 1993). Barger (1993a) suggests sustainable control will be achieved by combined use of drugs, vaccines, biological control agents and genetically resistant hosts.

One option of a non-chemical control method of ruminant parasitic nematodes is biological control, which is operationally defined as the action of natural enemies which maintain a host population at levels lower than would occur in the absence of the enemies (Waller and Faedo, 1996). Biological control does not assume to be a substitute for chemotherapy where the expectation, if not the reality, is that parasites may be eradicated by the frequent use of drugs with efficacies approaching 100%. Biological control agents rarely eliminate the target organism, but reduce the numbers to acceptable levels and maintain a balance between the pathogen and the antagonist. In contrast also to chemical control of nematode parasites which are directed entirely at the parasitic stage within the

host, biological control almost certainly will be focused on the free-living stages on pasture. Within this environment the pre-parasitic stages of nematodes are subjected to a variety of abiotic (temperature, humidity and oxygen) and biotic (coprophilic fauna and flora) factors which profoundly influence their development and survival. Among the vast assemblage of biota which may exploit nematodes as nutrient source are bacteria, protozoa, and invertebrates such as collembola, mites and predatory soil nematodes (Waller, 1997a). However, the most important amongst these, and from which may emerge a biological control agent of the free-living stages of nematode parasites of livestock, are the nematophagous fungi (Waller and Larsen, 1993; Grønvold *et al.*, 1993a).

Nematophagous fungi are a diverse and ubiquitous group of microfungi which exploit nematodes either as their primary source of nutrients, or opportunistically to supplement their normal saprophytic existence. They belong to the class *Deuteromycetes*, or Fungi Imperfecti, and include those which are trapping predators, endozoic parasites or egg parasites of nematodes (Barron, 1977). Considerable progress has been made, in both laboratory and plot studies, in defining the nematode-destroying capabilities of a range of fungal species, but interest is now turning to the ability of these organisms to survive gut passage in ruminants. This is because it is recognized that to become a feasible control proposition, fungi need to withstand the harsh environmental condition of the gut so that they can be deployed in feed additives or intraruminal sustained release devices (Waller, 1997b). Certain fungal species selected because of their superior nematode-destroying capabilities *in-vitro* have also been shown to be capable of surviving ruminant gut passage and exert a significant nematode killing effect in dung (Larsen *et al.*, 1991; 1992; Waller *et al.*, 1994). By reducing the number of the infective stage of the parasitic nematodes to a minimum, the transmission of the parasite to new hosts is expected to be restricted to levels where production losses can

be avoided (Larsen, 1991).

Recently, workers in Denmark have shown that daily feeding of yearling calves with microfungus *Duddingtonia flagrans* during the first 2 months of the season led to a lowered herbage infectivity and a reduced acquisition of *Ostertagia* spp. and *Cooperia* spp. later in the season and in addition, the procedure delayed the onset of clinical disease (Larsen *et al.*, 1995a). Simulation studies of sheep nematodes also suggest that the use of fungi is a viable alternative to chemotherapy (Barnes *et al.*, 1995).

Biological control has several advantages over other non-chemotherapeutic means. Firstly, it will be applicable to a wider range of helminth parasites both between and within species of domestic animals. Secondly, it will provide the opportunity for farmers to capitalise on the increasing public concern about chemical residues in animal products and in the environment (Strong, 1993). Thirdly, it is also difficult to envisage the development of resistance mechanisms as has occurred with anthelmintic drugs (Waller and Larsen, 1993; Prichard, 1994).

The Kenyan Ministry of Agriculture, Livestock Development and Marketing estimates the present national livestock population to be composed of ten and three million head of indigenous and grade cattle, respectively; 19 million sheep and goats, 22 million birds, 110,000 pigs, 900,000 camels and 250,000 rabbits (KARI, 1994). There are 1.5 million and 200,000 traditional and Kenya top bar beehives, respectively. The sector produces a total of 2.5 billion kg of milk; 310,000 tonnes of meat, a billion eggs, 20,000 tonnes of honey, 2,000 tonnes of bees wax and 857 tonnes of greasy wool clip. Besides these direct products, the livestock sector produces hides and skins, manure which is used to improve soil fertility for better harvests, draught power for haulage and land preparation, conversion of crop waste and by-products into useful economic products (KARI, 1994).

The above national production levels from the livestock sector need to be increased considerably if the policy of broad self-sufficiency in food supplies is to be attained and sustained in the face of an increasing human population. Moreover, the demand for livestock products tends to have a higher income elasticity and will also increase in response to higher levels of average health education and the knowledge of the role of livestock food products in balanced nutrition of growing children and nursing mothers (KARI, 1994). To achieve an increase in production, there is a need to improve both management practices and the control of production-limiting diseases like helminthosis. However, to promote sound management practices (i.e. pasture preparation, stocking rate, strategic drenching etc.) requires a thorough knowledge of the epidemiology of the parasites (Murrell, 1994).

Between January 1985 and December 1995, the Veterinary Clinical Services in Kiambu District treated an average of 2916 GI helminthosis cases per month in cattle compared to 1713 mastitis and 906 tick-borne disease cases. During the same period, an average of 1407 pimply gut cases and 4723 cases of liver condemnation due to liver flukes were reported annually in various abattoirs in the district (Anonymous, 1996). These statistics show in a broad view the significance of GI parasites in animal health in this area.

The aim of the present study was to define the seasonal prevalence and importance of GI nematodes in weaner-yearling dairy cattle and to further elucidate epidemiological events of these parasites over a four year period. This study was based on the following hypotheses:

1. That nematode infections of the GI tract of young cattle is widespread in the area of study and is the cause of considerable economic loss which is derived primarily from the failure of parasitised cattle to grow at a satisfactory rate.

2. That climatic factors like rainfall, temperature and relative humidity have a significant influence on the development of GI nematode larvae on pasture and good knowledge of these factors is essential for planning effective control strategies.
3. That a sustained release anthelmintic bolus could be highly effective in controlling GI nematodes of set-stocked yearling cattle in the tropical environment of Kenya.
4. That nematode-destroying capabilities of the microfungus *D. flagrans* may be enhanced by increasing fungal concentration and nematode larval densities.

The above hypotheses were tested with the aim of achieving the following objectives:

1. To determine the identity, prevalence and intensity of GI nematodes in dairy cattle in Kiambu District of Kenya in relation to seasonal weather factors, sex, age structure of the host and management practices on the farms.
2. To determine seasonal patterns of development and survival of *Haemonchus*, *Trichostrongylus*, *Cooperia* and *Oesophagostomum* eggs to infective larvae (L₃) on pasture and relate environmental factors to their abundance and availability.
3. To assess and compare the efficacy of morantel sustained release trilaminar bolus and conventional anthelmintic treatment in controlling GI parasitism in naturally infected grazing yearlings.
4. To quantify the effects of *D. flagrans* on free-living stages of *H. placei*, *T. axei*, *C. oncophora* and *O. radiatum* in faecal cultures. The effect of increasing doses of nematode eggs admixed to the faecal cultures, was also examined.

-Chapter 2-

LITERATURE REVIEW

2.1 Farming systems and livestock populations in Kenya

One of the most important goals for Kenya as a developing country is to become self-sufficient in food production which is clearly expressed in the National Food Policy Paper No. 4 of 1981 and the sessional paper No. 1 of 1986 (Anonymous, 1986). Ruminants play an important role in the agricultural sector in Kenya, contributing approximately 20 % of the total agricultural production. Only 25% of land in Kenya is suitable for arable farming and the current demand for meat and milk is estimated to exceed production by approximately 32% and 23%, respectively (Wafula *et al.*, 1994). With the rapid increase in human population, it is becoming increasingly important to maximise agricultural production through improved management practices and control of production-limiting diseases such as helminthosis of domestic ruminants.

Domestic animals are found in all parts of the country often as mixed species on any individual farm. Cattle, sheep, goats, pigs and poultry are kept under various production systems to produce meat, milk and eggs as the main economic products. Other animals which are farmed include camels, rabbits, donkeys and bees. The livestock component of a farm provides food for subsistence and/or animal products for sale to generate farm income. At the national level, the livestock industry is expected to contribute towards the attainment of the national goals and objectives including production of sufficient products to meet the domestic demand, creation of gainfull employment and utilization of national productive resources of land, capital and entrepreneurship efficiently to warrant the continued availability of these resources for livestock production. The latter process should be carried

out on environment friendly and sustainable basis. The livestock sector thus has an important role to play in development of the rural areas. Its economic importance varies considerably depending on the alternative enterprises competing for land use and availability of other non-land production factors. Thus, livestock constitutes the preeminent source of livelihood for communities in the range and arid lands; accounts for more than 60% of household revenue in dry farming areas and a significant source of income for mixed small scale and other farms (KARI, 1994).

At the macro level, livestock industry contributes a tenth of the recorded national gross domestic product and provides a half of the employment in the agricultural sector. It has been estimated that nearly 48% of the land used for food crop and livestock production is accounted for by dairying in wetter districts (KARI, 1994).

2.1.1 *Farming systems*

Most statistics on livestock populations published by the Ministry of Agriculture, Livestock Development and Marketing are aggregated by district, which allows a broad subdivision into production systems. It is hypothesised that production goals of livestock owners differ and influence their offtake strategies which depend on the functions that livestock fulfil. Pastoralists, who almost entirely rely on subsistence and cash from their herds, have sales policies that deviate from small-scale dairy farmers in the highlands and from large-scale ranchers in the semi-arid rangelands. Therefore, a simple classification in four major producers groups was adopted consisting of pastoralists, low-input smallholder mixed farmers and small-scale dairy producers; large-scale producers (either engaged in beef, milk production or both) are grouped under the umbrella term of ranching (de Leeuw and Reynolds, 1994).

Although few of the districts can be entirely allocated to one single system of production, they have been grouped according to the dominant system as shown in Table 2.1. The four major groups roughly coincide with the agro-ecological zonation from arid to highland environments. As infrastructures, distance from and access to markets vary, geographical subdivision created further subgroups.

The rangelands of Kenya are areas of marginal agricultural potential which comprise about 80% of the country's land surface (Field, 1986). Using the percentage distribution of Too *et al.* (1986) the range population of cattle, sheep, goats and camels is about 76% of the total in the country. The Maasai, Rendille, Samburu and Turkana who are the principal inhabitants of this zone, practice pastoralism as their main activity. The pastoral sector plays an important role in the national economy through provision of livestock and their products to the heavily populated crop/livestock farming systems of the high potential areas. Mbogoh (1984) and ILCA (1984) have analysed and discussed meat and milk production from the Kenyan rangelands, including economic indices of productivity and experiences with group ranches. Despite suggestions for improvements, productivity has remained low in the range/livestock ecosystem. In 1983 the offtake rate from pastoral lands was estimated at 12% from about 3.8 million zebu cattle (Anonymous, 1983). Reasons for this include a harsh environment that is as yet little understood (Carles, 1986) and a complicated social and cultural organization that is often sceptical of the advantages of new innovations (Maranga, 1990). Kenya's high population growth rate of 4% per annum (Anonymous, 1986) has also resulted in increased migration of mixed farmers from arable to rangeland districts especially into the dry season grazing reserve areas (Abate *et al.*, 1995). Considerations of the subject are many and recent ones include those of Amuyunzu (1990), Maranga (1990) and Njanja (1991).

Table 2.1: Livestock populations by zones and production system in 1990

Zone	Production system	Population ('000)				Percent increase ^b
		Cattle	Sheep	Goats	TLU ^a	Cattle
Arid	1.1 Pastoralists, Northeast	1002	1499	1398	0.18	82
	1.2 Pastoralists, Northwest	2045	3011	1342	0.38	59
Semi-arid	1.3 (Agro)-pastoralists	1666	2778	1689	0.25	55
Subtotal		4713	7288	4429	0.27	65
Semi-arid and subhumid	2. Small-holdings					
	2.1 East and ranching	409	1608	1281	0.16	21
	2.2 West	1434	1383	2695	0.10	53
	3. Small-holding (low input/dairying) and ranching	750	1380	998	0.21	45
Subtotal		2593	4371	4974	0.16	40
Highlands	4. Small- and large-scale dairying and ranching	1575	3292	781	0.1	36
Total		8881	14951	10184	0.20	50

Sources: Sloane (1986); Reynolds (1993). ^aTLU = Tropical livestock unit of 250 kg: zebu cattle 0.7 TLU/head; dairy cows 1.0; hair sheep and goats 0.1; wool sheep 0.15 TLU/head. ^b1990-1 versus 1981-83.

Aggregation of districts: 1.1: Mandera, Wajir, Garrisa, Tana River, Lamu; 1.2: Isiolo, Marsabit, Samburu, Turkana; 1.3: Kajiado, Narok, 2.1: Kilifi, Kwale, Taita Taveta, Kitui, Machakos; 2.2: Laikipia, West Pokot, Western and Nyanza provinces; 3: Meru, Embu, Baringo, Marakwet; 4: Central Province, Kericho, Nandi, Uasin Gishu, Trans Nzoia.

In Table 2.1, populations have been separated into cattle, sheep and goats, excluding camels, which are rarely traded outside their arid realm. Three pastoralist groups are distinguished; two represent the arid zone in the north, while the two Maasai-dominated districts constitute the third group. In 1990, this amalgam of pastoralists owned (or managed) one third of Kenyan cattle and more than half of its small ruminants (de Leeuw and Reynolds, 1994).

The second major grouping consists predominantly of small-scale mixed farmers engaged in low input livestock enterprises in the wetter parts of the semi-arid zone intermingling with larger-scale commercial and cooperative ranches in the drier parts. Over 35% of the cattle are owned by this group, most of which are zebu. In the better-watered areas, a third group of farmers predominates, keeping either zebras or grade cattle, but often both in mixed herds (Tessema *et al.*, 1989). In these districts (Meru etc. see Table 2.1), grade cattle in small-scale dairy operations increased from 0.4 million in 1981-83 to 0.7 million in 1990, or about 40% of the total. Due to the importance of cattle for dairying and as oxen for traction, small ruminants are less numerous, constituting only 14% of the overall livestock mass and about one third of the national flock.

The fourth grouping joins together all production systems associated with the highlands and is characterized by a predominance of grade cattle, most of which are used for intensive milk production; overall, they represent three quarters of the total dairy herd, ranging from 90% of all cattle in Central Province to 60% in Nakuru District. Small ruminants are unimportant, but in contrast to most other production systems, sheep are more numerous than goats.

In Kenya, smallholder dairy farms account for between 75 and 90% of all milk produced (Mbogoh, 1984; Brumby and Gryseels, 1985). However, most of the increased

production in the smallholder sector has been due to increased use of land and livestock resources rather than from higher individual cow productivity (Walshe *et al.*, 1991). A number of pressures (including rapid population growth, and limited land resources) already have pushed the smallholder to more intensive dairy production (Christiansen, 1989; Walshe *et al.*, 1991). In Kenya, approximately 10% of cattle are stall-fed for the greater part of the year (Goldson and Ndeda, 1985). Such intensification will require improved management and increased resources per cow. An important step in evaluating potential development alternatives is to identify the major constraints (including helminthosis) and opportunities for increased productivity on smallholder dairies (Gitau *et al.*, 1994a). Major disease constraints in smallholder sector are diarrhoea, mastitis and helminth infections (Anonymous, 1993). A recent study conducted in Kiambu District by Gitau and others (1994b) showed that diarrhoea was the most common cause of calf morbidity and mortality of 27% and 22% per year, respectively.

2.1.2 *Population dynamics*

The census data for 1990-91 compiled by Ministry of Agriculture, Livestock Development and Marketing (Anonymous, 1993) have been compared with those from 1981-83 as summarized by Sloane (1986). In the aggregate, cattle numbers rose from 10.3 million to 13.2 million (+ 28%) as compared to a 50% increase in small ruminants climbing from 13.8 million to 20.8 million. Growth varied between systems: the most rapid growth occurred in the northern districts; where cattle increased from 1.8 million to 2.8 million (+64%) and small stock from 4.5 to 7.6 million (+66%); thus 36% of all sheep and goats are kept by pastoralists in the north. Upward changes in the other systems have been less dramatic and diminish inversely with land potential and population diversity, being lowest

in the highlands. However, in all the systems there appears to be an overall trend of faster growth in smallstock than in cattle numbers (Table 2.1).

As a result of these shifts between species, the composition of the overall livestock mass has changed in favour of small ruminants, from 15% to 17% of the total biomass. This change is lower than that suggested by the population data, because, although cattle numbers increased less, the shift from zebu to grade cattle boosted mass per head.

2.1.3 *Offtake estimates by enterprise*

Except during the 1984/85 drought, Kenya has been self-sufficient in milk; during the drought about 16,000 metric tonnes (MT) of skim milk powder and 2,500 MT of butter oil had to be imported (Mbogoh and Ochuonyo, 1992). During 1980-87, domestic milk output rose by 2.7% (Mbogoh and Ochuonyo, 1992) increasing to 4.2% during 1988-91 (Reynolds, 1993). Reynolds (1993) estimated an overall milk offtake for human consumption of 1.2 million MT per year from 13 million cattle, 90% of which was produced by grade dairy cattle. Muriuki (1992) and Mbogoh and Ochuonyo (1992) quoted total milk yields of the cattle herd of 2.0 million MT for 1990 and 1.6 million MT for 1988. In 1990, 0.8 million MT of milk was marketed, satisfying an estimated demand of 730,000 MT for liquid milk (Reynolds (1993). The output of meat from the national herd and flock is less transparent than that of milk (de Leeuw and Reynolds, 1994).

Upton (1990) divided the Kenyan livestock sector into six major groups: three for cattle (small and large scale dairy, and traditional systems) and three for small ruminants (wool and traditional sheep, goat production). For each, output of milk and meat was presented and when multiplied with the estimated population the monetary contribution to the Kenyan economy was calculated. Total output was close to 20 billion Kenya shillings per

year, 60% of which was generated by dairy products. Sloane (1986) distinguished four major systems: dairying and pastoral enterprises for cattle, sheep and goats. However, output of each was assessed at three levels of inputs and management intensity.

Both Sloane (1986) and Upton (1990) provided a useful segregation of offtake by sex/age groups as determined by enterprise production goals. The dairy sector produces mainly culled females as a by-product, which are sold for slaughter. Surplus grade heifers destined for sale, may not enter the terminal market; instead, they remain in the system as milk cows until culled. The same happens to male stock; they are either slaughtered as young calves or sold for further growing out elsewhere or to be used as traction oxen in mixed farming systems for up to 10 years of age before being sold for slaughter. Offtake rates expressed as percentage of total mass varied because of different culling rates of females (11-23%) and weights (300-400 kg). The offtake documented by Reynolds (1993) of 4.2% was more conservative than that of Upton (1990) of 6.1% (Table 2.2).

Of equal interest to overall marketing are the mixed systems, which produces both milk (mainly for subsistence) and animals for sale. These producers keep mainly zebus, but at the upper end of the input level range may also have grade cattle. These enterprises produce immature males and heifers, bulls for breeding, but only steers and culled females for slaughter. It is assumed that most of the culled females will enter local trade circuits ending up in abattoirs and butcheries, representing mostly unrecorded slaughter. Due to high population densities combined with a large urban sector dispersed in small and medium size towns, local demand is likely to absorb most of this supply.

Projections for dairy herds are summarized in Table 2.2. Culled females produce all potential meat which, expressed as kg liveweight per head, increases with the level of milk yield per cow, as intensification of dairy production usually involves higher culling rates and

Table 2.2: Herd structures, and herd offtake of dairy and beef herds at three levels of inputs

Parameters	<u>Dairy herds input levels</u>			<u>Beef herd input levels</u>		
	Low	Medium	High	Low	Medium	High
<i>Herd structure (%)</i>						
Cows	48	42	52	46	43	35
Calves	34	15	22	15	17	18
Heifers	18	38	26	23	21	22
Steers/males	-	2	-	10	13	20
Bulls	-	3	-	6	5	5
Calving %	72	86	85	50	50	67
Milk offtake l/cow/yr	1040	1740	2020	89	97	116
<i>Offtake, cows</i>						
Culling %	11	25	17	7	9	14
% LW of herd	7.0	12.2	10.3	4.4	5.5	7.1
% of total N in herd	5.3	10.5	8.8	3.1	3.8	5.0
<i>Offtake, males</i>						
% LW of herd	-	-	-	5.3	7.4	11.2
% of total N in herd	-	-	-	3.1	4.3	6.4
Total, kg LW/herd	15.6	30.6	35.2	17.0	22.6	30.2

Source: Adapted from Sloane (1986) and Upton (1990).

heavier cows because of upgrading to pure exotic breeds. The output of 30-35 kg liveweight per head is higher than the projections given by Reynolds (1993) of 20 kg liveweight per head.

In beef systems the output range is similar to that of dairy herds, despite the fact that both culled females and mature males contribute to sales. The lower outputs represent subsistence-oriented herds of pastoralists, containing mainly females (up to 82 % of the total), whereas high outputs relate to more commercially managed herds aimed at marketing male stock and showing herd structures similar to Maasai herds (de Leeuw and Reynolds, 1994); these herds produce closest to the level projected by Reynolds (1993) (15 % offtake 125 kg carcass or 37.7 kg liveweight per head) and by Upton's (1990) estimate of 35 kg liveweight per head of traditionally managed zebu cattle. The unresolved problem of how to partition beef production systems according to the projected offtake levels remains.

Using the offtake parameters by Reynolds (1993), ruminant meat output in 1990-1 totalled 282,000 MT, 68% being supplied by zebu cattle, 11 % by grade cattle and 21 % by small ruminants. Adding illegal imports of cattle at 100,000 head (or 12,500 MT meat) and the 18,500 MT from poultry and pigs, the total supply amounted to 313,300 MT or 11.8 kg per caput for a human population of 26.5 million people; this output is 8 % above the earlier consumption rate estimated by ILCA (1993) for 1985-87.

2.2 Epidemiology of helminth infections of ruminants in the tropics

Helminthosis remains one of the world's most prevalent and economically important parasitosis of man and his livestock. This is particularly the case in the developing countries, many of which lie within the tropics, where, for most part, systems of livestock production, environmental and socio-economic conditions are highly conducive for the development,

maintenance and transmission of infection (Chiejina, 1994). Helminth infections in ruminants are characteristically chronic and insidious in nature and in East Africa, in particular, have attracted very little attention, including research funds, when compared with viral, bacterial and some protozoal diseases (FAO, 1991). This is inspite of the fact that they undoubtedly exert a heavy toll on the health and productivity of this important livestock resource, with obvious implications for the rural and national economies of the African continent (Fabiya, 1987; Carles, 1992).

Parasites, along with viral, fungal, and bacterial diseases, are major contributors of economic losses in developing countries (Murrell, 1994). In Africa, for example, trypanosomosis causes an estimated annual loss of US\$ 5 billion (excluding milk and hide losses) and theileriosis kills a million cattle yearly across eastern and southern Africa, and causes losses worth more than US\$ 165 million (Murray and Gray, 1984; S.K. Mbogo, personal communication, 1996). Although less well publicized, toxoplasmosis is responsible for considerable abortion and mortality among newborn lambs and pigs in developing countries (Dubey and Kirkbride, 1989).

The problem of helminth infections in animals, as in humans, is of paramount importance in the tropics. Bovine cysticercosis in Africa may account for more than a billion US\$ in lost revenue from beef production, especially to the export sector (Murrell, 1991). In addition, diseases caused by liver and stomach flukes result in major economic losses in cattle, buffaloes, sheep and goats amounting to more than US\$ 3 billion per year (FAO, 1994). In Kenya, annual losses due to fasciolosis in cattle, sheep and goats have been estimated at approximately US\$ 326 million (Agricultural Research Foundation Report, 1986). Among the economically important helminth infections of ruminants in the tropics are parasitic gastroenteritis, fasciolosis, and lungworm infection (Arambulo and Moran, 1981).

Gastrointestinal (GI) nematodes have been identified as one of the causes of production losses, which arises primarily through mortality, severe weight loss, poor meat, milk and wool production, carcase and offal condemnation and impaired reproductive performance (FAO, 1991). In addition, the cost of obtaining anthelmintics, a primary tool for the control of GI nematodes, places additional stress on the already strained foreign exchange of developing countries.

2.2.1 *Epidemiology of nematodosis in Kenya*

The epidemiology of GI nematodes is heavily influenced by weather conditions. In Kenya, different agro-ecological zones have been identified, which have relatively different climatic conditions (Stotz, 1979). Available information indicates that GI helminths occur in all the zones, and production and economic losses may be high due to both clinical and subclinical infections (Round, 1962, Mango *et al.*, 1974; Allonby and Urquhart, 1975; Carles, 1992).

Until recently, little information on the epidemiology of GI nematode infections of cattle in Kenya was available. Several studies have, however, been conducted in recent years in the arid and semi-arid lands, and in the medium to high agricultural potential areas.

The prevalence of nematode infections in cattle in the arid area of Marsabit District was studied by Omara-Opyene (1985). Marsabit District has a typical arid climate with low rainfall (<250 mm annually) occurring in two short rainy seasons (April and November), which are separated by dry 4-5 months. Faecal strongyle egg counts were high in the sub humid mountainous areas but low in the arid lowlands, and the frequency of strongylosis was highest during the dry season compared to the rainy seasons. The rainy seasons in this area are very short and it was postulated that animals became infected during the short rainy

season but high strongyle egg counts were detected during the following dry season (Omara-Opyene, 1985). Studies undertaken in Samburu District have shown that, the main effect of GI nematodes, is during the dry season when nutrition is very poor (Kariuki, 1997).

A prevalence survey conducted in Maasai group ranches in Kajiado District (semi-arid area) showed that strongylosis was the most prevalent GI parasite infection, and the rate of infection correlated with age in cattle. Most of the cases occurred in calves, which also suffered heavier parasitism (Ndarathi *et al.*, 1989).

In a medium to high potential area of Kenya, Gatongi and colleagues (1988) examined the influence of weather factors on the level of pasture infectivity in Tetu Division, Nyeri District. It was observed that larval population increased with rainfall peaks. Immediately following the onset of rains after a dry season, a high pasture larval population was detected, presumably due to reappearing of L₃ on the grass mat after having burrowed into the soil during the preceding dry season. Alternatively, these larvae may have come from the faecal pats after being softened by the rains (Gatongi *et al.*, 1988).

2.2.2 Bovine parasitic gastroenteritis

2.2.2.1 Aetiology, mode of transmission and species spectrum

Naturally-occurring bovine parasitic gastroenteritis (PGE) is a gastroenteropathy caused by mixed infections with several species of GI nematodes. It is the commonest helminth polyparasitism of ruminants in the tropics (Troncy, 1989), typically of young animals and may be acquired through a variety of ways (Lyon *et al.*, 1970; Soulsby, 1982a). *Toxocara vitulorum* is unique in being parasitic exclusively in young animals (Pandey *et al.*, 1990), particularly, young buffalo calves and patent infections can only be acquired through prenatal and lactogenic routes (Gupta, 1986; Swain *et al.*, 1987).

Gastrointestinal (GI) nematode fauna of cattle does not differ significantly between regions of the tropics, as animals are usually infected with a range of different species, and the following parasites seems to be representative for the mixed infection of cattle and buffaloes. The trichostrongylids *Haemonchus* spp., *H. placei*, *H. contortus* and *H. similis* are found unless the climate is too dry. The *Ostertagia* and *Nematodirus* species are not very common due to the fact that most species prefer a temperate climate (FAO, 1992). Of the other trichostrongylids, *Trichostrongylus axei* is common as are the *Cooperia* spp., *C. pectinata*, *C. punctata* and *C. oncophora*. Other common nematodes are the strongylid *Oesophagostomum radiatum*, the hookworm *Bunostomum phlebotomum*, the rhabditoid *Strongyloides papillosus* and the trichuroids *Trichuris* spp. The wide spread nature of the important parasitic diseases and their impact on livestock production in the developing countries is illustrated in Table 2.3.

Although several species of nematodes can contribute to PGE syndrome, only a few are primarily responsible for disease outbreaks under field conditions. For example, disease in cattle is caused mainly by *H. placei*, *H. contortus*, *C. pectinata*, *C. punctata* and *O. radiatum* (Fabiya *et al.*, 1979; Chiejina, 1986; Waruiru *et al.*, 1993a). *B. phlebotomum* is occasionally important in muddy unhygienic environments (Troncy, 1989), while, clinical strongyloidosis and toxocarosis are mainly diseases of neonates and juveniles (Ikeme, 1971).

2.2.2.2 *Nematode life cycles*

The life cycles of trichostrongylid nematodes, *Oesophagostomum* and *Bunostomum* are simple and direct, each adult worm being derived from an infective larva separately

Table 2.3: Distribution of major parasites of large herbivores and their relative importance (rank) within the region*

Nematode species	Latin America	North Africa	Equitorial Africa	India and South West Asia	Central East Asia	South East Asia
<i>Haemonchus</i>	1	-	1	2	2	1
<i>Cooperia</i>	2	1	2	2	2	2
<i>Trichostrongylus</i>	2	2	2	1	2	3
<i>Ostertagia</i>	-	4	-	-	-	-
<i>Toxocara</i>	-	3	4	1	1	1
Lungworms	-	-	5	3	-	-
Hookworms	3	4	4	2	-	2
<i>Oesophagostomum</i>	4	2	3	3	2	3
<i>Mecistocirrus</i>	-	-	-	1	2	1

*From FAO report AGA-815 (1991).

acquired from pasture. Adult nematodes inhabit the GI tract. Eggs produced by the female are passed out in the faeces and given appropriate environmental conditions, hatch in the faecal deposits to become first-stage larvae (L_1). The L_1 feed on bacteria, grow and moult to second-stage larvae (L_2), shedding their protective cuticle in the process. The L_2 larvae moult into third-stage larvae (L_3) but retain the cuticle from the previous moult. These double-cuticled L_3 is the infective larvae. The time required for the eggs to develop into infective larvae depends on temperature. Under optimal conditions (i.e. high humidity and warm temperatures), the development process requires 7 to 10 days (Hansen and Perry, 1994).

The parasitic phase of the life cycle begins with the ingestion of L_3 . The protective sheath is shed in response to specific stimuli such as temperature, CO_2 concentration and pH which occur within the organs preceding the site where parasitic development is completed. The third moult takes place within a few days after exsheathment and fourth stage larvae (L_4), either closely applied to or within the mucosal surface, undergo major changes in morphology, differentiate sexually and increase considerably in size. Further growth and maturation occurs after the final moult to the adult form and shortly afterwards female worms commence egg laying to complete the cycle. Generally, parasitic development in most trichostrongylids is completed within 3 to 4 weeks after infection but for *O. radiatum* a longer period of 5 to 6 weeks is required (Anderson and Bremner, 1983).

There are some exceptions to the general pattern described as in species of *Nematodirus*, development to the L_3 stage occurs entirely within the egg; the larva then hatches and is infective to the host. Infective larvae of hookworms (*Bunostomum*) and *Strongyloides* can also infect the host by penetrating its skin; the worms reach the intestine via the blood stream and lungs. The infective larval stage of *Trichuris* is contained within the

egg and is only released after the egg is ingested by the host (Hansen and Perry, 1994).

2.2.2.3 *Parasitic development and tissue changes caused by strongylid nematodes*

2.2.2.3.1 *Haemonchus* spp.

Considerable evidence has been advanced in favour of *H. placei*, the species usually associated with cattle, being distinct from *H. contortus*, a common parasite among sheep and goats (Whitlock and Le Jambre, 1979; Zarlenga *et al.*, 1994). Both species infect cattle, *H. contortus* occurring frequently when sheep and cattle are grazed together (Whitlock and Le Jambre, 1979). *Haemonchus* spp. are the largest of the worms found in the abomasum of cattle and measure 14 to 25 mm in length. Adult worms are readily seen on the mucosal surface and the females have a barber's pole appearance due to the spiral intertwining of a blood filled gut and white egg filled uteri.

Thirty-six hours after infection, exsheathed L₃ can be found in the pits of the gastric glands, some penetrating to the deeper parts. Except where inhibition of development occurs, 4th stage development is completed by the 10th day and is accompanied by a progressive inflammatory reaction, hyperaemia and fluid exudation. The fourth moult takes place around 12 to 14 days after infection and the immature adult worms attach to the mucosa for feeding, giving rise to circumscribed erosions on the mucosal surface and the formation of a coagulum of blood, mucus and desquamated epithelial cells. Infiltration at the base of the glands by mononuclear and eosinophil cells is slight during the 4th stage of development but intensifies when adult worms are present (Bremner, 1956).

Pathophysiological changes and disease signs are all referable to the haemorrhagic anaemia caused by the voracious blood sucking of the adult worms. The severity of the

changes and their speed of onset being determined by the numbers of larvae initially ingested (Hunter and Mackenzie, 1982).

2.2.2.3.2 *Trichostrongylus* spp.

Trichostrongylus spp. are slender, hair-like worms 3 to 6 mm in length. *T. axei* is the smallest of the worms found in the abomasum of cattle. *T. colubriformis*, a predominant pathogen of small ruminants is the smallest of the intestinal species; and only occasionally gives rise to a significant infection in cattle (Seddon, 1967).

One week after infection, *T. axei* larvae have entered the 4th larval stage of development and a further week is required to complete this phase before the final moult to the adult stage. During this period the heads of the developing larvae are located beneath the layer of cells lining the upper half of the gastric glands in the abomasum and their bodies lie free in the lumen of the glands. Displaced cells and those in the vicinity are destroyed. Cells lining deeper parts and others in adjacent glands lose their specialised secretory function causing decreased gastric acid production which in heavy infections leads to an increase in the pH of abomasal contents.

Within 2 weeks after infection, white raised circular plaques up to 1 cm in diameter can be observed in areas where aggregations of worms have occurred. Between 3 and 8 weeks after infection inflammatory changes intensify, plaques are enlarged and become more clearly defined and shallow ulcers appear in the mucosa, especially on the free edges of the abomasal folds (Ross *et al.*, 1971).

Experimental inoculations showed that calves dosed with 100,000 L₃ were not associated with signs of disease although, decreased albumin levels and moderate increases in plasma pepsinogen concentrations were detected 3 to 4 weeks after infection. With higher

doses (range 150,000 to 1.5 million L_3) however, young calves died within 3 to 8 weeks after infection. Acute and severe inflammation of the abomasal mucosa with oedema of the folds and patches of white necrotic debris on the mucosal surface was typical of these high levels of infection (Ross *et al.*, 1968).

2.2.2.3.3 *Cooperia* spp.

Three species of the genus *Cooperia* (*C. pectinata*, *C. punctata* and *C. oncophora*) are commonly found in cattle in the tropics and are regularly associated with outbreaks of PGE in calves (Seddon, 1967; Fabiyi *et al.*, 1979). There are small differences between species in the time needed for parasitic development and in the size of mature worms (4 to 7 mm in length) but all are found in the anterior half of the small intestine (Winks, 1974; Bremner, 1982).

During the first 2 days after infection, most *C. pectinata* larvae are recovered from the abomasum where some enter the gastric glands and from day 4 onwards, 85% of the worms are located in the proximal 40% of the small intestine. The parasitic phase of development is relatively short, with the 3rd moult to the 4th stage occurring about 2 days after ingestion of L_3 . Adult worms are found from the 8th day of ingestion onwards and eggs first appear in the faeces between days 13 and 17. Fourth stage larvae (L_4) are found either deep in the intervillous crypts or coiled around the tips of the villi. Adult worms lie in close contact with the mucosal surface frequently intertwined among the villi.

Destruction of the tips of the villi, an intense inflammatory response and exudation of plasma proteins are the main pathological changes induced by *Cooperia* infections. The severity of the lesions and the associated diarrhoea are directly related to the level of infection. Resistance to the establishment of infective larvae develops rapidly after moderate

to heavy initial infections (Winks, 1974).

2.2.2.3.4 *Oesophagostomum radiatum*

The large white adult (nodular) worms of cattle, 14 to 22 mm in length, are found in the lumen of the caecum and large intestine regardless of the site of larval penetration.

Within 2 days of infection, most of the exsheathed L₃ penetrate the wall of the posterior part of the small intestine and a few enter the wall of the caecum and colon. The moult from L₃ to L₄ takes place about 9 days after infection, a period marked by profound gut inflammation even in animals with primary infection (Elek and Durie, 1967). The final moult commences on the 19th day and eggs can be detected in the faeces between days 32 and 42 after infection (Roberts *et al.*, 1962).

Extensive tissue changes occur as a consequence of *O. radiatum* infections. In the posterior small intestine the points of larval entry are clearly defined as circular, slightly raised dark red patches. During the first week, larvae are surrounded by a clear serous fluid in a cavity outlined by dislodged muscle cells and the resulting nodule protrudes into the lumen where erosion of its surface occurs forming small crater-like apical ulcers. Vasculitis of the small blood vessels occurs in the vicinity of nodules. On the serosal surface, nodules are associated with circular blister-like lesions with bright red centres and protruding above the surface. Five to 6 weeks after infection most nodules appear as slight circular elevations consisting of dense fibrous plaques in the submucosa. A few large abscessed nodules persist after adult worms reach maturity; most contain calcified remnants of larvae (Elek and Durie, 1967).

During the first 2 weeks of infection, the mucosa of both caecum and colon become increasingly congested and oedematous, and an abundant slimy turbid mucus is produced,

and haemorrhages are common in the submucosal tissue. The net effect is a marked thickening of the wall of the caecum and proximal colon. The acute catarrhal inflammation persists while worms are present and is frequently associated with diarrhoea and a decrease in food intake. During the repair of abscesses, granulomata containing multinucleated giant cells of various shapes and sizes appear in the mucosal crypts and extensive fibrosis occurs in the gut wall. Periglandular lymphatic tissue, without obvious germinal centres, increases to form a continuous sheet in the colon. Infiltration by eosinophils is intense at all sites of infection (Elek and Durie, 1967).

Experimental infection of calves with *O. radiatum*, even at relatively low numbers of worms per animal, results in severe weight loss, anorexia, anaemia and diarrhoea (Bremner, 1961; Gasbarre and Douvres, 1987). Doses of 100,000 L₃ produced a severe haemorrhagic necrotic enteritis which resulted in the death of calves before maturity of the parasites (Elek and Durie, 1967; Bremner, 1969). Anaemia due to *O. radiatum* infection may be caused by a worm product that either directly affects haematopoiesis (Andrews and Maldonado, 1943), or induces severe inflammation that results in ulceration of the gut and subsequent blood loss (Elek and Durie, 1967). Alternatively, anaemia may develop due to blood loss resulting from mechanical disruption of the intestinal mucosa by the feeding adults (Bremner and Keith, 1962).

Reinfection of previously infected animals gives rise to an allergic enteritis which causes elimination of most of the infective larvae within 24 h. This reaction is associated with oedema, hyperaemia and hypersecretion in the intestine and is often followed by a transient but intense bout of diarrhoea. The infiltration of the oedematous intestinal tissues with eosinophil and neutrophil leucocytes and the vasculitis in the vicinity of histotropic larvae, and the subsequent proliferative granulomatous reaction with the appearance of

plasma cells, conform with descriptions of the Arthus type of immediate hypersensitivity (Elek and Durie, 1967).

Although the nodular worms of ruminants are severe pathogens in young calves and lambs, older animals are much less affected due to the strong protective immunity elicited by these parasites. Primary infection with as few as 1000 L₃ protects calves against reinfection (Roberts *et al.*, 1962).

2.2.2.4 Factors influencing epidemiology

The transmission, incidence and intensity of GI nematode infections are determined by several interacting factors. These include extrinsic factors of climate, weather and methods of husbandry and systems of livestock production and intrinsic host factors of age, heredity and physiological state (Barger *et al.*, 1983; Chiejina, 1995). A general account of the role of each factor in determining the dynamics of worm populations found in grazing cattle is outlined herebelow:-

2.2.2.4.1 Extrinsic factors

2.2.2.4.1.1 Climate and weather

Parasitologically, climate may be defined as the synthesis of the day-to-day values of the meteorological elements affecting a locality, expressed as seasonal or annual values, while weather describes the day-to-day meteorological conditions which constitute the climate (Thomas, 1974). Thus, climate (the long term pattern of atmospheric events which is used to divide areas of the world into regions with similar characteristics) influences the general epidemiological pattern, while weather (which characterises the seasons) determines the timing and magnitude of specific events that make up that pattern. The effects of weather are

direct, acting on the rate of development, migration and survival of the free-living stages of parasites and indirect, by regulating the growth, abundance and maturation of herbage (Barger *et al.*, 1983).

2.2.2.4.1.1.1 *Bionomics of free-living stages*

Pasture provides the micro-environment in which preparasitic development and survival of nematodes must take place, worm eggs and larvae being distributed in an unpredictable manner on pasture (Crofton, 1963; Thomas, 1974). Pasture infectivity depends on the independent and interactive influences of several factors in the macro- and micro-environment, and these include temperature, moisture, humidity, sunlight and oxygen supply (Andersen *et al.*, 1970; Waller and Donald, 1972), structure of the soil, growth and composition of pasture herbage (Collis-George, 1959; Knapp, 1964), as well as size and consistency of faeces (Silverman and Campbell, 1959; Young and Anderson, 1981; Chiejina and Fakae, 1989).

Field and laboratory studies have shown that: (i) Preparasitic stages of nematode parasites of livestock differ in their response to environmental conditions, especially temperature and moisture. These differences exist within and between nematode species. Generally, L₃ and embryonated eggs are the least susceptible to adverse environmental conditions, followed by unembryonated eggs, L₁ and L₂ in that order. (ii) High moisture levels are a general requirement for larval development and migration (Dinaburg, 1944; Young and Anderson, 1981). *Bunostomum* spp. larvae generally require moist, slightly sandy soil and temperature range of 23°C to 30°C for optimum development and activity (Soulsby, 1982a; Troncy, 1989). On the other hand, excessive moisture or sustained torrential rainfall adversely affects development of eggs and pasture larval densities through rapid

disintegration of faeces and washing away of eggs and larvae by rain and flood water (Ikeme *et al.*, 1986; Fakae and Chiejina, 1988; Bryan and Kerr, 1989) and also possibly through reduced oxygen tension in water-logged faeces (Silverman and Campbell, 1959). In contrast, because of the high moisture content of cattle dung pats, the hatching of nematode eggs and subsequent development to L_3 is mainly limited by ambient temperatures, and can be completed within the dung pat, even during drought (Young and Anderson, 1981; Barger *et al.*; 1984). (iii) Warm conditions and the presence of moisture films both within the dung pat and on herbage is essential for the migration of L_3 to sites making them available for ingestion by cattle (Silangwa and Todd, 1964; Soulsby, 1982a). Thus, the amount and distribution of rainfall is therefore a major determinant of the availability of L_3 and also of the occurrence of PGE. Larval dispersal in pasture may be aided by various mechanical agents such as farm machinery, implements and foot-wear of farm personnel. Biotic factors such as microfungi and psychodid flies, have been shown to transport the larvae of *C. punctata* and *T. colubriformis* (Bizzell and Ciordia, 1965) and *Oesophagostomum* and *Ostertagia* spp. (Jacobs *et al.*, 1968; Tod *et al.*, 1971), respectively. Earthworms and dung beetles may aid the distribution of the parasites by moving faecal material mechanically (Grønvold, 1979). (iv) Once on herbage the longevity of L_3 is extremely limited under hot dry tropical conditions (Dinnik and Dinnik, 1961; Chiejina *et al.*, 1989). In the Trichuridae and Ascarididae, the infective egg can survive in a warm humid environment for several years (Troncy, 1989) but their longevity is also severely curtailed during the hot dry season and by direct sunlight.

2.2.2.4.1.1.2 *Seasonal patterns of infection*

Two distinct patterns of pasture infestation may be encountered in the tropics,

depending largely on the distribution of rainfall. The first is seen in the dry tropics and in the subhumid zones having clearly defined favourable and unfavourable seasons for preparasitic development. The latter season is sufficiently hot, dry and prolonged to cause complete cessation of development and survival of preparasitic stages. Thus, except in irrigated or other permanently wet pastures, transmission of PGE in such areas is restricted to the wet season (Ogunsusi, 1979; Charles, 1989) and the only means of carry-over of infection from one rainy season to another is through animals harbouring adult worms and/or arrested (hypobiotic) larvae (Chiejina *et al.*, 1988; Kaufmann and Pfister, 1990; Ndao *et al.*, 1995).

In the less dry parts of the subhumid and wet tropics, faecal reservoirs of L_3 are also a very important additional source of the early rains herbage infestation in dry season-contaminated cattle pastures (Chiejina and Fakae, 1989; Fabiyi, *et al.*, 1988). Such larvae develop and accumulate inside faecal pats throughout the dry season but do not appear on herbage until adequate moisture conditions return at the start of the succeeding rainy season, when a spontaneous and synchronous translation of the entire faecal larval population takes place, within 24 to 48 h of the first substantial rainfall of the season (Fakae and Chiejina, 1988; Chiejina and Fakae, 1989).

During the rainy season, there is a continuous cycle of infection between the host and pasture and herbage larval densities, and worm populations in animals fluctuate considerably throughout the season (Chiejina and Emehelu, 1984; Fakae and Chiejina, 1988). This is in response to variations in the size of contamination, grazing intensity and frequency, short term fluctuations in weather conditions and host responses to infection. The relative short survival of L_3 on herbage during the wet season is the net result of several contributory factors such as constant heavy rainfall which, causes accelerated disintegration of faecal pats

and disappearance of free-living larval stages from pasture. Also, rapid breakup and burying of faeces in the soil by dung beetles, earthworms and termites may contribute to the fast depletion of pasture larval population (Grønvold, 1987; Bryan and Kerr, 1989). Predacious fungi, which play a similar role in temperate climates (Hashmi and Connan, 1989) have yet to be identified in the faeces of tropical livestock.

The second major infection pattern is non-discontinuous and occurs under continuously favourable climatic conditions in the humid tropics. In this zone there is no clear-cut seasonality in the pattern of larval availability in pasture (Okon and Akinpelu, 1982; Ikeme *et al.*, 1986; Gupta *et al.*, 1987) and several larval peaks and generations of parasites develop in pasture and animals, respectively throughout the year.

Acquisition of infective eggs of *T. vitulorum* may also follow a seasonal pattern in the dry tropics while in the permanently warm and humid zone infection may be constantly present both in the environment and in calves in endemic areas. The pattern of lactogenic and congenital infections is however, determined primarily by the dynamics of populations of dormant L₃ in maternal tissues, the reproductive state of the dam and methods of calf husbandry (Roberts, 1989). Somatic larvae can probably survive in maternal tissues for several months or years (Soulsby, 1982a).

2.2.2.4.1.1.3 *Livestock production systems and husbandry practices*

Livestock production systems and managerial practices influence the accessibility and transmissibility of infection to a susceptible host population by creating opportunities for contact between host and parasite. The methods of management of domesticated ruminants in the tropics are varied (Payne, 1970; Chiejina, 1986; Upton, 1987; Ndamukong, *et al.*, 1989) and are linked with local traditions and beliefs (Payne, 1970; Ndamukong *et al.*,

1989). The likelihood of disease increases with the intensity of grazing. Thus, the concentration of infective stages in the environment is very low in those systems which utilise extensive grazing areas, as they are thinly spread over a large territory. Nevertheless, relatively intensive traditional methods of village production of small and large ruminants such as tethering and other forms of confinement, including those which rely on zero-grazing or other cut-and-carry feeding regimens (Reynolds and Adediran, 1987), may sometimes be associated with clinical PGE (Ademosun, 1987). Similarly, true nomadic and, to a lesser extent, transhumant herds harbour generally low infection levels (Pullan, 1980) except if they accidentally encounter heavily contaminated foci, such as permanent swamps, during their migration (Onyali, 1989) or if they camp in, and themselves contaminate a given area for an extended period (Chiejina, 1991).

Certain management practices favour the transmission of parasitic infections and hence increase the likelihood, and severity of disease. These practices are primarily concerned with the density of stocking in relation to the availability of pasture, season of year and class of livestock.

Intensified cattle grazing is often associated with high stocking rates and segregation of stock into age classes whose nutritional requirements often exceed those provided by areas allocated to them. Common examples of this practice include the permanent calf paddock on many dairy farms, set stocking of replacement heifers on less productive areas, grazing young animals on the same area in successive years and the maintenance of bulls on small paddocks for most of the year (Banks and Mitton, 1960; Anderson *et al.*, 1965; Hotson, 1967). To reduce the risk of parasitic disease, higher stocking rates should be matched by appropriate increases in pasture production or supplementary feeding. Failure to do this forces cattle to graze close to the ground and closer to dung pats than they would by choice,

thus ensuring high intake of L_3 . Weaned dairy calves and beef yearlings magnify this risk because their rate of pasture contamination is much greater than that of mature cattle. High levels of infection on pasture often coincide with periods of inadequate nutrition, and measures to avoid this situation are a feature of effective control strategies (Barger *et al.*, 1983).

2.2.2.4.2 *Intrinsic factors*

2.2.2.4.2.1 *Nutrition*

Host nutrition influences the outcome of nematode infections in man and animals (Whitlock, 1949; Reveron and Topps, 1970; Coop, 1995). It is well established that inadequate intake or lack of proteins, minerals and vitamins, leads to the lowering of body resistance and specifically to impaired cellular immune responses to infections (McGee and McMurray, 1988). In helminth infections, this manifests as increased establishment, survival and pathogenicity of the parasite and hence production losses in the host (Gordon, 1964; Abbot *et al.*, 1988). Recently, Shaw *et al.* (1995) demonstrated that the development of acquired immunity to *Haemonchus* infections in young sheep was enhanced in grazing animals which received an additional supplementation with a protein rich concentrate. It is likely that the supplement counterbalanced the parasite induced protein deficiency.

An area of interest which has received attention recently is the question of whether animals infected with GI nematodes can alter their diet selection to ameliorate the pathological consequences of parasitism. Sheep continuously infected with *T. colubriformis* larvae and offered a free choice of low or a high protein ration increased the proportion of the high protein consumed (Kyriazakis *et al.*, 1994; 1996). This selection period coincided with the commencement of a reduction in serum albumin and phosphorus concentrations,

which have been shown to be associated with the damage of the small intestine in *Trichostrongylus* spp. infections (Poppi *et al.*, 1985; 1986).

The crude protein content of most native tropical grasses is adequate for only moderate levels of animal production for a few months of the year when the grasses are young (Reynolds and Adediran, 1987). Consequently, there is usually severe seasonal shortage leading to wide spread malnutrition and sometimes heavy parasitism, particularly in the semi-arid and savanna zones (Schillhorn van Veen, 1974; Charles, 1989). The full extent of malnutrition and its impact on GI nematode infection in tropical livestock are at present impossible to assess accurately. However, it is reasonable to conclude, based on the widespread occurrences of infections and undernutrition in animals that host nutrition is a major contributory factor to the incidence and production effects of helminthosis in general and PGE in particular in ruminants in the tropics (Schillhorn van Veen, 1974; Kaufmann and Pfister, 1990).

2.2.2.4.2.2 *Host age, sex, acquired resistance and genotype*

Age influences the susceptibility and resilience of animals to helminth infections, young animals being more susceptible than adults (Soulsby, 1979). Comparisons between mature cattle and calves have shown that older animals are better able to limit the number of worms establishing from an infecting dose. When cattle reared worm-free were dosed with larvae, burdens of *O. ostertagi*, *Cooperia* spp., *Nematodirus helvetianus* and *O. radiatum* were significantly less in animals over 1 year old than in calves less than 6 months of age (Herlich, 1960; Bremner *et al.*, 1976). Under field conditions, disease outbreaks are more likely to occur in young than in adult ruminants, and the latter usually harbour chronic, low-level infections and, acts as a constant source of infection for more susceptible animals.

However, clinical PGE is known to occur in adult small and large ruminants (Hotson, 1967; Wedderburn, 1970; Selman *et al.*, 1976). Such outbreaks usually occurred either under nutritional stress and intercurrent infection (Schillhorn van Veen, 1974) or as a result of poorly developed and waning immunity. Age resistance is highly effective in *T. vitulorum* infections in which expulsion of adult worms commences as early as 38 days after birth (Soulsby, 1982b) and is completed at 3 to 6 months of age.

Sex, pregnancy and lactation have long been known to affect worm populations in a wide variety of hosts (Dunsmore, 1965; Connan, 1976; Copeman and Hutchinson 1979; Gibbs and Barger, 1986; Bundy, 1988). Copeman and Hutchinson (1979) reported that bulls had higher nematode egg counts than steers and both were higher than those of heifers in an experiment where young bulls, steers and heifers of the same age and breed grazed together. The weight gain response to anthelmintic treatment was in the same rank order.

A temporary loss of acquired immunity to nematode parasites at around the time of parturition and during lactation has been extensively documented in ewes and to a lesser extent in cows (Lloyd, 1983), and recently, goats (Rahman and Collins, 1992). This loss of immunity typically involves a periparturient rise in faecal egg counts (PPR) in lactating animals, often accompanied by clinical signs of PGE. All of the parasitological manifestations of acquired immunity to nematodes appear to be compromised (Barger, 1993b). The phenomenon is associated with lactation (O'Sullivan and Donald, 1970) rather than pregnancy, although it may begin in late pregnancy, and can be abolished by removal of the sucking young (O'Sullivan and Donald, 1973). Its cause has been variously ascribed to poor nutrition, stress, lack of antigenic stimulation, and hormonal suppression of immunity, of which the latter is overwhelmingly favoured.

The net result of PPR is a substantial increase in host worm burden and pasture

contamination with worm eggs. The rise, which also occurs in cattle (Michel *et al.*, 1972) and goats (Okon, 1980; Rahman and Collins, 1992) is of considerable economic importance in sheep in temperate climates where it is sometimes the sole source of the first major wave of worm infections in lambs during the summer (Boag and Thomas, 1971). There are no reports of its occurrence in large ruminants and its epidemiological significance in the tropics is less well known.

Parasite populations in the host are regulated primarily by mechanisms and processes associated with either natural or acquired resistance, the latter being under genetic control (Wakelin, 1988). It is a result of natural resistance that some nematode parasites which are well adapted to one host species are unable to establish and reproduce successfully in another host, and this forms the basis for using mixed and alternate grazing of different species of animals for the control of animal helminthoses (Arundel and Hamilton, 1975). Acquired resistance, on the other hand, occurs more widely and helps to control parasite populations in field infections. Of its many manifestations (Michel, 1968a, Chiejina and Sewell, 1974a), resistance to establishment of new infections, reduced worm fecundity and worm rejection are of epidemiological significance.

The self cure phenomenon, is the best-known immunologically-mediated worm rejection mechanisms in field infections (Stoll, 1928; 1939; Lee *et al.*, 1960; Van Geldorp and Schillhorm van Veen, 1976). This periodically results in spontaneous exponential rejection of worm infections and is sometimes also accompanied by protection from further challenge infection (Gordon, 1968). However, self-cure is not always immunologically mediated, as it has been observed in non immune *Haemonchus*-infected sheep grazing worm-free lush pasture. The stimulus responsible for inducing the latter type of response is believed to be either an anthelmintic or an allergic substance present in freshly growing grass or as

yet undetermined physiological alterations in the abomasum (Allonby and Urquhart, 1973). Michel (1963; 1970) also described a non-immunologically-mediated mechanism for regulating burdens of *Ostertagia* spp. in calves in which worm populations were maintained by a balance between existing worms and the acquisition of new infection. However, this mechanism may not apply to all host-parasite systems (Chiejina and Sewell, 1974a; b).

The genetic basis of resistance of animals to parasites, especially helminth infections is now widely accepted based on work in laboratory animal models (reviewed by Wakelin, 1991) and in farm animals (reviewed by Windon, 1991). Generally, indigenous tropical breeds are more resistant than their exotic counterparts (Knight *et al.*, 1973; Piper and Barger, 1988), while crosses between the two are intermediate in their response, particularly with regard to establishment of worms in the host, faecal worm egg output and pathogenicity of infections, all of which are of epidemiological importance. The recognition of the potential value of this genetic characteristic in livestock selection and improvement schemes and in parasite control has prompted the search for suitable markers such as haemoglobin polymorphisms, lymphocyte antigen types (Altaif and Dargie, 1978; Stear *et al.*, 1988; Douch and Outteridge, 1989) and, more recently, DNA markers (Rohrer *et al.*, 1991) for predicting resistance to nematode parasites.

2.2.2.4.2.3 *Arrested larval development*

Inhibition, arrested larval development and hypobiosis are synonymous terms used to describe the cessation of development at an early phase of parasitic existence in the host. This temporary cessation of development is important in many GI nematodes of domesticated ruminants in which the L₄ or sometimes the L₃ is mostly affected (Michel, 1974; Schad, 1977). The factors which are known to induce larval hypobiosis are many and of these,

exposure of L₃ to adverse environmental conditions is the most important stimulus in GI nematodes of ruminants. This is consistent with the observation that the phenomenon occurs mostly in cold temperate (reviewed by Armour and Duncan, 1987) and in hot dry climates (Hart, 1964; Chiejina *et al.*, 1988). In both climatic zones, hypobiosis is highly seasonal in its incidence and sets in just before the commencement, and terminates at the end, of the unfavourable season, which corresponds to the autumn/winter and the dry season in temperate and tropical climates, respectively. In the dry savanna zone of Nigeria, for example, the highest and lowest incidence are observed during the dry and wet seasons, respectively. Thus, hypobiosis may be considered as an adaptation by the affected parasites which enables them to survive in their host at a developmental stage which is unaffected by the host immunological responses and at a time when environmental conditions are unfavourable for free-living development and survival (Taylor and Michel, 1953; Waller and Thomas, 1975). Consequently, it serves to synchronise the development of the parasite with events in the host and the environment (Soulshy, 1982a).

Reports from Kenya (Allonby and Urquhart, 1975; Gatongi, 1995), India (Gupta *et al.*, 1987), Malaysia (Ikeme *et al.*, 1987), Egypt (El-Azazy, 1990) and Nigeria (Chiejina *et al.*, 1988; Fakae, 1990) indicate that only limited hypobiosis or none at all occurs in those climatic zones where environmental conditions are permanently suitable for preparasitic development and survival.

In the dry tropics, hypobiosis is the most important means of survival and carry-over of infection in the host, with species such as *Haemonchus* and *Cooperia*, from the end of one rainy season to the beginning of another (Hart, 1964; Ogunsusi and Eysker, 1979; Ndao *et al.*, 1995). Its onset also serves to limit further increase in the number of adult worms (Schad, 1977), which are the more pathogenic stages, at a time when pasture larval density

is still high. However, synchronous development of large numbers of hypobiotic larvae can sometimes occur well before the end of the dry season, thereby resulting in a sudden increase in the numbers of pathogenic stages which may lead to dry season outbreaks of PGE (Fabiya *et al.*, 1979).

2.2.2.4.2.4 *Concurrent infections*

The capacity of an animal to control a given parasitic infection may be markedly compromised by concomitant infection with another related or unrelated parasite or pathogen (Urquhart *et al.*, 1973; Phillips *et al.*, 1974; Bell *et al.*, 1984). Laboratory studies have shown that this phenomenon is subjected to host strain-dependent variability which is, in turn, genetically-determined (Murray *et al.*, 1973; McElroy and Befus, 1987).

The laboratory findings have been confirmed by field observations on concurrent *Trypanosoma congolense*-*H. contortus* infections in indigenous and exotic breeds of goats in East Africa (Griffin *et al.*, 1981). This and other species of trypanosomes have been shown to suppress host immune responses to various important bacterial vaccines used in domestic animals, including livestock, in the tropics (Holmes *et al.*, 1974; Mackenzie *et al.*, 1975). Immunodepression in concurrent infections in tropical livestock is likely to be of epidemiological importance in trypanosome-endemic areas where concurrent trypanosome-helminth infections are common (Chiejina, 1987). These infections are frequently associated with nutritional and climatic stresses, which are known to influence host resistance to infections (Gordon, 1964; Abbot *et al.*, 1988).

2.2.2.5 Pathophysiology and clinical effects of nematode infections

2.2.2.5.1 Pathophysiology

Adverse impacts of parasitism on productivity are expressed in a number of ways which include loss in body weight (Sykes and Coop, 1977; Abbott *et al.*, 1986), reduced milk production (Michel *et al.*, 1982; Kloosterman *et al.*, 1985) and poor quality and quantity of wool in sheep (Steel *et al.*, 1982). Virtually all of the detrimental effects of the nematodes of major economic importance in cattle can be accounted for by decreased feed intake; gut motility, digestion and absorption, protein, energy and mineral metabolism as well as water and electrolyte balance.

2.2.2.5.1.1 Decreased feed intake

Irrespective of the site of location, most GI nematodes, except possibly *H. placei* (Anderson and Bremner, 1983), cause a reduction in voluntary feed intake, the severity of the effect being related to the level of intake of L₃, the species of nematode and the composition of the feed (Parkins and Holmes, 1989). The cause of reduction in feed intake is unknown but current evidence suggests that it is likely to be multifactorial (Coop, 1995).

Many GI nematode infections are associated with marked pathological changes and it is possible that, in addition to pain or discomfort (Gibson, 1955), alterations in the rate of passage of ingesta even in the absence of diarrhoea could be associated with changes in voluntary feed intake (Gregory *et al.*, 1985). Others have suggested that the increased levels of GI hormones such as gastrin and cholecystokin observed in parasitized ruminants, may be responsible (Symons and Hennessy, 1981; Titchen, 1982; Fox *et al.*, 1989; Fox, 1993). Attempts have been made to study the impact of parasite infection on the central neural control at the hypothalamus. The data of Dynes *et al.* (1990) from sheep continuously

infected with *T. colubriformis* suggest that the resulting depression in feed intake could be alleviated by inhibiting the satiety effect at the ventromedial hypothalamus, suggesting that central satiety signals may play a role.

2.2.2.5.1.2 *Gut motility, digestion and absorption*

In addition to reductions in voluntary feed intake, studies have shown that GI parasitism reduces the efficiency of feed utilization by interfering with GI motility, digestion and absorption.

Experiments conducted on GI motility in parasitized ruminants have shown that infection of the abomasum, small intestines and possibly the large intestine can seriously disturb the normal pattern of GI motility and digesta flow, even in the absence of diarrhoea (Gregory, 1985). Where studied, the rate of flow through the gut was reduced rather than increased, an effect which appears to be partly due to reduced feed intake and partly to the influence of the parasite *per se*. With clinical infections, the onset of diarrhoea is preceded by the disruption of migrating myoelectric complexes (MMCs) and severe inhibition of the reticulo-rumen and abomasum, at which time there may be an increase in the bacterial populations which could contribute to occurrence of the diarrhoea (Holmes, 1994).

Numerous attempts have been made to determine whether impaired digestion and absorption are major causes of poor utilization of feed by parasitized animals. Although some studies have clearly shown depressed absorption of substrates in parasitized portions of the intestinal tract, absorption may be enhanced in non-parasitized portions and, as a result, absorption overall may not be affected (Symons, 1976; Castro, 1981). Generally, these studies have indicated that impaired digestion and absorption are not important causes of the poor utilization of nutrients by parasitized ruminants, rather, it is the increased metabolic

demands on the host as a result of the parasites activities (Holmes, 1994). However, there are situations in which malabsorption may be significant, especially, with mixed strongylid infections in ruminants.

2.2.2.5.1.3 *Protein and energy metabolism*

In GI parasitism, substantial protein losses occur in terms of exfoliated epithelial cells, plasma, mucus and red blood cells (Bremner, 1969; Holmes and Mcleans, 1971; Abbott *et al.*, 1986; Poppi *et al.*, 1986). Reduced nitrogen retention has been shown to be a characteristic feature in helminth infections and has been associated with depressed growth rates and other factors of productivity (Parkins *et al.*, 1973; Roseby, 1977). It has further been demonstrated that protein synthesis is reduced in skeletal muscles of parasitised animals (Symons and Jones, 1975). The overall effect of infection with GI nematodes can be summarised as diversion of amino acids away from productive processes such as meat and milk production into processes which sustain the integrity of the GI tract and maintenance of homeostatic mechanisms which are essential for life (Symons, 1985; Coop, 1995). The protein requirements of the parasitised ruminant are consequently increased (MacRae, 1993).

The increased synthetic rates of protein in the liver and the gut tissue have been found to draw heavily on the digestible energy (Jones and Symons, 1982; Symons and Jones, 1983) and this has been shown to be substantially reduced in parasitised animals (Sykes and Coop, 1977; MacRae *et al.*, 1982; Entrocasso *et al.*, 1986a).

2.2.2.5.1.4 *Mineral metabolism*

A number of studies have shown that muscle growth and mineralisation are impaired in parasitised ruminants (Reveron *et al.*, 1974; Sykes *et al.*, 1977; 1979). Deposition of bone

calcium and phosphorus has been shown to be reduced by as much as 65 % in infected lambs compared with worm-free lambs (Sykes *et al.*, 1977). The net result of this has been calcium and phosphorus deficiency which leads to stunted growth (Poppi *et al.*, 1985).

2.2.2.5.1.5 *Water and electrolyte balance*

Diarrhoea is commonly seen among grazing cattle and often, without the necessary justification, directly attributed to parasitism. However, phases of persistent or intermittent diarrhoea occur during moderate to heavy infections of all important GI parasites except *H. placei* (Anderson and Bremner, 1983). With *Ostertagia* spp. infections, diarrhoea coincides with maturation of larvae into young adults and this also corresponds with the time that pathological changes of inappetance, plasma protein losses and negative nitrogen balance are pronounced (Holmes and Mcleans, 1971; Parkins *et al.*, 1973). During this stage of infection, there is a marked increase in water turnover in faeces to as much as 20% greater than normal average (Holmes and Bremner, 1971). Furthermore, potassium losses in infected calves have been found to be 10 times that in non-parasitised calves (Parkins *et al.*, 1982) which is an indication of massive sloughing of intestinal epithelial cells.

Although water loss through diarrhoeic faeces in parasitised animals has been shown to be higher than in non-infected animals, their water loss through urine has been found to be considerably below normal (Bremner, 1982). This increased water retention by parasitised animals indicates that tissue loss attributable to parasite infections cannot be strictly determined from losses in body weight alone (Halliday *et al.*, 1965; Abbot *et al.*, 1986; Entrocasso *et al.*, 1986b).

Gastrointestinal (GI) parasitism is associated with a range of clinical signs, including a failure to gain weight, inappetance and usually diarrhoea. Also, alterations in body composition occur and these can be of considerable importance in judging the impact of GI parasitism. Changes in body composition as a result of trichostrongylosis have been reported in cattle by Halliday *et al.* (1965) and Entrocasso *et al.* (1986b).

There is evidence that milk production may be reduced in ruminants infected with trichostrongylids (Bliss and Todd, 1976; Thomas and Ali, 1983). In addition, GI parasite infections can also reduce conception and pregnancy rates, and delay the onset of puberty in cattle (Oakley *et al.*, 1979; Kumar and Sharma, 1991).

Many of the pathological findings reflect the clinical signs, for example, poor body condition and weight loss, while others relate to specific changes within the GI tract. The lesions associated with *Haemonchus* spp., are broadly similar to other trichostrongylid infections of the abomasum, although it is the haematophagic habit of the L₄ and adults following their emergence from gastric glands which are most detrimental to the host. *Haemonchus* is the most pathogenic of the blood suckers and infections with large numbers of this parasite often result in severe anaemia in the host. Blood losses from *Bunostomum* and *Oesophagostomum* infections may add to the severity of the anaemia. In trichostrongylid infections of the small intestine, the main damage results from activity of the adult worms. Heavy infections are associated with severe enteritis and diarrhoea.

Migrating larvae of *T. vitulorum* may cause damage of the liver and lungs. The presence of the adult parasites in the small intestine is often associated with diarrhoea and reduced weight gain.

Impact of nematodosis on livestock production

Production losses due to PGE are high whenever adequate moisture is available for prolonged periods of the year and where animals are being grazed at high densities (Fabiya, 1987). Mortalities of over 30% were reported on heavily stocked farms in Burma (Griffiths, 1957) and on irrigated pasture in wet tropics of North Queensland (Copeman and Hutchinson, 1979), respectively. In western Nigeria, helminthosis was found to be an obstacle of prime importance to the cattle industry as it caused severe mortality, abortion and stunted growth, whenever animals were kept under high stocking rates (Lee, 1955).

Severe losses are known to occur in non-humid climatic areas including semi-arid zones, although this is generally associated with poor nutrition and over stocking around water holes. Thus, as high as 10-20% mortality was reported in Senegal (Vassiliades, 1974) and extensive deaths, and up to 30% carcase condemnation due to severe emaciation was recorded in Botswana (Carmichael, 1972); these are semi-arid areas. In a survey of GI parasitism under nomadic management in Marsabit District of northern Kenya, strongyles were found to be the most important parasites contributing to morbidity. *Haemonchus* spp. was the most important parasite and was a major constraint to cattle production in this area (Omara-Opyene, 1985).

The nodular worm, *O. radiatum*, do cause additional losses by rendering intestines, which are important items of diet in tropical Africa, unfit for human consumption. Thus in Swaziland alone up to 28.1% sets of intestines have been condemned annually, amounting to 60 tons of protein rich food (Mitchell, 1974). In a limited survey, condemnations in cattle due to *Oesophagostomum* spp. (pimply gut) was 1.5% of all cattle slaughtered in five abattoirs around Nairobi (Githigia *et al.*, 1995).

While strongylids are the GI nematodes commonly involved in production loss, *T.*

vitulorum too can be important in wet areas. In untreated cases and heavy infections, the mortality rate may be up to 35-40% of infected animals, and it is believed to be the most serious disease of buffalo calves in Southeast Asia (FAO, 1992). Parasitological studies carried out in Central African Republic (Vercruysse, 1980), Nigeria (Tekdek and Ogunsusi, 1987), Tanzania (Makundi, 1994) and Kenya (Kanyari *et al.*, 1995) revealed that *T. vitulorum* and *S. papillosus* were the main causes of diarrhoea and mortality in calves.

Economic estimates of the losses due to multiple nematode parasite infection of ruminants are difficult to obtain. However, some examples are available. In Uruguay it is estimated that helminth infections account for losses in cattle of upto 50 kg body weight with a total loss of US\$ 220 million per year (FAO, 1991), and the estimated annual costs to the Australian sheep industry were A\$ 400 million in 1984 and A\$ 309 million in 1985, owing to production losses and A\$ 50 million and A\$ 53 million, respectively, for the use and administration of anthelmintics (Gray, 1987; Windon, 1990). Estimates of yearly costs owing to GI nematode and lungworm infections in dairy cattle in The Netherlands amounted to about US\$ 100 million. The estimate included losses from growth depression in calves and yearlings, lower milk production in cows and the cost of lungworm vaccine, anthelmintic treatments and preventive pasture management (Ploeger and Kloosterman, 1988).

There are few published estimates in Africa, but production losses are generally high especially for small ruminants. Graber (1965) calculated an annual loss of 11.3% of the total economic value of sheep and goats in Chad due to GI nematodes. Akerejola and colleagues (1979) estimated an annual loss of over US\$ 40 million due to GI nematodes in the Kano area of northern Nigeria, and annual mortality rates of 60% in lambs and 30% in ewes have been reported (Eysker and Ogunsusi, 1980). In Zaire, Brito (1947) estimated an annual mortality rate of 54% due to GI trichostrongylosis alone and an additional 12% due to the

combined effects of helminth and coccidial infections. In Kenya *H. contortus* infection in sheep is estimated to cause an annual loss of US\$ 26 million in the agricultural sector (Preston and Allonby, 1979).

2.2.2.7 **Diagnosis of parasitic gastroenteritis**

2.2.2.7.1 *Clinical diagnosis*

The presenting signs in a typical case of PGE depend on the predominant pathology and parasites. They include poor body condition, anorexia, diarrhoea and anaemia. Anaemia and its sequelae are associated with cases in which either of the following: *Haemonchus*, *Oesophagostomum*, *Bunostomum* spp. or, sometimes, *Trichuris* spp. (Georgi *et al.*, 1972) are the dominant nematodes. By contrast, anorexia, poor body condition and diarrhoea characterise infections dominated by *Trichostrongylus* and *Cooperia* spp. The occurrence of these signs in several animals, taken together with a good history and seasonal factors, is sufficient to suspect PGE. However, differential diagnosis of PGE is important in all suspected cases since it can easily be confused with, and indeed compounded by, malnutrition, chronic fasciolosis, coccidiosis, intestinal paramphistomosis (Butler and Yeoman, 1962) and trypanosomosis. Thus, where applicable laboratory diagnosis should be encouraged.

2.2.2.7.2 *Laboratory diagnosis*

Laboratory procedures and findings arising from them should be considered as aids of diagnosis. The most useful of these procedures include (1) qualitative and quantitative faecal worm egg counting procedures, (2) faecal culture for differential strongylid larval counts, (3) pasture larval counts (4) haematological and serum biochemical examinations for

the investigation of anaemia and for pepsinogen estimation, respectively and (5) post mortem examination for quantitative differential worm counts and for gross pathological observations.

The most widely used laboratory technique is faecal worm egg count. However, only quantitative techniques such as the McMaster technique and its numerous modifications (Whitlock, 1948; Thienpont *et al.*, 1979; MAFF, 1986; Hansen and Perry, 1994) as well as quantitative flotation techniques (Jackson, 1974; MAFF, 1986) should be employed for diagnostic purposes in ruminants.

The clinical importance of hypobiotic larvae in ruminants is now well recognised and their isolation and counting depends on standard procedure for making post mortem mucosal analysis (Herlich, 1956; MAFF, 1986).

2.2.2.7.2.1 *Faecal egg counts*

The use of faecal egg counts as an ante mortem means of diagnosing naturally acquired GI nematode infections of domestic livestock has been practised for many years. This procedure is well developed, standardized and has recently been presented in a FAO technical manual (Hansen and Perry, 1994). Although it is possible to derive a correlation between faecal egg counts and total burdens of parasitic nematodes of sheep and calves, the correlation is poor for cattle of more than one year (Rubin, 1967; Michel, 1968b; Brunsdon, 1971; Ploeger *et al.* (1994). To obtain reliable counts, particular attention must be paid to the sampling of adequate numbers of animals as well as to the methods of collection, transportation, storage and the actual examination of the samples. As suggested by Gordon (1967), samples should be collected from some of the heaviest, best conditioned animals and some of the lightest, poorest specimens; a comparison of egg counts between the two groups is often useful. Moreover, the clinician or parasitologist must be thoroughly familiar with the

numerous factors which influence the accuracy and diagnostic significance of faecal worm egg counts, usually expressed as eggs per gram of faeces (epg) (Soulsby, 1982a; MAFF, 1986).

2.2.2.7.2.1.1 *Factors affecting egg counts*

Factors limiting the significance of faecal egg counts includes:-

- (a) Resistance of the host animal can result in both depression or suspension of egg production by adult worms. Also, immature worms do not betray their presence by laying eggs, yet the immature stages of a number of species are highly pathogenic.
- (b) The number of eggs produced per adult female worm also varies considerably between species, i.e. a few *Haemonchus* or *Chabertia* may produce a similar total count to that produced by many thousands of *Trichostrongylus* (Brunsdon, 1970). This can make interpretation of individual strongyle egg count difficult, though counts in excess of 1,000 epg of faeces are generally considered an indicator of heavy infections and those over 500 of moderate infection (Urquhart *et al.*, 1987).
- (c) Eggs of *Nematodirus*, *Trichuris* and *Capillaria* can be identified whereas eggs of major strongylids cannot be differentiated readily from each other, and they are generally referred to as typical strongyle eggs when doing total egg counts.
- (d) A fairly regular diurnal fluctuation in faecal egg count has been shown to occur, and it is recommended that sampling should be undertaken at the same time of the day (Spedding, 1952; MAFF, 1986).
- (e) The number of epg varies considerably depending on the consistency of the faeces. Fluid faeces due to diarrhoea will have less epg whereas, fasting reduces faecal output which in turn increases epg count (Gordon, 1967).

- f) Inherent deficiencies in egg counting techniques and in the rectal methods of faecal sampling arising from the fact that the eggs are not evenly distributed through the faeces can influence the accuracy *per se* of individual egg counts. However, this source of error is not unduly great.

2.2.2.7.2.1.2 *Faecal cultures*

Differentiation of typical strongyle eggs can be achieved by the use of faecal cultures. These provide an environment suitable for the hatching of the strongyle eggs and larval development to the L₃ which can be identified to genus level. The cultures can be left at room temperature for 14-21 days, by which time all the larvae should have reached the infective stage.

It is important to remember that faecal cultures indicate only which parasites are present and not their relative numbers either in the host or in the original faecal sample. This is because (a) the biotic potential (i.e. egg-laying) of parasites varies; and (b) different parasite eggs have different optimum conditions for hatching, development and survival (MAFF, 1986). Faecal larval counts, like egg counts, should only be used as an aid to the diagnosis of PGE.

2.2.2.7.2.2 *Pasture larval counts*

Larval recovery from pasture samples may be required in (a) epidemiological studies (sometimes as part of anthelmintic control programmes); (b) the diagnosis of parasitic diseases in grazing animals; and (c) establishing whether pasture of unknown grazing history is safe for young stock, even though, absence of L₃ at certain time of year does not necessarily mean that a pasture is worm-free.

Pasture larval counts may be influenced by several factors such as, season, weather, and host immunity. Of major importance is stocking density, as higher densities result in heavier pasture contamination and, therefore, greater numbers of larvae on pasture (Armour, 1980).

2.2.2.7.2.3 *Blood parameters*

Traditionally, the diagnosis of GI nematode infections relies largely on faecal egg counts. In the case of abomasal infection, diagnosis can also rely on serum pepsinogen and serum gastrin measurements as indicators of abomasal changes.

2.2.2.7.2.3.1 *Pepsinogen*

An increase in pepsinogen concentration is mainly a reflection of development and emergence of larval stages of *Ostertagia* with subsequent mucosal damage. *Ostertagia* infection causes hypo- and metaplasia of the parietal cells resulting in a decrease in acid production and a subsequent reduction of the pepsinogen transformation into pepsin. The accumulated pepsinogen may escape into the blood between the broken cell junctional complexes (Mylrea and Hotson, 1969).

The importance of serum pepsinogen linked to ostertagiosis was first reported by Anderson and colleagues (1965). Although considerable variations in serum enzyme activity are observed in naturally infected animals, the value of this parameter in diagnosing gastroparasitic disease is widely accepted (Berghen *et al.*, 1987; Williams *et al.*, 1987). Also, a good correlation between increasing levels of infection and pepsinogen concentration has been reported (Jennings *et al.*, 1966; Mylrea and Hotson, 1969; Snider *et al.*, 1981). However, the relationship between pepsinogen and the actual internal worm burden is not as

strong as that of the egg count in moderate infections (Murrell *et al.*, 1989).

Other parasitic or non-parasitic diseases may be responsible for a moderate rise in pepsinogen concentration. The abomasal parasites such as *T. axei* and *Haemonchus* spp. produce inconsistent rises in blood pepsinogen (Ross *et al.*, 1967; Bourdeau, 1985). Other non-abomasal parasites such as *Cooperia* spp. and *D. viviparus* cause minor rises of serum pepsinogen (Kloosterman and Frankena, 1988).

The only studies on variations of pepsinogen levels throughout ovine haemonchosis were carried out by Mapes and Coop (1970) and Coop (1971). These authors showed that massive infection with one million L₃ of *H. contortus* caused lesions in abomasal mucosa and rise in pepsinogen level above 1500m μ tyrosine compared to 300 to 600 in uninfected animals. Under natural conditions, worm load is never so high; therefore pepsinogen level is not a reliable parameter in assessment of pathophysiological changes due to haemonchosis.

2.2.2.7.2.3.2 *Gastrin*

The use of the gastrin assay as a diagnostic tool for demonstrating ovine and bovine ostertagiosis was mentioned by Anderson *et al.*, (1981), Entrocasso *et al.* (1986c), Fox *et al.* (1987) and Hilderson *et al.* (1992).

As demonstrated for pepsinogen, other abomasal or intestinal parasites can result in gastrin increase (Reinemeyer *et al.*, 1981; Fox *et al.*, 1988). Fox and colleagues (1991) demonstrated increased levels of gastrin and pepsinogen in *H. contortus* experimentally infected Malaysian goats. The magnitude of the blood gastrin response was significantly greater than that of pepsinogen during the period that both blood values were elevated. Other effectors of fluctuations in the level of bovine gastrin are lactation, abomasal lesions such as ulcerations, sand impaction and abomasal leucosis (Luthman *et al.*, 1979; Schillhorn van

Veen, 1988).

Several authors have confirmed the usefulness of determining pepsinogen and gastrin levels for confirming clinical disease in calves during their first grazing season (Entrocasso *et al.*, 1986c; Dorny *et al.*, 1988; Berghen *et al.*, 1990). However, the value of these parameters for detecting subclinical parasitism is questionable (Berghen *et al.*, 1993). These parameters are not really suitable for use by diagnostic laboratories because they are labour intensive and expensive methods. Therefore, they have so far mainly been used for research (Ploeger *et al.*, 1994; Eysker and Ploeger, 1995).

2.2.2.7.2.3.3 *Albumin*

Many authors report a sharp decrease in total proteins and albumin after an infection with GI nematodes in ruminants (Kuttler and Marble, 1960; Ross and Armour, 1960a). This decrease has to be related to an increase in tissue permeability and a plasma leak towards lumen of the gut and a rise in the albumin catabolism. A decrease appeared as early as the 5th day after the lambs were infected with *Teladorsagia (Ostertagia) circumcincta* (Holmes and McLeans, 1971). Ross and Armour (1960a) showed that serum albumin and packed cell volume percentage were useful measures of pathogenicity of *H. contortus* in sheep, when considered in conjunction with a series of differential faecal egg counts.

2.2.2.7.2.3.4 *Immuno-diagnosis (serodiagnosis)*

Western blot and enzyme-linked immunosorbent assay (ELISA) have been used to detect diagnostic antigens in *O. ostertagi* (Cross *et al.*, 1988), *D. viviparus* (Leeuw and Cornellissen, 1991) and *H. placei* (Schallig *et al.*, 1995) infections in cattle. ELISA using crude *Ostertagia* and *Cooperia* antigens correlate better with exposure levels when production

losses are likely to occur. In particular, crude *Cooperia* antigens appear to be useful because a correlation with infection levels is observed from approximately six weeks after the beginning of exposure onwards (Ploeger *et al.*, 1994). The ELISA is far less labour intensive and can even be automated. Nevertheless, it has not yet been used for other purposes than research.

The availability of serological methods for the estimation of nematode infection in cattle and small ruminants in the tropics would be very useful, particularly for *Haemonchus* spp. the most important nematode in most tropical regions. These methods should be used for herd monitoring and not for diagnosis in individual animals. The latter is probably not feasible considering the wide variation in the humoral response against worm antigens (Eysker and Ploeger, 1995).

2.2.2.7.3 *Biotechnology and the diagnosis of nematode infections*

Strongylid infections have mainly been diagnosed by determination of faecal egg counts. However, the eggs of most species (except for *Nematodirus*) are morphologically indistinguishable at the generic level (Georgi and McCulloch, 1989); hence their identification requires faecal culturing to produce L₃. Even then, it is difficult to accurately identify the larvae of some species using morphological characteristics (Hubert and Kerboeuf, 1984; Berrie *et al.*, 1988). The development of rapid and sensitive molecular techniques for the identification of nematode species is of significance for their control.

Recently, DNA sequences specific and sensitive for the four common cattle nematode genera, *Haemonchus*, *Ostertagia*, *Cooperia* and *Oesophagostomum* were identified by "Shortgun" cloning, to develop a rapid, reliable, and reproducible DNA-based test for ante mortem identification of these parasites (Christensen *et al.*, 1994). At present, the major

drawback of this technique is that parasite eggs still must be isolated from faeces; however, the preparation need not be highly purified because these probes do not cross react with host DNA (Zarlenga, 1994).

A second method, polymerase chain reaction-linked restriction fragment length polymorphism (PCR-RFLP) of ribosomal genes has been used to discriminate between closely-related species of trematodes (Anderson and Barker, 1993) and cestodes (Wachira *et al.*, 1993). Techniques of this nature have been developed for identification and differentiation of the morphologically similar *H. contortus* and *H. placei* (Zarlenga, *et al.*, 1994). Prior to the development of PCR primers, *H. contortus* and *H. placei* could be differentiated with confidence only when a population of worms was examined, because of the overlapping range of variation in the morphological characteristics.

As it is possible to amplify ribosomal genes from single nematode eggs (Gasser *et al.*, 1993), the PCR-RFLP approach has potential to develop a rapid and sensitive diagnostic system. It will be a useful experimental tool to identify female nematodes of different species, where morphological criteria are insufficient to delineate between species (Gasser *et al.*, 1994).

However, to Eysker and Ploeger's (1995) opinion, DNA probes in dot blots or in the PCR will not be very helpful for routine diagnosis of GI nematode infections because they will not give useful quantitative information. These methods would certainly allow a more accurate speciation of nematode eggs in the faeces, giving a more accurate differential faecal egg count. A more promising use for DNA technology may be in monitoring development of anthelmintic resistance (Kwa, 1994).

Principles of helminth control

Eradication of most helminth infections is not practical and, generally, such a course is not required in order to control economically important helminth diseases of livestock (Gordon, 1957; Spedding, 1969). Rather, the aim of control is to ensure that parasite populations do not exceed levels compatible with economic production. This objective may be achieved by using one of three interrelated approaches, namely chemotherapy involving the use of anthelmintics at selected times, grazing management and provision of the animals with a certain degree of immunity. Potentially, the most efficient control requires the complete integration of all the three facets. This is possible only on the basis of a full understanding of the epidemiology of helminth infections (Brunsdon, 1980).

In developing countries of Africa, there are hardly any set plans of prophylactic control of GI helminths. This is largely due to lack of awareness of the value of routine disease control measures, other than those for the well known killer diseases. Anthelmintic use in small-scale and nomadic production systems is limited and, treatments are carried out largely curatively only as livestock owners do not see the need to control until animals are in *extremis*. The results are often unsatisfactory and not cost effective (Fabiya, 1987). Organised worm control is practical only in modern systems of production found in large privately-owned as well as in institutional farms and ranches. Parasite control under the latter situation is carried out against a background of grossly inadequate epidemiological data, unreliable backup veterinary advice and diagnostic services, lack of competent farm management and organisational skills necessary to understand and implement modern control strategies, and chronic shortage and high cost of essential anthelmintics.

2.3.1 *Control of parasitic gastroenteritis in cattle*

There are three practical methods of controlling PGE, namely, pasture management and grazing hygiene, anthelmintic medication and the integrated method, which combines limited anthelmintic treatments with other measures such as grazing management (Brunsdon, 1972; Downey, 1973; Michel, 1976).

2.3.1.1 *Pasture management and grazing hygiene*

The essential features of this method of control which has been well perfected in modern intensive systems of production in other parts of the world, were outlined and discussed by Michel (1976, 1982). It requires, among other things, detailed knowledge of the seasonal dynamics of infection in pasture and animals as well as of the infestation status of all available pastures throughout the grazing season. Based on such detailed epidemiological information, various managerial options have been devised which achieve clearly defined objectives under given husbandry situations.

It is evident that these relatively sophisticated management practices cannot be easily implemented under our local conditions without modifications, especially in the humid zones. In these zones, free-living development is very rapid, transmission of infection occurs all the year round, resulting in several parasite generations per annum, and heavy pasture infestation can build up soon after turn-out. Thus, "clean" and "safe" pasture, as defined by Michel (1982), may be difficult to provide under intensive system of management in the humid zones. It would probably be necessary for pastures to remain free of stock for at least 6 to 8 weeks before they can attain a "safe" pasture status (Fakae and Chiejina, 1988). On the other hand, "clean" pastures occur naturally at the start of the rains in the dry tropics and such pastures could be utilised to device appropriate grazing management programmes in

those localities where the relevant epidemiological data is available.

2.3.1.2 *Control based on anthelmintic usage*

Anthelmintic drenching may be empirical, curative or preventive. Empirical drenching is not based on any strategy. Whereas, in curative drenching, treatment is often delayed until pastures are heavily infested and clinical signs or death occur (Herd, 1988). It has the disadvantage that considerable production losses have already been incurred by the time clinical signs are visible in the host (Michel, 1976), and reinfection takes place directly after drenching unless the animals are removed from the infested pasture at the time of treatment. Despite the disadvantages, curative drenching is favoured by some, as it causes a relatively low selection pressure for resistance, and because it is considered unnecessary to drench adult animals routinely (Brunsdon *et al.*, 1983).

Preventive drenching is most important for forestalling excessive contamination of the pasture with free-living stages of the worms, and thus minimising the exposure of susceptible hosts to nematodosis. Preventive drenching may be applied in the form of strategic or tactical drenching.

2.3.1.2.1 *Strategic drenching*

Strategic drenching comprises treatment at predetermined intervals, based on seasonal fluctuations in the prevalence of specific worm species; on managerial considerations such as weaning; or on combination of these. Thus, it can be regarded as a regular drenching programme and when applied in conjunction with other methods of control such as pasture spelling, the system can be termed integrated strategic worm control.

Most often, strategic drenching consists of a series of drenches at the start of the

worm season, when the worm species concerned commence egg production, and conditions become favourable for the development of free-living worm stages on pasture. This ensures that worms do not accumulate excessively at the beginning of the season, and constitute a threat to susceptible animals later.

Strategic drenching in the "off-season" when conditions are unsuitable for worm development on pasture and the worms that do occur in the animal are usually hypobiotic is referred to as offensive or "extended" drenching (Reinecke, 1983). Another variation of preventive treatment is suppressive drenching, also termed "protective" drenching. In this approach, nematodosis is suppressed when it becomes an immediate threat to the animal. The most important difference from other methods of strategic drenching, is that treatment is delayed until worms have substantially contaminated the pasture and are accumulating in the host. Treatment therefore suppresses the parasite in the host, before it can do significant damage.

The suppressive treatment is commonly applied as continuous low-level administration of the drugs (in licks, or by means of slow-release devices) or as repeated drenchings at intervals as short as every 2-3 weeks while the worm threat remains. This form of control can be very effective (Brunsdon, 1980), but is dependent on the intensive use of drugs, thus considerably increasing the chances of selection for anthelmintic resistance (Martin, 1985).

2.3.1.2.2 *Tactical drenching*

Tactical drenching consists of preventive drenching when an excessive accumulation of worms is to be expected, e.g. after good soaking rains when the climate is suitable for development of the preparasitic worm stages on pasture. Usually, tactical drenching is used to support the strategic drenching programme for those periods when the set drenches are not

sufficient for the expected worm challenge (Van Wyk, 1990a). Depending on the timing, tactical drenching can be either preventive (e.g. at the time that the climate first becomes favourable for the accumulation of worms on pasture), or suppressive, after considerable numbers of L₃ have already become available on pasture.

No hard and fast rule can be laid down for the timing of tactical drenches. Thus, it is advisable to drench tactically after rains have fallen over a period of 15 to 30 days and then to move the animals to safe pastures if possible. The drenching should commence about 3 to 6 weeks after the start of the good rains, and the course of the infection in the animals should be monitored by means of the faecal worm egg counts, to guard against overwhelming infections (van Wyk, 1990a).

2.3.1.2.3 *Anthelmintic treatment strategies in the tropics*

This is by far the most popular and in some cases such as the ranching system, the only realistic control measure. It relies exclusively on the use of drug treatment to control worm infections. Theoretically, a single treatment of all stock during the dry season, in the dry tropics, with one of the broad spectrum anthelmintics effective against adult worms and arrested larvae should ensure that all animals are free of worms at the start of the succeeding rainy season when all new pastures should also be clean. In such a situation, pastures should remain relatively "safe" for the rest of the rainy season. However, this requires detailed study and evaluation, particularly with regard to the optimum frequency and timing of the treatments, the associated pasture changes and their effects on pasture contaminations, worm burden and performance of animals.

Worm control by anthelmintic treatment alone in the humid zones and in those parts of the savanna areas with a long (6 to 7 months) rainy season is problematic and relatively

expensive since several treatments spread throughout the year are likely to be required and the frequency and timing of such treatments have not been properly determined. However, Chiejina and Emehelu (1986) evaluated three anthelmintic programmes in intensively managed N'dama cattle in the Nigeria derived savanna and found that at least one dry season and two wet season treatments were necessary to prevent significant infection and obtain satisfactory growth rates in calves. Unfortunately, these studies do not provide a basis for a firm recommendation of treatment programmes for use in other situations.

2.3.1.3 *Integrated worm control*

Integrated worm control denotes different forms of rotational grazing, with or without anthelmintic drenching at the time of withdrawal of the livestock from the pasture. It is mainly based on two phenomena: the adult worm does not have long life span in the host, and the worm's free-living stages on the pasture, develop at different rates during the various seasons of the year (Van Wyk, 1990b). This control method demands even more careful planning as well as experience and skill in the timing of treatments and movement of stock to clean or safe pastures. Potentially, it offers the best option for economic utilisation of anthelmintics and available pastures as well as reduces the risks of anthelmintic resistance. It is, therefore, ideally suited to modern intensive systems of production but could be adapted to small scale semi-intensive traditional systems such as those involving rotational tethering or other forms of confinement of relatively large numbers of small ruminants to limited grazing plots.

Pasture spelling or rotational grazing, the cornerstone of integrated control, comprises the resting of the pasture until the free-living worm stages no longer constitute an immediate threat to the host. There are various ways in which this reduction in pasture infectivity can

be achieved: classical pasture spelling; alternation of different host species on pasture; alternation of susceptible and insusceptible hosts of same species; alternation of pasture and crop aftermaths; combined alternation of crops and different animal species; and creep grazing for young animals (Van Wyk, 1990b).

The effectiveness of rotational grazing system involving only cattle is questionable (Michel, 1969a; 1976; Morley and Donald, 1980). Under the rotational grazing system part of the pasture will be left ungrazed over a shorter or longer period, and the development and/or survival of free-living parasite stages will be favoured under the shelter of growing herbage. In contrast, continuous grazing of set-stocked animals on pasture eliminate the herbage covering the free-living stages with eventual increased harmful exposures to dessication, sunlight etc. Brunsdon (1980) suggested that production of "safe" pasture by spelling would require an interval of 3 months or more before regrazing. On the other hand, efficient pasture utilization would normally dictate that grazing interval in a rotation could be no longer than 6-8 weeks. Grazing susceptible stock on such pasture after this interval may well subject them to peak levels of larval availability (Bisset *et al.*, 1991).

Better control has been achieved by integrated rotational grazing with different age categories, i.e. the so-called "leader/follower" or "dilution" systems (Leaver, 1970). However, alternate or mixed grazing with two host species, i.e. sheep and cattle, is a feasible control measure in areas with mixed husbandry systems. Both of these methods, which are based on parasite-host specific relationships, will usually reduce pasture infectivity of a given species and show benefits for both host species (Southcott and Barger, 1975; Helle, 1981).

The control of helminth infections in Kenya is largely based on the use of anthelmintics and pasture management is rarely practised. In the high rainfall districts, internal parasite control is often based on repeated curative treatment during times of high

transmission and there has been no attempt to control by strategic, preventive anthelmintic treatment. Drenching is normally done at irregular intervals without following the epidemiology of the parasites. The most common practice is to treat animals, especially young cattle at intervals of approximately 3 months. A large proportion of animals in the arid/semi arid zones is probably never treated with any anthelmintic during their lives because anthelmintics are not affordable or available to all farmers. However, in some areas, peasant farmers and pastoralists are using large amounts of anthelmintics (Kinoti *et al.* 1994). Attempts should be made to test strategic control schemes based on the epidemiological investigations by Allonby and Urquhart (1975) and Straat (1979).

2.3.2 Availability and distribution of anthelmintics

Although there are increasing efforts to develop sustainable control programmes that can reduce the dependence on anthelmintics, it is highly unlikely that these will be completely replaced by alternative farming systems, vaccines or biological control methods. For the short term, at least, protection of the effectiveness and availability of these modern anthelmintics is critical as they have a great potential role to play in the control of helminthosis in the tropics. However, the availability of anthelmintic drugs at prices that are locally acceptable, from values and marketability of animals and animal products, vary much from country to country and from one husbandry system to the other (FAO, 1991).

Anthelmintic treatment against helminth infections in the tropics is faced with two major obstacles, namely, cost of drugs and their proper use at farm level. The first problem may be solved by the manufacturing and marketing of cheaper versions of the efficient anthelmintics (i.e. benzimidazoles) introduced from the 1960's and no longer protected by the original invention patent. Whereas, the second problem is linked with the educational

levels, community infra-structures and activities of agricultural and veterinary extension services (Nansen, 1991).

2.3.2.1 *Dosing methods*

Anthelmintics used against GI nematodes are given by parenteral, oral, topical or intraruminal routes. Oral formulation involves the use of tablets, gels, pastes, drenches or granules or powders for inclusion in-feed or in-water. Accuracy of dose is best achieved by oral or parenteral administration because in-feed or in-water applications are notoriously inaccurate since the greedy animals consume more than others. It is also important to realise that infections often suppresses appetite resulting in low uptake of the drug by infected animals (Abbott *et al.*, 1985).

In-feed methods also run the risk of promoting the development of resistance since overdosing will lead to high selective pressure on resistance genes. In spite of these drawbacks, since handling and dosing are expensive on time and labour, in-feed preparations will continue to be attractive to the farmer. Studies are being done on the incorporation of fenbendazole into molasses "blocks" (Miller *et al.*, 1992; Sanyal *et al.*, 1995).

Oral drenching of ruminants carries with it the risk that portions of the drench will bypass the rumen via the oesophageal reticular groove and hence pass directly to the omasum/abomasum. In some animals this oesophageal groove reflex persists after weaning and will occur in response to drenching. To circumvent this problem a special gun is now available in some countries e.g. Australia (Bogan and Armour, 1986) and Britain (Coopers Autoworm injector) which delivers oxfendazole through the skin and directly into the rumen. This allows the treatment of large numbers of cattle over a short period of time.

Working with oxfendazole, Ali and Hennessy (1993) have shown that anthelmintic

efficacy in sheep can be elevated by temporarily reducing feed intake. In practice, the temporary feed restriction could be achieved by holding animals in a paddock which contained little feed prior to and after drenching. After treatment, this paddock would also serve as a quarantine area which would retain nematode eggs as they are passed in faeces from the treated animals.

Only levamisole and ivermectin are currently available as pour-on topical applications. Levamisole is used for the treatment of GI nematodes of cattle. However, it does not give as good a protection as that of the subcutaneous or oral routes (Sievers and Guzman, 1991). Furthermore, the uptake and hence its efficacy varies considerably depending on the climatic conditions at the time of administration with lower absorption at low temperatures (Forsyth *et al.*, 1983).

2.3.2.1.1 *Slow and pulse release methods*

For the control of endoparasitic nematodes of ruminants and cattle in particular, the development of sustained-release bolus technology for the administration of anthelmintics is viewed as an important advance (Zimmerman and Hoberg, 1988). The use of a bolus allows a measured quantity of drug to be delivered directly to the rumeno-reticulum over an extended time period. This allows for a reduction in labour costs to the farmer and handling stress to the animal while giving sustained anthelmintic cover. These bolus delivery systems are based on either continuous or intermittent release of the anthelmintic drug (McKeller, 1988).

Morantel has been incorporated into two sustained-release devices for cattle. The first was a ruminal bolus (MSRB; Paratect[®] Bolus, Pfizer Inc.) designated to provide delivery of drug for 60-90 days (Jones, 1983). The second, more recent, device (MSRT; Paratect Flex[®]

Bolus, Pfizer Inc.) is a laminated ethylene vinyl acetate sheet containing 11.8g of morantel tartrate. The bolus is administered rolled in a cylinder with a cellophane retaining tape. Following oral administration the tape and the sheet is retained in the reticulo-rumen by virtue of its geometry (McKeller, 1994).

The Paratect Flex^R bolus releases morantel tartrate at a controlled and sustained rate for 90 days (Cardinal *et al.*, 1988) through holes punched in the laminate. It has a more consistent release profile than the solid Paratect^R bolus and has been shown to reach steady state zero-order rate after 10 days (Boettner *et al.*, 1988). The abomasal and ileal concentrations of morantel tartrate remain at approximately $1.0 \mu\text{g ml}^{-1}$ for 98 days after administration (Lanusse *et al.*, 1992). The release profile has been shown to confer protection of first year calves throughout a 90 day grazing period (Vercruysse *et al.*, 1989). A similar device (chronomintic) is also available for delivery of levamisole in the reticulo-rumen of calves (Probert, 1994). Ivermectin is now available as a sustained release bolus for cattle. The Ivomec^R SR bolus (Merk & Co.) is based on the principle of an osmotic pump and has been developed to deliver approximately 8 mg or 12 mg day⁻¹ (for 200 kg or 300 kg animals, respectively) over a 135 day period. The bolus is held within the rumen by high specific gravity conferred by an iron densifier at one end. Steady state plasma concentrations of ivermectin are achieved within 14 days of administration of the bolus and the release-plasma concentration relationship is linear (Pope *et al.*, 1985). The ivermectin bolus has been found to be highly effective against GI nematode and lungworm infections in first-year grazing calves (Schneider *et al.*, 1996).

Bolus preparations are available for the convenient administration of benzimidazoles to cattle and sheep. Weighted benzimidazole boluses for cattle have been designed to release therapeutic doses of drug at time intervals approximately equal to the prepatent period of the

common parasitic nematodes. The oxfendazole pulse release bolus (Repidose, Autoworm, Coopers Animal Health) is designated to release 750 mg or 1.25 g tablets of oxfendazole for 100-200 kg and 200-400 kg cattle, respectively (Mitchell, 1987; Herbert and Probert, 1987).

The most sophisticated pulse release bolus is the E-Bolus (SmithKline Animal Health Products) which is an electronic device delivering three doses of albendazole at 31 days intervals to cattle. Contact with the ruminal fluids activates a timing device run by alkaline batteries which after 31 days interval generates a gas (mainly carbon dioxide) which drives out the dose of anthelmintic (Gyurik and Bagnall, 1986). A biodegradable fenbendazole sustained release bolus is now available for use in cattle. Approximately 0.2-0.4 mg of fenbendazole per kilogram body weight per day are released over 130 days. This bolus has been shown to prevent dictyocaulosis and heavy infection with GI trichostrongylids, thus conferring a production benefit (Downey *et al.*, 1992).

The effect of slow release formulations on the development of resistance has yet to be assessed but it seems likely that it will be enhanced by their use (Anderson, 1985; Donald, 1985). On the other hand, the successful use of Paratect^R for more than ten years has yet to be associated with reports of resistance (Coles *et al.*, 1994). Donald (1983) suggested that these devices may not be hazardous in this respect provided the total release time is less than the maximum life span of the parasite's free-living stages, there is high kill rate and the release rate declines rapidly to zero at the end of its life. The device has to be infrequently used (Probert, 1994). Sustained release devices are now being offered in the developing countries at prices that are competitive with the oral formulations (FAO, 1991).

2.3.2.2 *Anthelmintic characteristics of morantel and albendazole*

2.3.2.2.1 *Morantel*

Morantel and pyrantel are anthelmintic compounds that belong to a family classified as tetrahydropyrimidines (Lynch and Bartolucci, 1982). Morantel (1-4-5-6-tetrahydro-1-methyl-2-[trans-2-(3-methyl-2-thienyl) vinyl] pyrimidine) is a methyl ester analogue of pyrantel and is formulated as the tartrate salt for the control of nematode infections in domestic animals.

Studies investigating the mechanism of action of morantel show that the drug inhibits the enzyme, fumarate reductase, which plays an important role in energy metabolism of parasites; the enzyme is not present in the tissues of the vertebrate host (Behn and Bryant, 1979). Additionally, morantel affects the nervous system of nematodes causing contraction and death (Coles, 1977).

Morantel is a broad-spectrum group 2 anthelmintic highly effective against most nematodes of ruminants (Jones *et al.*, 1978; Pott *et al.*, 1979). Morantel tartrate at 10 mg had high efficacy against adult and immature stages of the GI nematodes, including *Nematodirus battus*, in cattle and sheep. Also, low level administration in feed (1.5 mg/kg day⁻¹) resulted in reduced faecal egg counts and post mortem worm burden in cattle and sheep (Jones *et al.*, 1978). In lactating grazing adult dairy cows given a morantel sustained-released bolus, Bliss *et al.* (1982) reported a highly significant improvement in milk production, milk fat and protein content compared with untreated animals.

2.3.2.2.2 *Albendazole*

Albendazole (methyl[5-(propylthio)-1H-benzimidazole-2-yl] carbamate is a potent member of the benzimidazole group of anthelmintics with broad spectrum activity against GI

nematodes including larval stages, tapeworms, liver flukes, and lungworms in many host species (Theodorides *et al.*, 1976; Williams *et al.*, 1977; Wescott, *et al.*, 1979).

Albendazole has been widely used in veterinary medicine and human clinical medicine as a safe anthelmintic with high activity against larval and adult stages of many helminth parasites. In addition to its vermicide and larvicide properties, albendazole is also ovicide and attention has focused on its use in systemic helminth infections such as hydatid disease, cysticercosis and systemic nematode infections of man (Horton, 1990). The mode of action in such parasitic infections is believed to derive from the inhibition of tubulin polymerization into microtubules, with a cascade of other metabolic effects resulting from this (Lacey, 1990). Table 2.4 shows anthelmintics available for the control of nematodes in cattle.

2.3.3 Anthelmintic resistance

Anthelmintic resistance in nematodes can be a problem in small ruminants, horses, cattle and swine. The most widespread resistance problems occur to benzimidazole anthelmintics in nematodes of sheep, goats and horses (Prichard, 1994; Waller *et al.*, 1996). In contrast to the situation in sheep and goats, resistance has been slow to develop in cattle (Craig, 1993). Barger (1993a) suggests that bovine dung-pats may provide a relatively larger refugia of susceptible infective larvae, and hence, reduce the proportion of the population exposed to anthelmintic selection. Alternatively, less frequent use of anthelmintics in this host may minimize selection pressure (Waller, 1994). It has been suggested (Eagleson and Bowie, 1986; Waller, 1993a) that selection for resistance in some parasites of cattle occurs in other species, such as goats, which can harbour species of nematodes common to both species. As noted in Table 2.5, few reports of resistance are available, but in many cases the parasite identified as being resistant is *O. ostertagi*. Since, with a single exception, all of the reports

of resistance to date are for levamisole/morantel or benzimidazole, both of which provide less than optimal protection against inhibited larval stages, it is possible that resistance has developed in response to what amounts to subtherapeutic dosing of inhibited larvae (Conder and Campbell, 1995). Populations of *Haemonchus* spp., *T. axei* and *C. oncophora* resistant to the benzimidazoles have also been identified sporadically. Boersema (1983) reported a strain of *D. viviparus* resistant to levamisole, and Watson (1993) has identified a *Cooperia* spp. resistant to ivermectin.

There is ample evidence that anthelmintic usage in certain regions of sub-Saharan Africa is high, particularly during the rainy seasons and this may lead to development of widespread, and possibly high levels of anthelmintic resistance (Waller, 1993b). This view is supported by recent reports of anthelmintic resistance and multiple anthelmintic resistance in Tanzania (Bjorn *et al.*, 1991), Cameroon (Ndamukong and Sewell, 1992), Nigeria (Mbah *et al.*, 1992) and Kenya (Njanja *et al.*, 1987; Mwamachi *et al.*, 1995; Wanyangu *et al.*, 1996). In Africa, the development of anthelmintic resistance and drug resistance in general, is further compounded by the use of drug preparations of questionable efficacy (Baker, 1995b; Wanyangu *et al.*, 1996).

2.3.3.1 *Factors associated with resistance*

The main factors associated with anthelmintic resistance are frequent usage of anthelmintics, underdosing and continuous use of anthelmintics from the same class, irrespective of whether different brands or formulations were used. Other factors such as the relative importance of parasite species, the percentage of the parasite population which is under selection pressure, animal management practices and the degree to which resistance has been specifically investigated, are also of significance in relation to the magnitude and

Table 2.4: Anthelmintic available for the control of nematodes in cattle^a

Chemical	Trade Name	Gastrointestinal		Nematodes		Dose (mg kg ⁻¹)
		Adults	Developing larvae	Inhibited larvae	Lungworms	
Thiabendazole	Thibenzole	+	+	±	±	66-110
Parbendazole	Topclip	+	+	-	-	20-30
Oxibendazole	Anthdworm	+	+	-	-	10
Cambendazole	Novazole	+	+	-	-	25
Fenbendazole	Panacur	+	+	+	+	7.5
Oxfendazole	Systemex	+	+	+	+	4.5
Albendazole	Valbazen	+	+	+	+	7.5
Febantel	Bayverm	+	+	±	+	7.5
Thiophanate	Nemafax	+	+	±	±	66-132
Levamisole	Nilverm	+	+	-	+	7.5
Ivermectin	Ivomec	+	+	+	+	0.2
Morantel*	Paratect	+	+	+	-	90 day ⁻¹ for 60days

+ = effective; ± = variable activity; - = inactive.

Febantel, fenbendazole, oxibendazole and albendazole are also active against tapeworms while albendazole shows variable activity against liveflukes and ivermectin shows activity against ectoparasites.

*Used as a slow release bolus.

^aBased on Marriner and Armour (1986).

prevalence of anthelmintic resistance (Waller, 1987). There are also justifiable grounds for concern that the trend to use anthelmintic sustained-release devices may accelerate the appearance of resistance in bovine parasites (Donald, 1985).

2.3.3.2 *Detection of resistance*

Several *in-vitro* techniques have been developed for the detection of anthelmintic resistance, but the procedure of choice for field survey investigation is the faecal egg count reduction test (FECRT) (Presidente, 1985; Waller, 1986; Johansen, 1989). FECRT involves the treatment of naturally infected animals and can be used with ruminants, horses and pigs, with all types of anthelmintics and with all species of nematodes in which eggs are shed in the faeces. To overcome obvious limitations and deficiencies of this procedure (Waller, 1986; McKenna, 1987; Lacey *et al.*, 1990), guidelines have been recommended for all phases of the procedure, and standardised microcomputer software has been developed so that data on the epidemiology of resistant nematodes can be easier to produce and results widely accepted (Coles *et al.*, 1992).

2.3.4 **Alternative control methods**

Resistance to anthelmintic drugs (Waller, 1987) and demand for lower levels of chemical residues in livestock products and in the environment (Strong *et al.*, 1996), together with diminishing prospects for new classes of drenches, has stimulated interest in helminth control methods which are less reliant on chemotherapy. In Africa the high cost and poor availability of effective anthelmintics are further limitations on their use. Thus, there is considerable incentive to develop alternative methods of control. This could be attractive if such alternatives could be effected at lower costs.

Table 2.5: Nematodes of cattle with reported resistance to broad-spectrum anthelmintics

Nematode	Country	Anthelmintic Class ^a		
		Benzimidazoles	Levamisole/Morantel/Pyrantel	Macrocylic lactones
<i>Haemonchus</i> spp.	Brazil	1	- ^b	-
<i>Ostertagia ostertagi</i>	Australia	2,3	2	-
	Belgium	-	4	-
	New Zealand	5	-	-
	United States	-	6,7	-
<i>Trichostrongylus axei</i>	Australia	8,9	-	-
	New Zealand	10 ^c	-	-
<i>Cooperia onchophora</i>	New Zealand	5,11	-	12 ^c
<i>Dictyoculus viviparus</i>	Belgium	-	13	1

^aReferences: (1) Pinheiro and Echevarria, 1990, (2) Anderson, 1977, Anderson and Lord, 1979, (4) Geerts *et al.*, 1987, (5) Hosking and Watson, 1991, (6) Williams, 1991, (7) Williams *et al.*, 1991, (8) Eagleson and Bowie, 1986, (9) Eagleson *et al.*, 1992, (10) McKenna, 1991, (11) Jackson *et al.*, 1987 (12) Watson, 1993, (13) Boersema, 1983.

^b-, No known report. ^cGenus but not species identified.

2.3.4.1 *Prospects of vaccines*

The only effective vaccines against parasitic nematodes in domestic animals depend on the use of irradiated larvae and whilst these have been successful against lungworms (Peacock and Poynter, 1980; Dhar and Sharma, 1981; Armour, 1987) and successful experimentally, although not commercially, against canine hookworm (Miller, 1978), they are not effective against nematodes as pathogenic as *H. contortus* in lambs. However, with the advent of DNA technology, considerable progress towards development of commercially available vaccines for the control of helminth parasites of domestic animals has been made for the last few years particularly with species that infect ruminants (Dineen, 1985; Emery and Wagland, 1991). The publication by Johnson *et al.* (1989), detailing the development of a vaccine against *Taenia ovis* infection in sheep, was the first to describe a highly effective recombinant vaccine against a parasite. The vaccine remains the most effective of the defined antigen vaccines described to date (Lightowers, 1994). Current work on a commercial recombinant vaccine against *F. hepatica* in ruminants appears to stand a good example for the development of recombinant vaccine in general (Zahner, 1994).

One approach towards the immunological control of blood-feeding nematodes like *H. contortus* has been to immunize the host with parasite gut proteins (Smith, 1993). Antigens associated with the gut of *H. contortus* have been shown to be capable of inducing host protective immune response in vaccinated sheep (Tavernor *et al.*, 1992a,b). Antigen H11, an integral membrane glycoprotein in the intestinal microvillar membrane has been characterised, and expressed in baculovirus (Munn *et al.*, 1993a; Newton, 1995). Gut associated antigens, rich in H11, have been tested experimentally in Merino Sheep in South Africa with high efficacy (Munn *et al.*, 1993b). Development and field-testing of a commercial product with suitable adjuvants is required (Reinecke, 1994). Vaccines will form

part of sustainable comprehensive control programmes and will integrate with drenching, grazing management, biological control methods and genetic selection to prolong the effective life of anthelmintics, protect highly susceptible young stock and reduce contamination of pasture in successive seasons and generations of grazing livestock (Emery *et al.*, 1993; Barnes *et al.*, 1995). The use of vaccines however, will require careful cost-benefit analysis, based on thorough epidemiological knowledge, prior to instituting vaccine-based control programmes (FAO, 1991).

2.3.4.2 Genetic resistance

Another potential source for additional helminth parasite control is the enhancement of genetically-based resistance to infection. Considerable research has been carried out on sheep, less so on cattle, and the result clearly demonstrated that there is considerable variability both between and within breeds in resistance to infection and its impact on certain production traits (Esdale, *et al.*, 1986; Owen and Axford, 1991; Baker *et al.*, 1993).

The most comprehensive research on breed difference has been carried out at the CSIRO Tropical Cattle Research Centre at Rockhampton, Australia (Vercoe and Frisch, 1992). It has been clearly documented that *Bos indicus* cattle (i.e. Brahman and Brahman crosses) are more resistant to both GI nematodes and tick (*B. microplus*) than *Bos taurus* breeds (Hereford and Shorthorn). In West Africa there is also some limited evidence that the trypanotolerant N'Dama cattle are more resistant to endoparasites than Zebu cattle (Claxton and Leperre, 1991). Ouedraogo *et al.* (1991) detected lower *H. contortus* burdens in Baole' than Zebu cattle in Burkina Faso.

It has been shown that variation in resistance to nematode infection within sheep breeds is as great as between breeds (Barger, 1989). In cattle, Ross and colleagues (1960b)

identified a Zebu-bull in Nigeria whose progeny showed much better weight gain and lower worm egg counts (epg) than-offspring of other bulls of the same breed. Much later, Kloosterman and colleagues (1978) in Holland and Australian workers have found significant differences in resistance to experimental infections to *C. oncophora* or to other worms within the same breed (Barger *et al.*, 1983). More recently, Kaufmann and colleagues (1990) made similar observations with N'Dama-bulls after experimental and natural infections with GI nematodes.

As genetic variation in resistance to nematode infections within breeds can be as great as that between breeds (Barger, 1989; Kaufmann *et al.*, 1990), Pfister (1991) suggested that breeding programmes in developing countries should concentrate on genetic improvement of local indigenous breeds. However, there is limited evidence on the amount of genetic variation within indigenous African breeds for resistance to endoparasites (Baker *et al.*, 1993). Thus, it is therefore likely that breeding programmes in Africa will utilize both between- and within-breed genetic variations for resistance to nematode infections (Baker, 1995).

Pfister (1991) posed a number of questions which need to be answered before breeding programmes for resistance to endoparasites in Africa can be implemented successfully. These include socioeconomic issues, profitability and the acceptability of programmes by local livestock owners. The breeding programme proposed by Pfister (1991) did not envisage stopping the use of anthelmintics, but the development of genetically resistant animals which would receive fewer anthelmintic treatments. Pfister's proposal was for on-farm recording and evaluation but this scheme could be increased in scope to include aspects of a group breeding system, including a nucleus herd as suggested by Cummins and colleagues (1991).

2.3.4.3 *Medicinal plants*

The effects of herbage, or plant extract, on parasites have been known for a long time and many traditional de-worming preparations currently used by livestock owners in the tropics and subtropics are based on such materials (Hammond *et al.*, 1997). Pasture plants such as legumes, have been reported to have anthelmintic properties, and at certain stages of growth, grasses and forage crops appear to act as vermifuges (Anderson *et al.*, 1987). Recently there has been considerable interest generated following the studies of Niezen and colleagues (1996) which suggests that forages containing condensed tannins provide sheep with the ability to withstand parasite infection. This may be due to direct anthelmintic properties of tannins, or most probably due to the role of tannins in protecting dietary protein from ruminal degradation and thus animals are on a better plane of nutrition (Waller, 1997a).

In Indonesia, extensive studies have been conducted to investigate materials from papaya trees for anthelmintic properties. A high efficacy of papaya latex was demonstrated against *Ascaridia galli* in chicken (Mursof and He, 1991), against *Ascaris suum* in pigs (Satrija *et al.*, 1994) and against *H. contortus* in sheep (Murdiati and Beriajaya, 1997).

In Kenya, medicinal plants are used especially among the pastoralists, who claim that certain plants eliminate tapeworms; their only evidence of helminthosis. However, the specific anthelmintic efficacy of these plants and herbal preparations has not been experimentally documented.

2.3.4.4 **Biological control**

Control of GI nematodes of domestic livestock is directed mainly at the parasitic forms in the host using anthelmintic products. However, to complete their life cycle, parasitic nematodes have to develop through a series of free-living stages on pasture. It is within this

nvironment that there is a wide variety of natural enemies that are constantly regulating their population. Bacteria, viruses, protozoa, other nematodes and fungi are among the principal natural enemies of nematodes in the soil (Tribe, 1980; Pryodko *et al.*, 1985). In the past, most interest have been focused on those organisms producing chemical toxins which have been developed as anthelmintics, for example, *Streptomyces avermitilis*/ivermectins (Benz and Ernst, 1979). However, the threats of development of anthelmintic resistance, public concern about chemical residuals in animal products and in the environment (Herd *et al.*, 1993; Strong *et al.*, 1996) has stimulated attempts to develop alternative methods of PGE control. One possible non-chemotherapeutic approach is biological control using nematophagous fungi.

2.3.4.4.1 *Nematophagous fungi*

The nematode-destroying fungi are natural enemies of nematodes, capable of capturing, killing, and digesting them. The nematodes have a due function in this relationship, of serving as prey and also, triggering fungal development in a way that infective structures (traps or conidia) are formed (Nordbring-Hertz and Jansson, 1984). They are commonly found world wide, occurring in natural and agricultural soils, in old faecal deposits and all kinds of decaying organic materials (Gray, 1983).

Nematophagous fungi consist of over 150 species which include the nematode-trapping, or predacious fungi and the endoparasitic fungi, being the most important groups. Others include fungal parasites of cyst and root-knot nematodes of plants which invade eggs or females by vegetative hyphae (Nordbring-Hertz, 1988), and fungi that produce metabolites which are toxic to nematodes.

2.3.4.4.2 *Endoparasitic fungi*

According to Barron (1977), endoparasitic fungi belong to the classes *Phycomycetes* (*Oomycetidae*, *Chytridiomycetidae*, *Zygomycetidae*), *Basidiomycetes* and *Deuteromycetes*. Endoparasitic fungi infect nematodes by means of spores. They have no extensive hyphal development outside the host except for fertile hyphae, such as evacuation tubes or conidiospores that release the spores.

2.3.4.4.3 *Nematode-trapping fungi*

Nematode-trapping fungi produce trapping organs such as constricting (active) or non constricting (passive) rings, sticky hyphae, sticky knobs, sticky branches or stick networks along the vegetative hyphal system. Predacious fungi are prevalent in the class *Deuteromycetes*, especially in the group *Hyphomycetales*. Others belong to the class of *Basidiomycetes* (Barron, 1977).

Building of trapping organs forms the basis for the activity of the predacious fungi. Some species form traps spontaneously while, most species only form traps when induced by extracts from nematodes, chemical compounds, soils or earthworms (Rosenzweig, 1984; Dowe, 1987). However, the living nematodes are still the most potent trap inducers (Nordbring-Hertz and Jansson, 1984).

Lectins (Nordbring-Hertz and Mattiasson, 1979; Borrebaeck *et al.*, 1985) as well as an adhesive compound (Tunlid *et al.*, 1991) have been shown to be involved in the actual capture of nematodes. *A. oligospora* secretes a nematotoxin which paralyses or kills trapped nematodes (Olthof and Estey, 1963). Recent studies suggest that in *A. oligospora*, serine proteases are involved in the process of immobilization of nematodes (Tunlid and Jansson, 1991).

The use of nematophagous fungi as a possible means of biological control of parasitic nematodes of domestic livestock has been postulated (Roubaud and Deschiens, 1941; Fernandez *et al.*, 1985; Pryadko and Osipov, 1986; Grønvold *et al.*, 1985; 1988; 1989; Ilyaletdinov and Pryadko, 1990; Larsen *et al.*, 1992).

In one of the earliest *in-vitro* studies conducted by Roubaud and Deschiens (1939), it was showed that the fungus *Dactylella ellipsospora* exhibited nematophagous activity against infective larvae of *Ancylostoma duodenale* and *Strongyloides* spp. These workers also demonstrated the value of predatory fungi, *D. ellipsospora* and *A. oligospora* in the control of *S. papillosus* and *Bunostomum* spp. (Roubaud and Deschiens, 1941).

As reported by Soprunov (1966), Russian workers examined the impact of *Arthrobotrys*, *Trichothecium* and *Dactylaria* spp. when added to faecal cultures derived from a strongyle infected horse. *Arthrobotrys* spp. was the most effective in reducing the number of larvae.

Parnell and Gordon (1963) reported that the predacious fungus, *Acrostalagmus verticillium* markedly reduced *H. contortus* larvae in faecal cultures derived from sheep. However, administration to sheep of material from faecal cultures containing the fungus gave inconclusive results.

Pandey (1973) examined the nematode-trapping capability of ten fungi (*A. oligospora*, *Dactylaria brochopaga*, *D. gamsospora*, *D. polycephala*, *D. thaumasia*, *D. vermicola*, *Monacrosporium (Dactylella) bembicodes*, *M. cionopaga*, *M. ellipsospora* and *Trichothecium cystosporium*) against the infective larvae of *O. ostertagi* and *T. axei*. Infective larvae were attacked by all the 10 microfungi and species producing adhesive networks and adhesive

branches were the more efficient predators than those forming constricting rings and adhesive knobs. The most efficient predatory fungus was *D. thaumasia* while, *D. vermicola* seemed almost without any effect (Pandey, 1973).

Charles and colleagues (1996) reported that the endoparasitic fungus *Harposporium anguillulae* which colonizes cattle pats, was 99.5% effective in reducing *H. contortus* L₃ in sheep faecal cultures. *H. anguillulae*, which can be produced artificially in pure culture (Aschner and Kohn, 1958) have an advantage in watery substrates when compared to predatory nematode-destroying fungi, as their spores are easily dispersed and have a high likelihood of being ingested by nematodes. Also, oral infection allows the fungus to spread to other locations in the habitat, and once the nematode population declines, *Harposporium* also dies off (Glockling, 1993). The above advantages are applicable to the control of cattle strongylid nematodes, because cattle faeces have a high watery content (Charles *et al.*, 1996).

Working with *A. oligospora*, one of the most common nematode-trapping fungi in Danish agricultural soils (Shepherd, 1961), Nansen and colleagues (1986) demonstrated that *A. oligospora* entrapped parasitic stages of *C. oncophora* and the free-living nematodes *Panagrellus redivivus* and *Rhabditis wohlgemuthi* with the same efficiency. The parasitic and free-living nematodes were comparable in their ability to induce trap formation in the fungus. The same workers examined the trap-inducing capabilities of L₃ of nine animal parasitic nematodes, and they revealed that the ability of L₃ of *C. oncophora* and *O. ostertagi* from cattle, *H. contortus*, *C. curticei* from sheep, and *Cyathostoma* spp. from horses to induce traps was high compared with L₃ of *O. dentatum* and *O. quadrispinulatum* from pigs and *Nematospiroides dubius* from mice. The trap-forming potential of the slow moving *Dictyocaulus viviparus* was poor, suggesting that nematode species with highly motile larvae are the most effective inducers of trap formation. It was also shown that larvae of all

parasitic nematodes were rapidly captured in pre-formed traps (Nansen *et al.*, 1988).

Recent studies conducted using *H. contortus* L₃ in sheep faecal cultures demonstrated that addition of 20,000 conidia of *Monacrosporium endermatum*, *A. oligospora* and *A. robusta* g⁻¹ of faeces caused a reduction of 95.7%, 98.3% and 10.1%, respectively, compared with the control group. Whereas, a 97% reduction was observed when combined conidia of the three fungi were used (Mendoza-De and Vazquez-Prats, 1994). The addition of the three fungi at 100,000 conidia g⁻¹ of faeces resulted in total reduction of the larval population.

The ability of *A. oligospora* and *A. flagrans* (syn. *Trichothecium flagrans*, *Duddingtonia flagrans*), to control the development of L₃ in faeces from naturally infected horses was recently assessed by Bird and Herd (1995). The two fungal species, significantly reduced the number of infective cyathostome L₃ in horse faeces at concentration of 10 and 100 spore egg⁻¹. These findings supported the work of Nansen and colleagues (1988) showing that L₃ were able to induce trap formation, become entrapped and killed by *A. oligospora*.

A series of controlled plot experiments conducted in Denmark have shown that admixture of *A. oligospora* directly to cattle faeces may confer a significant reduction in the numbers of L₃ that develop from eggs of *O. ostertagi* and *C. onchophora* (Grønvold *et al.*, 1987; 1988; 1989). In a field study, Grønvold *et al.* (1987) inoculated cow pats containing mycelial fragments and conidial spores of *A. oligospora* and observed a 10-fold reduction in the number of *C. onchophora* L₃ in inoculated cow pats, and surrounding herbage relative to the control fungal-free pats. Using conidial spores only, Grønvold *et al.* (1988) found that L₃ of *O. ostertagi* were also significantly reduced in inoculated cow pats, and surrounding herbage compared with fungal-free control pats. These results showed that the conidia of *A. oligospora* were able to germinate and develop trapping organs in cow pats. Working with *O. ostertagi*, Grønvold and colleagues (1989) studied the effects on parasite control by

monitoring calves grazing either on a control plot or a plot in which cattle faeces containing 0.25 g mycelial fragments of *A. oligospora* kg⁻¹ faeces was deposited. The calves on treated plots were exposed to a lower level of parasitism compared to calves on the control plots, as shown by lowered L₃ numbers on pasture, lower epg counts, lower pepsinogen levels and higher body weights (Grønvold *et al.*, 1989).

The results of Grønvold and colleagues (1987; 1988; 1989) indicate that the prospect of practical biological control of GI nematodes of ruminants could be enhanced markedly, if the nematophagous fungi could pass through the GI tract without loss of viability. Thus, the effect of introducing predacious fungi into fresh faeces by feeding fungal material to various hosts has been investigated by several workers. Gruner and colleagues (1985) and Peloille (1991) reported that the nematode-trapping fungi *Dactylaria candida*, *Candelabrella (Arthrobotrys) musiformis*, *A. tortor* and *D. flagrans* fed to sheep on millet grains successfully passed the alimentary tract of sheep. Investigations conducted in Russia have shown that *A. oligospora* grown on chopped corn passed the alimentary tract of donkey (Sopruncov 1966) and when *A. arthrobotryoides* and *A. flagrans* were fed to ewes over a period of 3 days, they were effective in reducing the number of GI nematode and lungworm larvae in the faeces (Pryadko and Osipov, 1986). Hashmi and Connan (1989) reported that conidia of *A. oligospora* given by mouth were passed alive in faeces of cattle. In contrast, Descazeaux and Capelle (1939) found no survival of *A. oligospora* and *Dactylella bembicodes* given to horses and guinea pigs, and in recent studies conducted in Denmark, *A. oligospora* was unable to pass the alimentary tract of cattle alive (Grønvold *et al.*, 1993b).

As a means of screening predacious fungi for gut survival capabilities, Larsen and colleagues (1991) developed an *in-vitro* assay designed to mimic environmental stresses of rumen and abomasal passage. This study proved valuable in identifying *D. flagrans* as being

superior to *Arthrobotrys* spp. which was confirmed in subsequent feeding trials in calves (Larsen *et al.*, 1992). In a semi-natural plot experiment, *D. flagrans* isolates when fed to calves resulted in a significant reduction in numbers of *O. ostertagi* L₃ transmitted to the herbage from deposited dung pats (Grønvold *et al.*, 1993a).

Several field studies have corroborated the findings of Grønvold and colleagues (1993b) on the effectiveness of *D. flagrans* as a potential biocontrol agent against GI nematodes of livestock, especially cattle. In an attempt to control *O. ostertagi* in calves exposed to natural pasture infection, it was shown that feeding of calves with a *D. flagrans* isolate reduced herbage infectivity and worm burdens in the animals (Wolstrup *et al.*, 1994).

In this experiment, where calves were fed daily with fungal material through the initial 2 months of the season, results comparable with those of anthelmintic strategies commonly applied in north-west Europe were obtained. In a subsequent investigation, it was shown that strategic feeding of first season calves with *D. flagrans* over the first three months of the grazing season was able to prevent severe clinical trichostrongylosis in the late summer.

The results showed that larval populations of *Ostertagia* and *Cooperia* were significantly reduced on the pasture grazed by fungal-treated calves. In contrast, the number of *Nematodirus* larvae seemed less affected (Nansen *et al.*, 1995). A study conducted in the 1993 grazing season with yearling calves exposed to a pasture with a natural mixed trichostrongyle larval infection, has shown that daily feeding with *D. flagrans* during the first 2 months of the season led to a lowered herbage infectivity and a reduced acquisition of *Ostertagia* spp. and *Cooperia* spp. later in the season. In addition, the procedure delayed the onset of clinical disease (Larsen *et al.*, 1995a).

In another experiment, a study was undertaken to examine the potential of *D. flagrans* to survive passage through the GI tract of horses and subsequently to destroy free-living stages of cyathostomes in faecal cultures. Results showed a positive relationship between dose level and reduction in the number of L₃. Fungi were recovered in faeces at times which

corresponded to high larval reduction (Larsen *et al.*, 1995b). Other recent studies have shown that *D. flagrans* is highly effective against GI nematodes of pigs (Nansen *et al.*, 1996), horses (Larsen *et al.*, 1996) and sheep (Githigia *et al.*, 1997). The effect of an artificial increase in nematophagous fungi in the environment is likely to be short lived due to ecological factors that limit fungal populations in the field (Cooke and Satchuthananthavale, 1968). This transience should prevent ecological problems that might be associated with the nonspecific, free-living nematodes in the field.

-Chapter 3-

PREVALENCE AND INTENSITY OF HELMINTH AND COCCIDIAL INFECTIONS: A FIELD SURVEY

3.1 Introduction

Helminth and coccidia infections in cattle in Kenya are thought to be widespread (Froyd, 1959; Mango *et al.*, 1974; Omara-Opyene, 1985; Ndarathi *et al.*, 1989). However, information on the prevalence and intensity of particularly subclinical infections are limited.

Haemonchus spp. has been reported as the predominant species in cattle under nomadic management in the arid Marsabit District of northern Kenya (Omara-Opyene, 1985). In more intensive grazing systems (agroclimatic zones 2 and 3, with medium altitude and bimodal rainfall), *Cooperia*, *Trichostrongylus* and *Haemonchus* spp. were found to be numerically dominant in Nyeri District (Gatongi *et al.*, 1987); and *Haemonchus* and *Trichostrongylus* spp. in Nyandarua District (Maingi and Gichigi, 1992). In an abattoir survey of parasitic worms of slaughtered cattle throughout Kenya, *Haemonchus* spp. was found to have the highest national prevalence followed by *Cooperia* spp. (Mango *et al.*, 1974). Elsewhere, the helminths listed above are known to depress growth rates in cattle when burdens are sufficiently high, and Falvey and Bambridge (1975) found that gastrointestinal helminthosis reduce liveweight gain in 6-month-old cattle grazing improved pastures. Apart from the limited observations reported, no detailed epidemiological study has been conducted in central Kenya. Thus, a preliminary survey was conducted to provide basic information on the identity, prevalence and intensity of helminth and coccidia infections in dairy cattle. The effect of age, sex, farm and season on the occurrence and distribution of these parasites was also determined as this information is important in formulating control strategies.

3.2 **Materials and methods**

3.2.1 *Study area*

3.2.1.1 *Location and size*

This study was conducted in Kiambu District (which included Gatundu and Thika divisions of the newly created Thika District), one of the seven districts in Central Province. It is located at the southern part of the province and has a total area of 2451 km². The district shares common boundaries with several districts both within and outside Central Province (Fig. 3.1a) and lies between 0° 25' and 1° 20' south of the equator 36° 31' and 37° 15' East. Fig. 3.1b shows the administrative divisions of the district.

3.2.1.2 *Topography and geology*

The district is divided into four broad topographic regions viz. upper highland zone, lower highland zone, upper midland zone and lower midland zone.

The upper highland zone is found in Lari Division and is an extension of the Aberdare Range and lies at an altitude of 1800 m above sea level. It is dominated by highly dissected ranges and the soils are of high fertility and well drained. The area has very reliable rainfall and is generally a sheep and dairy cattle zone though various food crops and fruits are also grown.

The lower highland zone is mostly found in Limuru and parts of Gatundu, Githunguri and Kikuyu divisions. The area is characterized by hills, plateaus and high level structural plains. The soils are of moderate high fertility, well drained, though in some places they are imperfectly drained. The area lies between 1500-1800 m above sea level and is generally a tea-dairy cattle zone though crops like maize, pyrethrum, horticultural crops, fruits and sheep farming are practised.

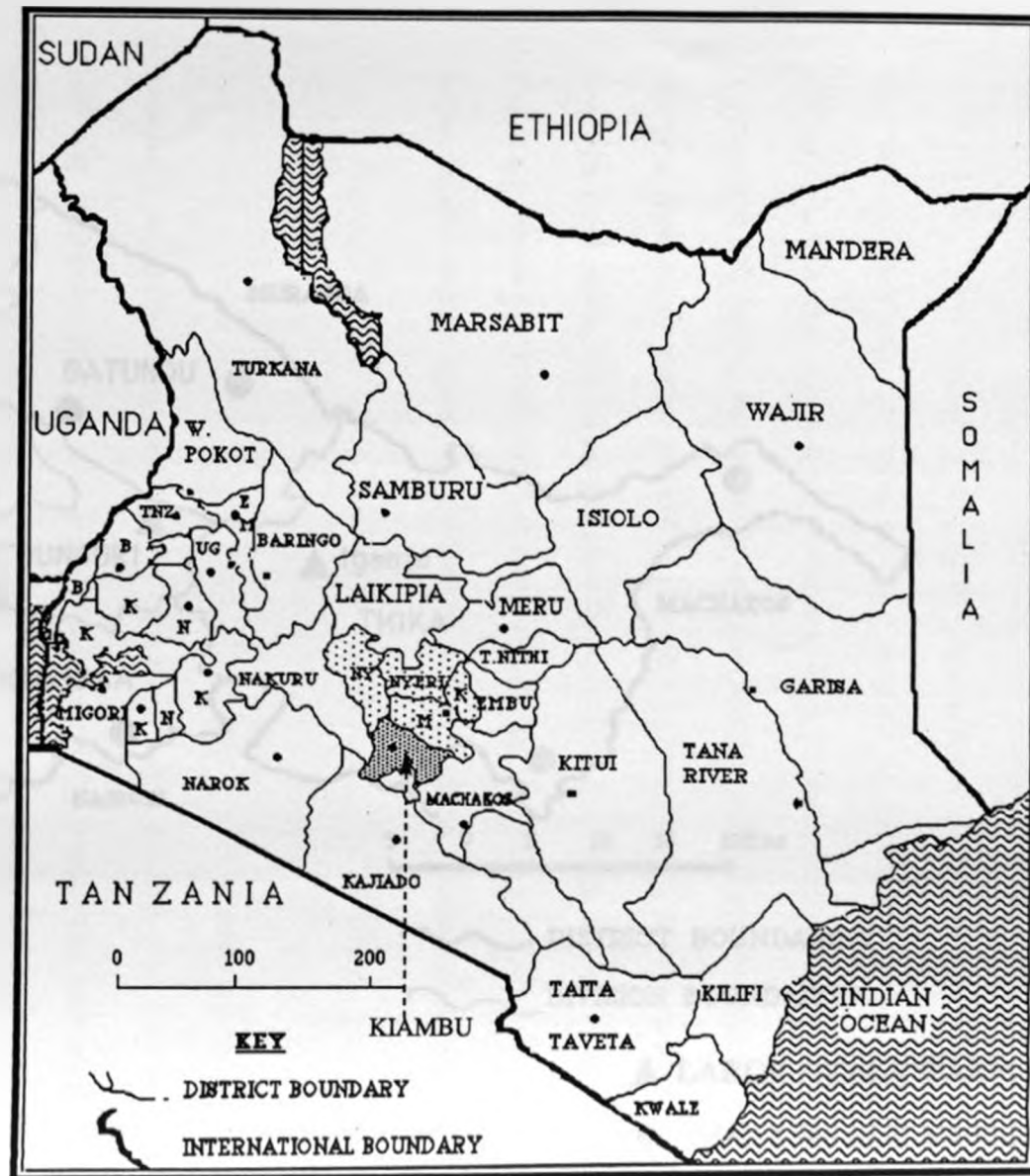


Fig. 3.1a. Sketch map of Kenya showing location of Central Province and Kiambu District

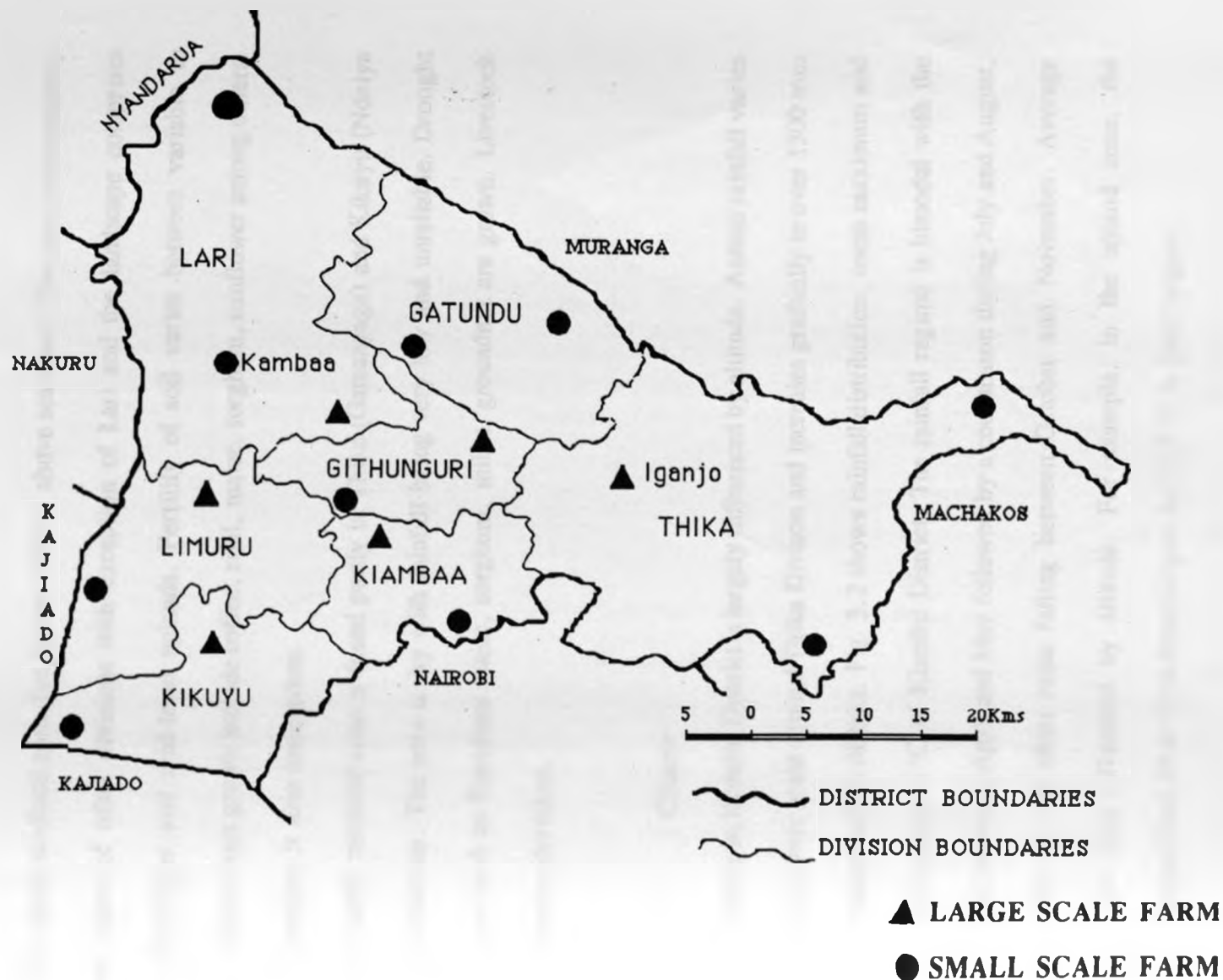


Fig. 3.1b. Sketch map of Kiambu District showing administrative divisions and approximate location of farms used for this study

The upper midland zone lies below 1500 m above sea level. The zone covers mostly Thika and parts of other divisions with exception of Lari and the landscape comprises volcanic footridges and mid-level uplands. Fertility of soil varies between variable to moderate. Main crops grown include coffee, sisal, maize, sorghum, sunflower among others. Livestock keeping is also undertaken.

The lower midland zone is found partly in Thika (Gatuanyaga) and Kikuyu (Ndeiya and Karai) divisions. The area is dry with rainfall being very low and unreliable. Drought resistant crops such as Katumani maize, sorghum, millet, groundnut are grown. Livestock keeping is also undertaken.

3.2.1.3 *Climate*

The climate in Kiambu District is largely influenced by altitude. Annual rainfall varies from 500 mm in lower areas around Thika Division and increases gradually to over 1300 mm in the upper regions of the district. Fig. 3.2 shows rainfall distribution, mean maximum and minimum temperatures in °C of Kiambu District. The rainfall regime is bimodal with the long rains falling between April and May followed by a cool season during July and August, which culminates to the short rains falling between October and November. Average temperatures are also influenced by altitude. For example, in the upland zone, the temperature ranges from 20.4 °C in March/April to 12.5 °C in July/August.

3.2.1.4 *Livestock production*

The majority of farmers in Kiambu District are smallholders, covering 94,820 ha and practising mixed agriculture, including livestock production, and food and cash crops. With a population of 1,012, 438 it has a population density of 463 persons km².

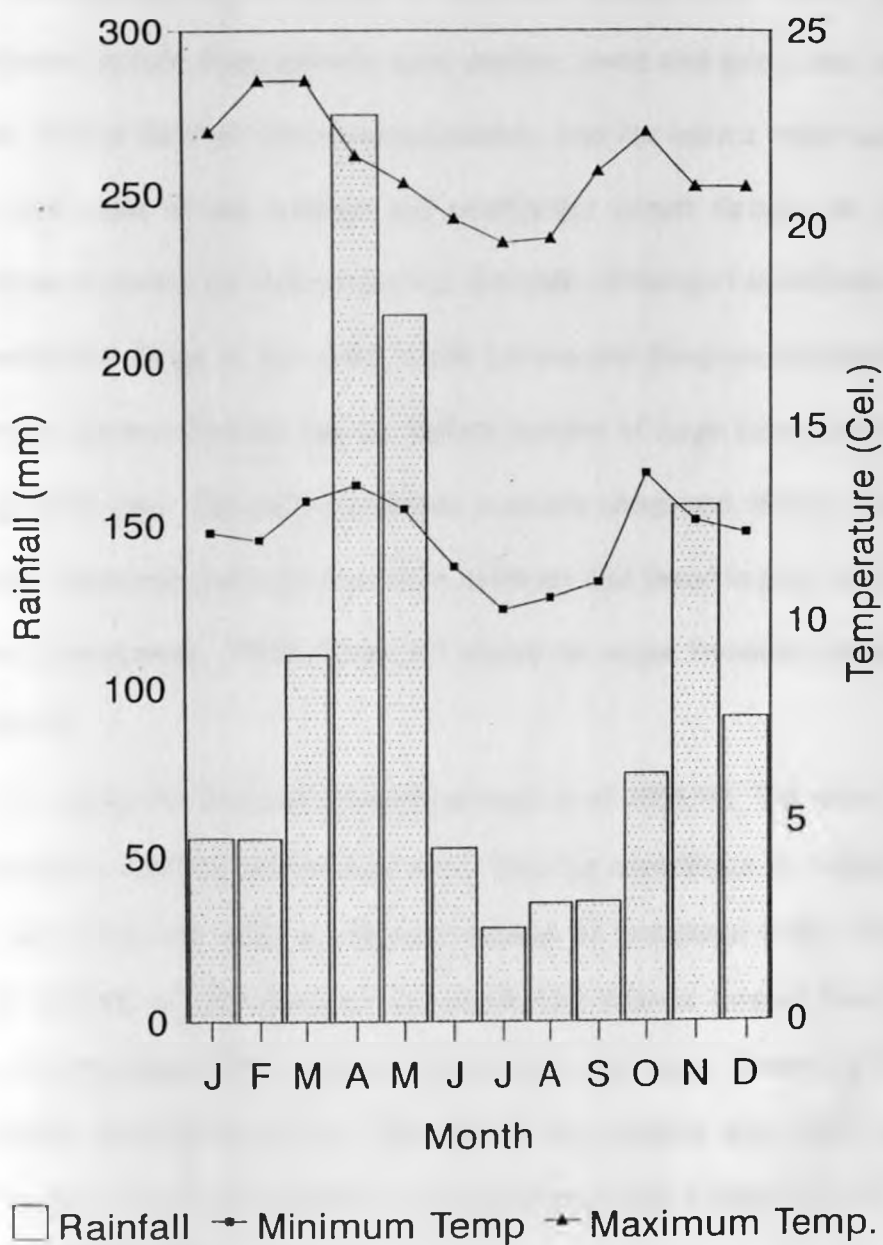


Fig. 3.2: Meteorological data for Kiambu District (at least 10 years of records up to 1995)

(Anonymous, 1994; Gitau *et al.*, 1994a)

Livestock production in Kiambu District is one of the enterprises that play a crucial role as far as development in the district is concerned (Anonymous, 1994). The major livestock enterprises include dairy animals, pigs, poultry, sheep and goats, beef in arid and semi-arid areas. Due to the ever increasing population, land has been a major constraint to all enterprises and most of the residents are smallholder mixed farmers for whom the livestock enterprise is mainly for milk production. Gatundu, Githunguri and Kikuyu divisions have most smallholder farms in that order while Limuru and Kiambaa divisions have the least. Conversely, Limuru Division has the highest number of large farms and the largest acreage of large farm land. The cattle population is mainly comprised of dairy cross breeds namely Fresians, Guernseys and Ayrshires. Zebu cattle are also found in parts of Limuru and Thika divisions (Anonymous, 1993). Table 3.1 shows the major livestock enterprises and population 1991/92.

Table 3.2 shows the livestock production trend as of 1991/92. The most important livestock enterprise is dairying followed by beef. Dairying contributes the highest income amounting to K£12,554,183. Milk is marketed through 14 functional Dairy Co-operative Societies, with 75-80% of milk produced by smallholder farmers owning fewer than ten milking cows (Anonymous 1994). Due to the small land size, most farmers produce milk under the intensive zero-grazing system. Beef and poultry keeping also contribute a high percentage of income to the local population and agricultural Gross Domestic Product (GDP) in general (Anonymous, 1994).

3.2.2 *Experimental design*

The farms surveyed were selected by stratified random sampling (Plews, 1979) and included 6 large (> 30 animals) and 10 small scale (< 20 animals) farms randomly

Table 3.1: Livestock enterprise and 1991/92 population

Enterprise	1991/92 population
Dairy	155,273
Beef-zebu	27,642
Dairy cows ATS (milk and meat)	47,510
Sheep (hair and wool)	81,851
Poultry (layers, broilers and indigenous)	765,945
Bee-keeping (hives)	5,801
Pigs	29,770
Rabbits	35,276
Geese, ducks and turkey	5,875
Total	1,154,673

Source: District Livestock Production Office Kiambu, 1993.

Table 3.2: Livestock production trend 1991/92

Enterprise	Products	Estimated production	Estimated value (income) 1991/92 K£
Dairy	Milk (litres)	61,129,080	12,554,183.0
Beef	Beef (No. slaughtered) Cattle hides (pieces)	177,201 178,217	1,560,608.2
Mutton	Mutton (No. slaughtered) Sheep skins (pieces)	20,802 40,163	659,269.8
Goats	Goat meat (No. slaughtered) Goat skins (pieces)	6,329 31,872	326,892.7
Pigs	Pork (No. slaughtered)	815	97,800.0
Bee Keeping	Honey (kg)	145,025	725,125.0
Rabbits	Rabbit meat (No. slaughtered)	34,603	86,507.5
Poultry	Poultry (No. slaughtered) Eggs	2,181,164 150,662,250	1,036,052.9 22,599,337.5

Based on Anonymous (1993).

distributed within the 7 divisions of the district. At least ten animals (range 10-20) in three age group cohorts of cattle, of mixed breeds and sexes (young, i.e. < 6 month of age; yearlings, i.e. 6-12 months old; and adults, i.e. > 12 months old at the start of the survey) were selected on each farm initially, individually ear-tagged and sampled. Rectal faecal samples were collected monthly during an atypically dry spell (September 1991 to January 1992) as there were no short rains in 1991 (Fig. 3.3), and during a wet period (March to July 1992).

The number of strongylid eggs and coccidian oocysts per gram (epg/opg) of faeces was determined for each sample by a modified McMaster technique (MAFF, 1986). The number of strongyle eggs counted in a McMaster slide chamber were assigned values, for example, zero (0) to 100 eggs were assigned 0 value and so on to an assigned value of 10 for 2000 to 2100 eggs (Fig. 3.8). Presence of tapeworm eggs was also noted. A sedimentation technique as described by Hansen and Perry (1994) was used to detect the presence of trematode eggs. The modified Baermann method as described by Rode and Jørgensen (1989) was used to search for lungworm larvae, but was discontinued after being negative for 2 months in the dry and wet periods, respectively. Faecal samples from animals of the same age groups on individual farms were pooled and cultured at 27°C for 14 days for differential larval counts (Keith, 1953; MAFF, 1986).

Identification of *Eimeria* spp. was carried out in pooled faecal samples where coccidian oocysts were concentrated by centrifugal flotation using saturated magnesium sulphate solution. The oocysts were sporulated in a solution of potassium dichromate (2.5 w/v) incubated for one week at room temperature and with constant aeration. *Eimeria* spp. were identified on the basis of morphological characteristics of the oocysts and sporocysts (Joyner *et al.*, 1966; Levine, 1973).

The infectivity of the pastures was tested using tracer bull calves of mixed breeds, 5-6 months of age and kept under worm-free conditions from birth. One calf was introduced monthly on each farm in the dry and wet season, respectively (i.e. a total of 10 tracers per farm) and grazed alongside the resident cattle population. After grazing for 28 days, tracers were held in confinement on concrete floor for 3 weeks prior to necropsy for parasite recovery. At necropsy, the gastrointestinal tract was removed from the carcass, and abomasum, small intestine and large intestines were separated, opened and washed within 5 min, according to standard procedures (MAFF, 1986). Worm counts were performed on 10% of the total washings. The species composition was determined as described by MAFF (1986). (see Appendix 3.1).

3.2.3 Statistical analysis

Faecal egg/oocyst counts were log-transformed $\ln(x+10)$ and examined by repeated measures analysis of variance to test the effects of season of sampling, age, sex, farm and their interactions. The differences between means were tested for significance using a Student's t-test and a value of $P < 0.05$ was considered significant. These tests are contained in SAS packages (PROC GLM; SAS, 1990; Schlotzhauzer and Littell, 1991). The period prevalence rates of parasite eggs/oocysts were defined as described by Durfee (1978) and Margolis *et al.* (1982), and the proportions of infected animals were compared using the χ^2 test.

3.3 Results

3.3.1 Meteorological data

The rainfall distribution of the study area is shown in Fig. 3.3. An atypical bimodal

rainfall of 76.9 and 500.1 mm was recorded during the short and long rains, respectively. This was below the expected average of about 200 and 800 mm during the two rainy seasons.

3.2 Parasitological findings

The prevalence rates calculated from 5 consecutive samplings from September 1991 to January 1992 (dry period) and from March to July 1992 (wet period) are presented in tables 3.3a and 3.3b. A total of 165 animals were examined monthly and comprised of 57 calves, 56 immature and 52 adult cattle. Strongylid followed by liver fluke eggs and coccidia oocysts were the most prevalent infections while, the prevalence of *Moniezia* was low. In all age groups, concurrent strongyle and liver fluke infections were most common, accounting for 25.9% and 10.2% of mixed helminth infections in the dry and wet periods, respectively. The prevalence of strongylid infection was significantly higher ($P < 0.05$) in the yearlings (94.0%) compared with either the young calves (87.6%) and adult cattle (75.9%) irrespective of season while, the prevalence of coccidian oocysts was significantly higher ($P < 0.05$) in the young calves (69.3%) relative to either yearlings (25.9%) or adult cattle (3.8%). In both seasons, the prevalence of liver fluke eggs was significantly higher ($P < 0.05$) in adult cattle (68.3%) compared with either the yearlings (24.1%) or young calves (9.7%). The wet season prevalences of strongylid and coccidia infections in calves and yearlings were significantly higher ($P < 0.05$) than for the dry season, whereas, for liver flukes, there was no significant influence of season on prevalences of age groups. Overall, the proportions of females and males shedding strongylid, liver fluke eggs and coccidian oocysts were not statistically ($P > 0.05$) different (Table 3.3a).

Nematodirus spp., *Strongyloides* spp. and *Trichuris* spp. eggs made minor contributions to faecal egg counts. Faecal samples examined for the presence of larvae of the

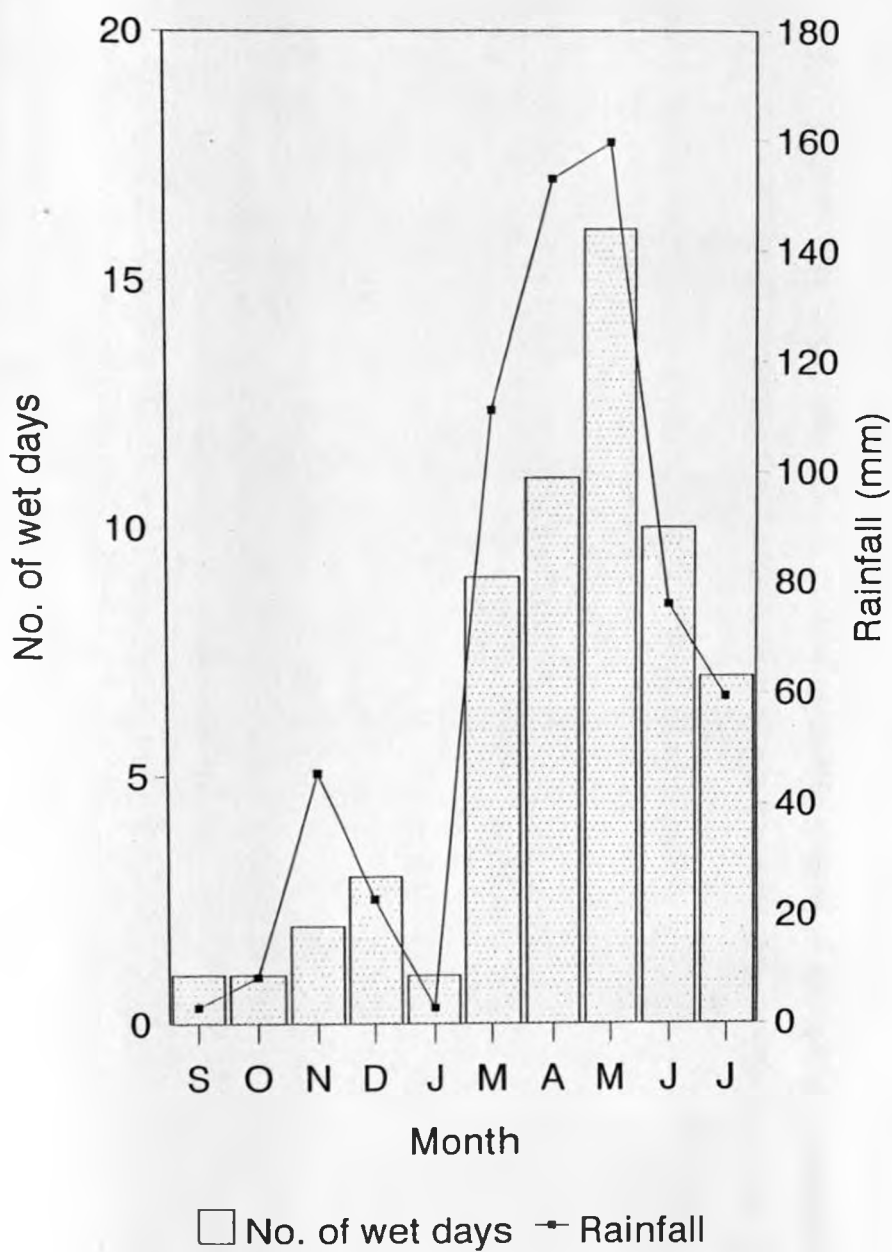


Fig. 3.3: Rainfall distribution in study area during 1991-92

Table 3.3a: The prevalence percentages (5 months sampling) of strongylid, liver fluke, tapeworm eggs and coccidial oocysts in faecal samples in relation to age group and sex of cattle on 16 farms in Kiambu District during the dry and wet seasons

		AGE GROUP			SEX	
		Calves n=57 ^a	Yearlings n=56	Adults n=52	Female n=100	Male n=65
Season	Parasite	% ^b	%	%	%	%
Dry	Strongylid	83.9	93.0	75.4	87.7	82.9
	Coccidia	61.4	10.7	3.8	23.0	30.8
	Tapeworms	10.5	5.4	3.8	7.0	6.2
	Liver flukes	10.5	23.2	71.2	39.0	26.2
Wet	Strongylid	91.2	95.0	76.5	87.4	89.7
	Coccidia	77.2	28.6	3.8	40.0	33.8
	Tapeworms	24.6	9.6	3.8	12.0	12.3
	Liver flukes	8.8	25.0	65.4	33.0	30.8

^aNumber of animals examined. ^b% number of animals diagnosed positive at least once during the 5 months period.

Table 3.3b: The prevalence percentages of eggs of strongylids, *Fasciola*, *Moniezia* and coccidial oocysts in faecal samples from all sampling locations during dry (D) and wet (W) seasons

Internal parasites	Age group	Season	Sampling location							Group prevalence
			Thika	Lari	Kikuyu	Limuru	Gatundu	Kiambaa	Githunguri	
Strongylids	Calves (*57)	D	76.6	88.1	83.9	83.6	85.0	87.9	82.2	83.9
		W	87.5	92.2	89.7	90.8	91.2	92.7	94.3	91.2
	Yearlings (*56)	D	95.6	93.7	94.5	92.3	90.4	97.1	87.4	93.0
		W	97.3	96.5	97.1	95.6	91.2	97.5	89.8	95.0
	Adults (*52)	D	68.6	71.1	76.5	80.4	66.8	87.7	76.7	75.4
		W	70.2	72.9	76.9	81.5	80.4	76.1	77.5	76.5
Liver flukes	Calves	D	1.6	25.8	15.8	17.7	3.6	6.2	2.8	10.5
		W	2.0	16.3	11.6	15.8	5.9	8.9	1.1	8.8
	Yearlings	D	18.9	42.2	32.4	32.8	11.6	9.4	15.1	23.2
		W	17.8	48.2	27.9	40.1	14.4	8.6	18.1	25.0
	Adults	D	60.7	96.9	82.9	95.7	53.1	33.8	75.3	71.2
		W	54.7	83.5	76.5	88.4	48.6	42.4	63.7	65.4
Coccidia	Calves	D	46.5	56.7	60.5	53.6	69.4	85.2	57.9	61.4
		W	63.8	78.9	90.8	72.6	87.8	79.4	67.1	77.2
	Yearlings	D	2.9	2.1	22.4	8.7	19.9	15.2	3.7	10.7
		W	23.8	10.6	20.1	17.7	49.8	42.1	36.1	28.6
	Adults	D	2.9	7.8	1.2	6.2	0	4.8	3.7	3.8
		W	1.7	5.9	2.3	7.4	1.6	5.2	2.5	3.8
Tapeworms	Calves	D	5.2	18.8	10.8	4.2	9.4	10.5	14.6	10.5
		W	22.4	24.9	17.7	20.4	29.4	21.3	36.1	24.6
	Yearlings	D	0	10.3	5.9	1.1	4.2	9.1	7.2	5.4
		W	2.8	12.3	15.8	5.6	13.1	6.0	11.6	9.6
	Adults	D	0	5.7	0	5.3	9.1	3.9	2.6	3.8
		W	1.5	2.4	1.8	4.2	10.3	5.2	1.2	3.8

^aNumber of animals examined per age group

lungworm, *Dictyocaulus viviparus* were all negative.

The mean epg/opg values for the two seasons are shown in Fig. 3.4. A repeated measures analysis of variance showed that yearlings had significantly higher ($P < 0.05$) strongylid epg than either young calves and adult cattle. However, in both calves and yearlings, strongyle epg counts increased significantly ($P < 0.05$) after the onset of the rains in March. Young calves had significantly higher ($P < 0.05$) opg compared to both yearlings and adult cattle in both sampling seasons, and as with the strongylids, opg counts increased significantly ($P < 0.05$) after the onset of the rains. Yearlings and adult cattle had significantly higher ($P < 0.05$) liver fluke epg than young calves, and epg did not differ significantly ($P > 0.05$) between the two former groups in both sampling seasons. For the three parasites, epg/opg did not differ significantly ($P > 0.05$) between the females and males during the two sampling seasons (Fig. 3.5).

There were significant ($P < 0.05$) farm effects on the intensity of strongylid, coccidia and liver fluke infections, even though analysis of variance showed that strongylid epg counts did not vary significantly ($P > 0.05$) between small- and large-scale farms (Fig. 3.6). Significant interactions ($P < 0.05$) were observed between farm/season, farm/age for strongylid and coccidia, and farm/season for liver fluke infections, respectively.

A scattergram showing the number of observations with strongylid epg relative to age during the months of September and May is presented in Fig. 3.7. On both occasions, the intensity of infection was higher in yearlings, which also formed the group with the largest number of infected animals. These were followed by calves and adult cattle, and in all age groups, the intensity was higher during the wet season (May) (Fig. 3.7). The observed frequencies of strongylid egg counts for the three age groups combined, are presented in Fig. 3.8.

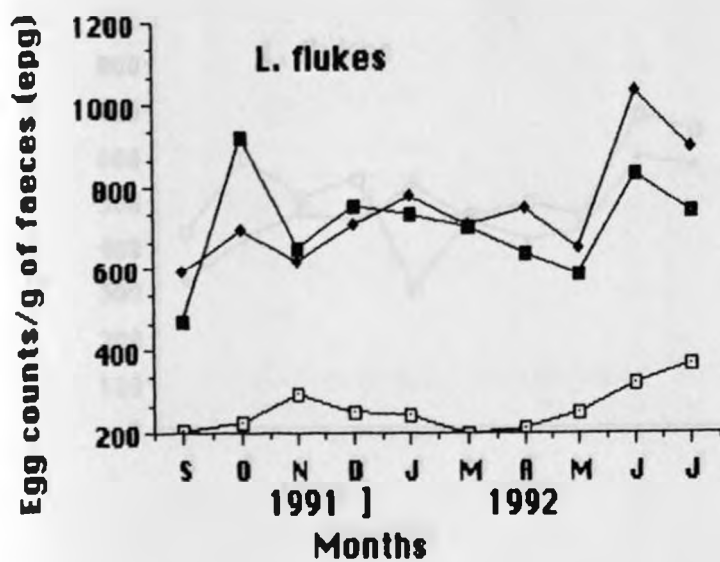
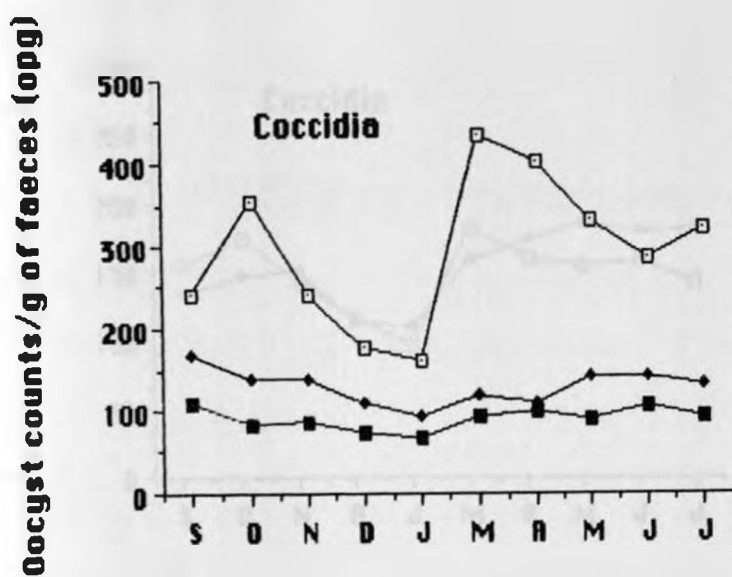
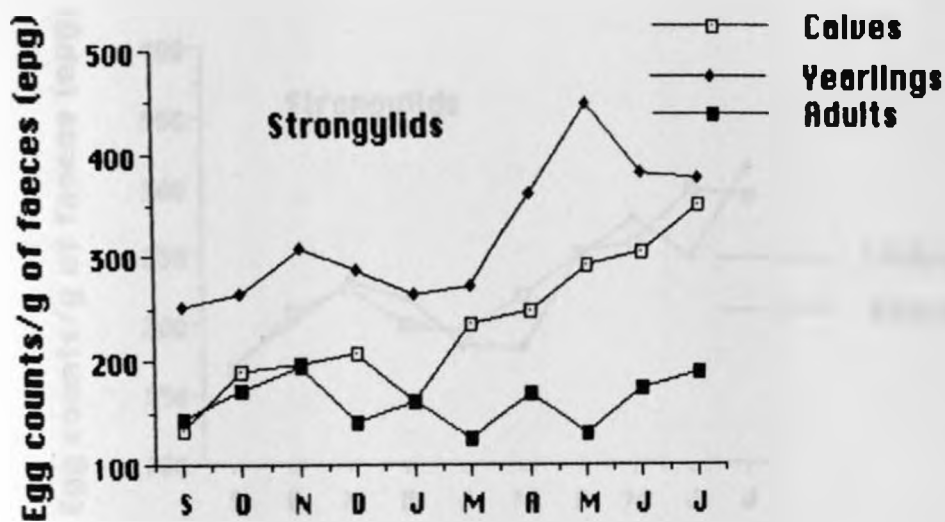


Fig. 3.4: Geometric mean strongylid, liver fluke eggs and coccidial oocyst counts of calves, yearlings and adult cattle

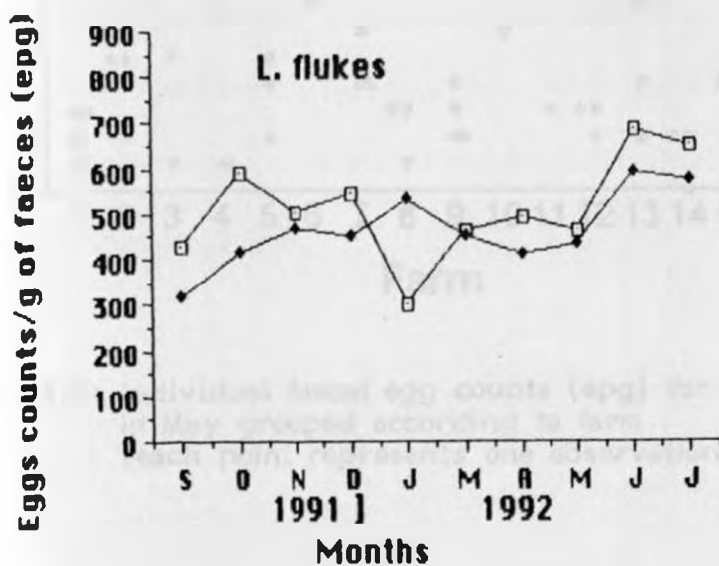
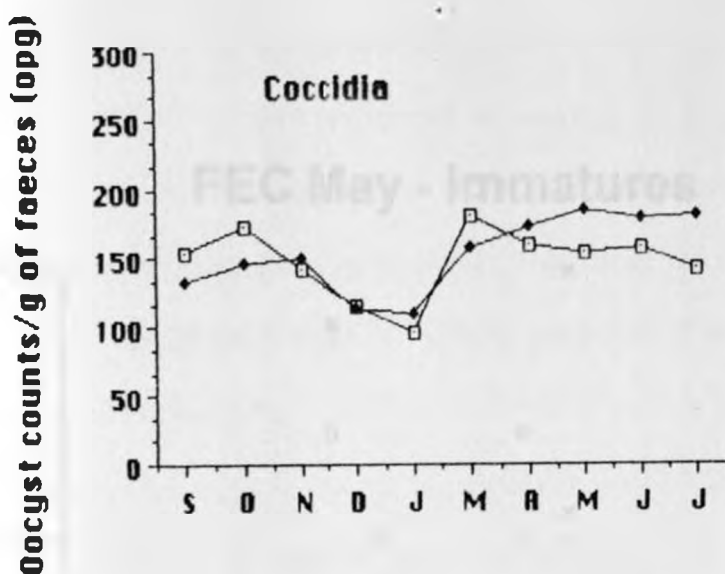
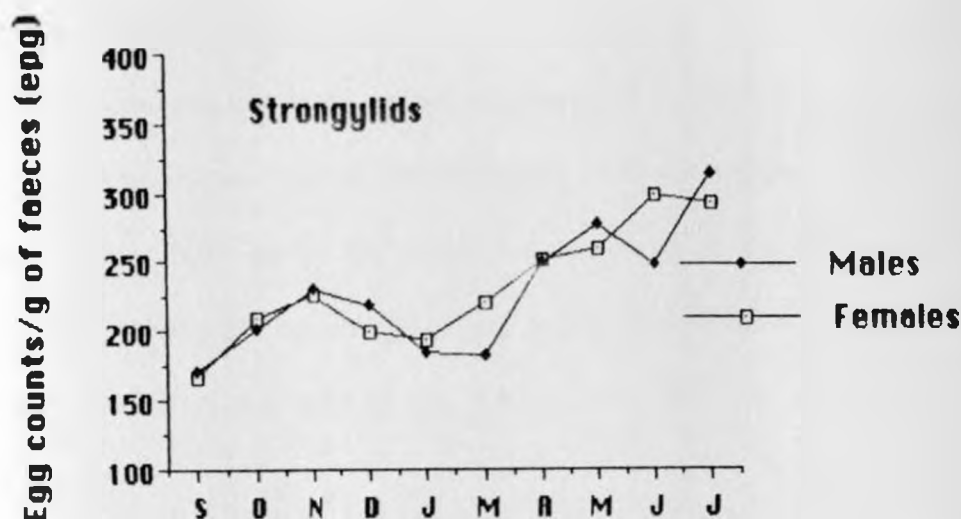


Fig. 3.5: Geometric mean strongylid, liver fluke eggs and coccidial oocyst counts of female and male animals

FEC May - immatures

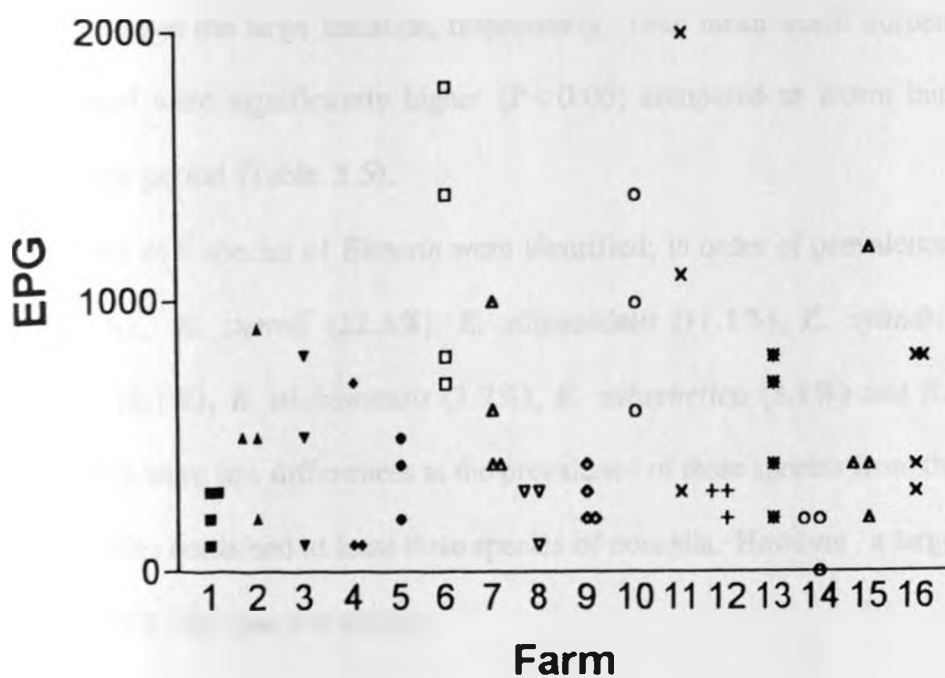


Fig. 3.6: Individual faecal egg counts (epg) for yearlings in May grouped according to farm (each point represents one observation)

The mean and overall larval distribution in the faecal cultures of young calves, yearlings and adult cattle over the study period are presented in Table 3.4 and Fig. 3.9, respectively. Third stage larvae (L₃) of *Haemonchus*, *Trichostrongylus*, *Cooperia* and *Oesophagostomum* were identified in the faecal cultures of all farms and age groups. *Haemonchus* was the predominant species in cultures followed by *Cooperia*, which became more important in animals > 6 months of age. *Strongyloides* spp. was only identified in cultures of calves.

The prevalence and intensity of infection with individual species of GI nematodes in tracer calves slaughtered during the dry and wet periods are shown in Table 3.5. Every calf examined was infected by more than one species of nematode. *H. placei*, *Cooperia* spp. (*C. punctata*, *C. pectinata*) and *O. radiatum* were the most prevalent species in the abomasum, small intestine and the large intestine, respectively. Total mean worm burdens of 10,433 in the wet period were significantly higher ($P < 0.05$) compared to worm burdens of 4,884 during the dry period (Table 3.5).

A total of 8 species of *Eimeria* were identified; in order of prevalence these were *E. bovis* (42.2%), *E. zuernii* (22.6%), *E. ellipsoidalis* (11.1%), *E. cylindrica* (8.9%), *E. auburnensis* (6.1%), *E. alabamensis* (3.7%), *E. subspherica* (3.1%) and *E. wyomingensis* (2.3%). There were few differences in the prevalence of these species from the 16 farms and positive samples contained at least three species of coccidia. However, a large proportion of the samples (76.0%) had 4-6 species.

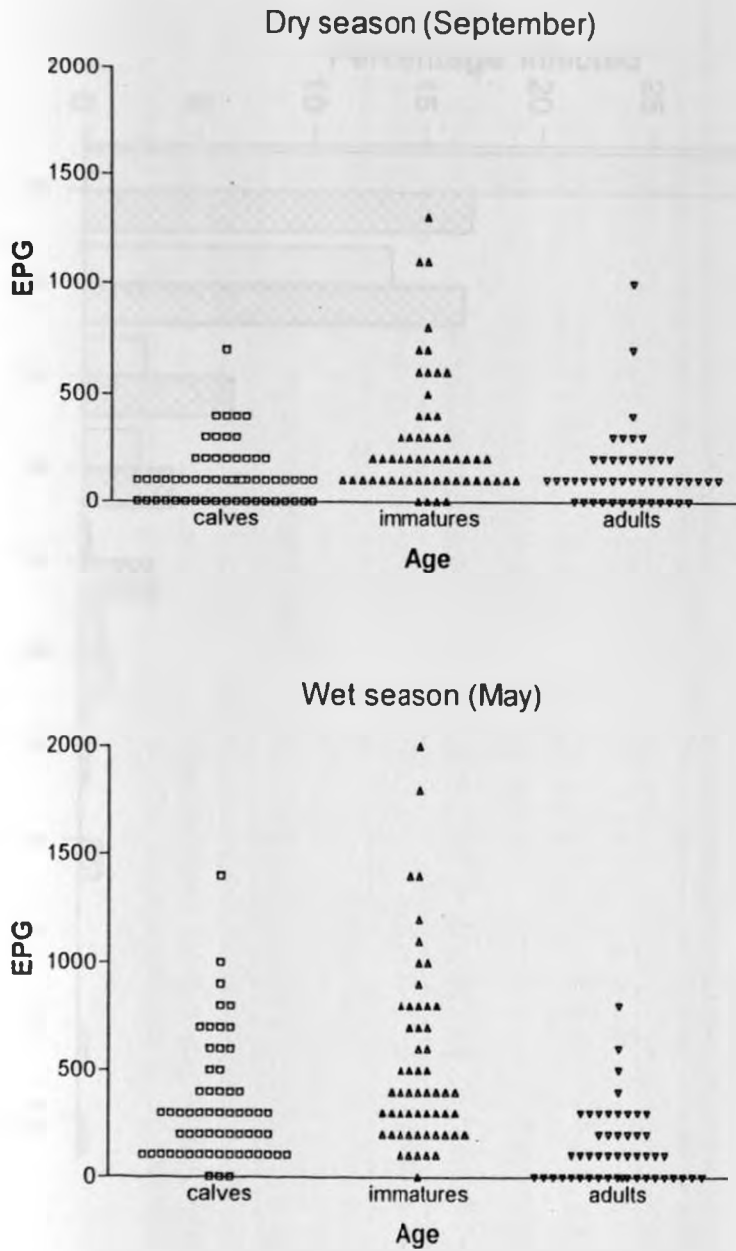


Fig. 3.7: The distribution of strongylid faecal egg counts in relation to age groups in the dry (September) and wet (May) seasons

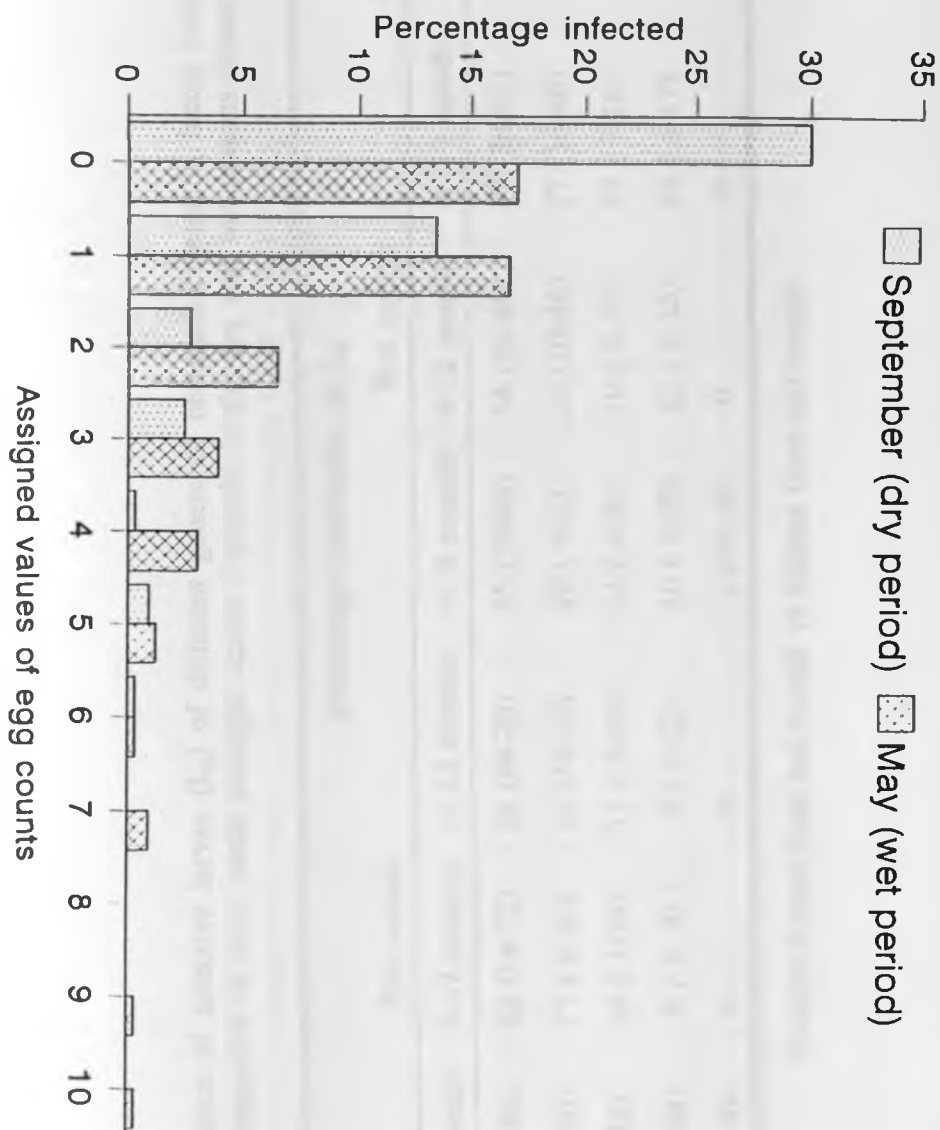


Fig. 3.8: Observed frequencies of faecal strongyloid egg counts in all age groups in the dry (September) and wet (May) seasons

Table 3.4: Percentage distribution of infective larvae (L_3) of different genera of nematodes found in faecal culture of pooled samples of three categories of dairy cattle averaged across 5 months in the dry and wet seasons (mean of farms and range^a)

Parasite	Percentage distribution of L ₃						Overall %
	Dry season			Wet season			
	< 6 month	6-12 months	> 12 months	< 6 months	6-12 months	> 12 months	
<i>Haemonchus</i>	61 (37-86)	63 (54-72)	56 (34-70)	55 (39-68)	54 (36-60)	49 (36-62)	56
<i>Cooperia</i>	14 (4-30)	17 (6-36)	25 (16-30)	19 (8-32)	24 (10-46)	27 (16-40)	21
<i>Oesophagostomum</i>	9 (4-18)	14 (1-19)	11 (5-20)	14 (4-30)	10 (2-12)	14 (7-16)	12
<i>Trichostrongylus</i>	12 (5-28)	6 (2- 8)	8 (2-12)	10 (5-20)	12 (2-35)	10 (4-17)	9
<i>Strongyloides</i>	4 (1- 8)	0	0	2 (0- 4)	0	0	2

^aRange=lowest farm and month vs highest farm and month.

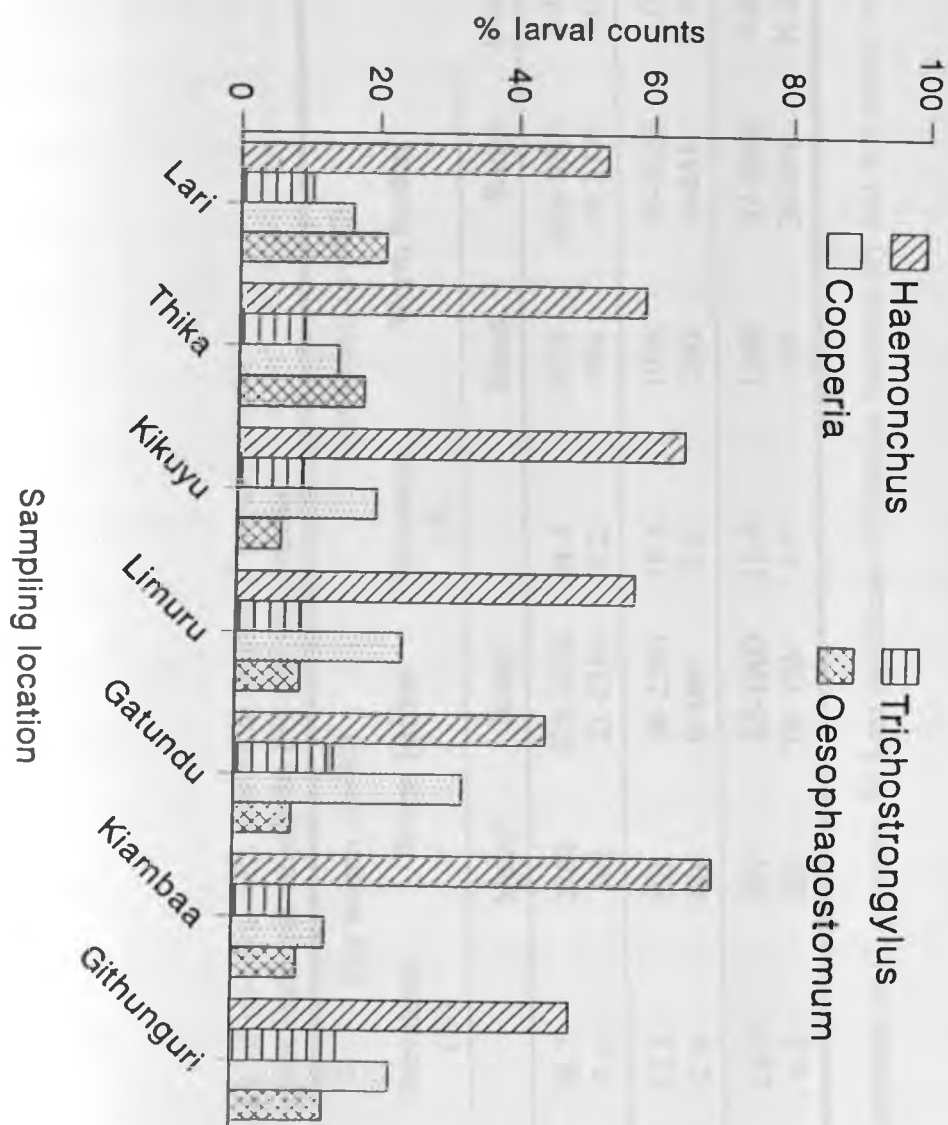


Fig. 3.9: Overall percentage distribution of strongylid genera found in faecal cultures

Table 3.5: Prevalence and intensity of infection with individual species of gastrointestinal nematodes in tracer calves examined during the dry and wet seasons

Location	Parasite	Dry season (n=80)			Wet season (n=80)			P-value
		Prevalence (%)	Worm burden		Prevalence (%)	Worm burden		
			Mean ¹	Range		Mean	Range	
Abomasum	<i>H. placei</i>	56.7	2768	375-5350	54.4	5674	606-9325	<0.05
	<i>T. axei</i>	7.4	361	21-1310	9.2	960	85-2325	<0.05
Small intestine	<i>Cooperia</i> spp ²	17.8	869	96-2100	18.5	1936	46-3850	<0.05
	<i>N. helvetianus</i>	2.9	144	0-389	2.8	290	6-611	N.S
Large intestine	<i>O. radiatum</i>	11.0	537	63-1900	13.4	1398	37-3960	<0.05
	<i>T. globulosa</i>	4.2	205	75- 755	1.7	175	26-810	N.S

¹Geometric mean. ²*Cooperia pectinata* or *C. punctata*. N.S-no significant differences between dry and wet season values.

3.4 Discussion

3.4.1 Strongylid infections

This study clearly shows that GI parasites and particularly strongylids, are prevalent in cattle within the study area. This is in agreement with other workers' findings in Kenya (Omara-Opyene, 1985; Ndarathi *et al.*, 1989; Waruiru *et al.*, 1993b). The spectrum of strongylids found in cattle in this study confirms observations by Mango *et al.* (1974); Gatongi *et al.* (1987) and Maingi and Gichigi (1992) in Kenya, and elsewhere in Africa (Sauvage *et al.* 1974; Assoku, 1983; Kaufmann and Pfister, 1990; Ndao *et al.*, 1995; Moyo *et al.*, 1996). The study revealed that *Haemonchus* was the most common genus and the level of infection was generally moderate. However, with heavy parasite burdens in some animals and in light of previous findings by Waruiru *et al.* 1993a, it would be considered to be a problem especially in yearlings and calves. Results of the present study demonstrate that *Cooperia* and *Oesophagostomum* must also be regarded as common parasites of cattle in the area of study.

The effect of weather on the prevalence of GI nematodes was evident as the level of strongylid infection was higher during the wet period than during the dry period. This was in agreement with earlier observations recorded in calves in Mathira Division, Nyeri District, with similar climatic conditions (Waruiru *et al.*, 1993b) but was in variance with the findings reported by Omara-Opyene (1985) under arid conditions, where a higher frequency of strongylosis and epg counts in calves occurred during the dry season. The rainy seasons in this area are very short and Omara-Opyene (1985) suggested that animals became infected during the short rainy season but high strongylid egg counts were detected during the following dry season.

The effect of age on the prevalence of strongylids was clearly demonstrated as

previously reported by Omara-Opyene (1985). Levels of strongylid eggs were highest in yearlings (6-12 months of age at the start of the study), followed by calves (0-6 months age group). Adult cattle were the least affected. Available evidence shows that, usually, only young cattle are affected clinically. Winks *et al.* (1983) reported that in the tropical and subtropical zone of Australia, the majority of strongylosis outbreaks among dairy cattle occurred from 4-12 months of age. This is in agreement with a recent report by Waruiru *et al.* (1993a) where an outbreak due to haemonchosis occurred in weaner calves at Iganjo farm (see chapter 4). In a study conducted in Ankole District, Uganda, the number of strongylid eggs in the faeces was highest for the calves (< 1 year) and lowest for adults (> 3 years) under all systems of husbandry (Sauvage *et al.*, 1974). In Zimbabwe, the lowest egg counts were found in steers and highest in calves (Pandey *et al.*, 1993). These findings suggest that strongylosis starts in young calves as they start grazing and then the rate of infection increases with both the age and pasture contamination with the peak in yearlings when the young stock become entirely dependent on grazing (Omara-Opyene, 1985). Those which survive develop some form of resistance so that only low levels of parasitism is later possible as shown by lower faecal egg counts and prevalence in adult cattle in the current study.

The sex of the host did not influence the prevalence or intensity of infection with nematode worms as was reported by Schad *et al.* (1984). This was in direct contrast with Asanji and Williams (1987) who noted that female animals harboured a higher worm load than the male host counterpart. Generally there is a tendency for males to be more susceptible than females to GI nematode infections (Herd *et al.*, 1992), and this can be attributable to the differential effects of gonadal steroid hormones on immunity and to grazing management (Barger, 1993b).

Strongylid egg counts differed between farms. However, no significant differences

ere observed between small- and large-scale farms. The farm to farm variations in epg counts are likely indicative of variations in farm management (including helminth control), local microclimatic conditions and other farm-level factors (Hassan Wan *et al.*, 1989). One farm-level variable associated with good animal husbandry is the farmers experience in dairy farming. It has been argued that farmers with more experience were better farm managers (Martin *et al.*, 1975). However, in a recent study, in the present study area, calves of more-experienced farmers had lower daily weight gains (Gitau *et al.*, 1994c). It was postulated that older farmers tend to develop more off-farm interests over the years, thus directing less attention to dairy farming. New farmers with all their investments in the farm, might pay closer attention to their cattle (Gitau *et al.*, 1994c).

The strongylid epg counts followed a typical overdispersed distribution. This observation was similar to that reported by Roberts and Swan (1982) and Maingi *et al.*, (1993) in sheep and goats, respectively.

The lungworm (*D. viviparus*) was not found in faecal samples examined. However, in an abattoir survey conducted at the Kenya Meat Commission, Athi River by Bwangamoi and Kagonyera (1971), 8.3% of the lungs examined had *D. viviparus*, but the origin of the slaughtered animals could not be ascertained. In a more recent study, *D. viviparus* larvae were found in cattle from the highlands of Tanzania (Thamsborg *et al.*, 1997). A definite statement concerning the absence of *D. viviparus* should be deferred until a larger number of animals from various regions of the country are examined, especially during the rainy season. The very low occurrence of *Nematodirus*, *Strongyloides*, *Trichuris* and *Moniezia* species would seem to suggest that they are not important problems of livestock production in the study area.

Most of the animals examined during the present survey had low to moderate

strongylid epg counts, suggesting that the infections are usually subclinical (Soulsby, 1965). This has been described as the most economically important type of helminthosis since it occurs in the majority of cases leading to retarded growth, reduced productivity and animals are more susceptible to other infections, and continually contaminate pastures (Craig, 1988). To maximize food nutrient intake and productivity of the animal, treatment of animals suffering from such infections should be undertaken. Such treatments should reduce the egg load and the chances of infecting the more susceptible young animals (Ndarathi *et al.*, 1989).

3.4.2 *Coccidia infections*

The prevalence of coccidian oocysts and opg counts were significantly higher in young calves and frequency of occurrence was higher during the wet sampling period. These findings are in agreement with those of other workers (Ward *et al.*, 1979; Omara-Opyene, 1985; Ndarathi *et al.*, 1989; Waruiru *et al.*, 1993b) showing that calves are the major source of pasture contamination. The *Eimeria* spp. and their prevalence as found in this study are in general agreement with those of other workers in Kenya (Munyua and Ngotho, 1990) and elsewhere (McKenna, 1972; Ernst and Benz, 1981; Parker and Jones, 1987), where *E. bovis* and *E. zuernii* were the most commonly encountered coccidia species of cattle. According to Levine (1973) these two species represented the most pathogenic of the bovine coccidia, with *E. zuernii* in particular being associated with both acute and chronic type of disease. In the current study, no cases of clinical coccidiosis were encountered.

3.4.3 *Liver fluke infections*

The results showed that liver fluke infections were widespread in Kiambu District. This is in accordance with observations made by Mango *et al.* (1974), that fasciolosis was

common in cattle in Central Province of Kenya, with a prevalence of 41.5%. This prevalence was comparable with 34.0% obtained in faecal examination of liver fluke eggs. Cheruiyot (1983) also reported that fasciolosis was endemic and of high prevalence in high rainfall and high altitude areas of Kenya. One of the important factors in the distribution of fasciolosis is the presence of the snail hosts. Brown (1980) showed that *Lymnaea natalensis*, the snail host of *F. gigantica* in Kenya has a limited distribution but is widespread in Central Province. The relative increase of liver fluke prevalence in the dry sampling period could be attributed to management practises of moving animals to low and swampy areas in search of fresh vegetation.

In accordance with reports of Castelino and Preston (1977) and Baldock and Arthur (1985), prevalence rates of liver flukes were higher in adult cattle than in either yearlings or young calves. It is reasonable to speculate that the prevalence increased with age because of the longer period that animals are exposed to infected pastures and give some support to the postulation that *F. gigantica* infections are not as well and rapidly expelled as *F. hepatica* infections (Hammond and Sewell, 1974). The sex of cattle did not influence the prevalence of liver fluke infection in the present study. This was in contrast with earlier reports where incidence in females was generally higher than in males (Asanji and Williams, 1984). However, in other studies, low prevalence in females of all ages was recorded, while there was an increase in prevalence in males with age (Baldock and Arthur, 1985).

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-Chapter 4-

FATAL HAEMONCHOSIS IN HEIFERS AT IGANJO FARM: A CASE STUDY

4.1 Introduction

Helminthosis causes economic losses in ruminants world-wide. *Haemonchus* spp. infection of sheep and goats and, to a lesser extent, of cattle is one of the most important causes of economic loss in Kenya (Allonby and Urquhart, 1975; Carles, 1992; Waruiru *et al.*, 1993a). Dairy calves are the most commonly affected group among cattle but steers and other young cattle up to 3 years of age may also be affected (Blood *et al.*, 1979). The disease is characterized clinically by severe anaemia and anasarca. A chronic wasting disease has also been described (Allonby and Dargie, 1973). Where the disease is unchecked, severe morbidity and high mortality are common; in other areas the cost of control in terms of labour and prophylactic chemotherapy is often a significant factor in production costs (Preston and Allonby, 1979; Urquhart *et al.*, 1987).

Haemonchus contortus is the species most commonly found in the abomasum of sheep and goats, and *H. placei* is the usual species in the abomasum of cattle. However, *H. contortus* may also be present in cattle but usually only when they are grazing the same pasture as sheep or goats.

Haemonchosis is for the most part a primary parasitosis. The predisposing causes for infection include overcrowding, lush pastures and hot, humid climatic conditions. The development of clinical illness is favoured by a fall in the plane of nutrition, particularly in calves. Under adequate nutritional levels, cattle may develop a subclinical infection but when the nutritional level subsequently declines the disease develops (Blood *et al.*, 1979).

Haemonchosis outbreaks due to *H. contortus* in sheep and goats are known to occur

in Kenya and are well documented (Allonby and Urquhart, 1975; Preston and Allonby, 1978). However, a review of literature indicates that only a limited number of studies have shown that haemonchosis is a serious problem in young stock of cattle of post-weaning age (Omara-Opyene, 1985). In this report, clinical haemonchosis in a herd of young dairy cattle, intensively managed is described.

4.2 *Case history*

A farm visit was undertaken to Iganjo farm in Thika Division to investigate the efficacy of triclabendazole and oxyclozanide against *F. gigantea* in naturally infected dairy cattle. However, on arrival at the farm the farmer related the following history. That 42 weaners which had not been drenched were released on to the field at the beginning of June, 1992. Four of these died and these losses occurred while the weaners (6-9 month of age) were confined to 1.5 acre "calf pasture". Those that died had all suffered severe chronic diarrhoea, sub-mandibular swellings and progressive emaciation but had continued to eat until shortly before death.

4.3 *Clinical observations*

On physical examination of most of the remaining weaners, the animals displayed signs of helminthosis such as being stunted, emaciated, dehydrated with sunken eyes and some were unable to stand. Some had sub-mandibular swellings, their hindparts and tails smeared with faeces, and their mucous membranes were pale. Faecal examination of the 37 surviving weaners revealed a moderate strongylid epg count of about 500 with a range of 100-2200 epg.

4.4 *Post mortem findings in calf No. 1625*

Post mortem examination of a freshly dead case revealed pallor of all body tissues, depletion of body fat reserves with serous atrophy of pericardial fat and gelatinous peritoneal fat. Other lesions were oedema of the abomasal folds, subserosal oedema of the abomasum, thickened and inflamed areas of abomasal and duodenal mucosae and, an accumulation of over 4 litres of straw-coloured fluid in the abdominal and thoracic cavities.

As shown in Table 4.1, massive *H. placei* adult and immature worms estimated to be in excess of 10,000 were recovered from the abomasum. Markedly fewer *T. axei*, *Cooperia* spp. and *O. radiatum* were recovered from the abomasum, small and large intestines, respectively.

4.5 *Treatment*

On advice, the farmer removed the 12 critically sick yearlings to the pens and they were put on supportive therapy which included glucose given intravenously, supplemented with iron dextran and multiple vitamins administered intramuscularly. The above 12 animals and 25 others were also drenched with albendazole at a dose rate of 7.5 mg kg⁻¹. The farmer was also advised to supplement the yearlings' diet with grain and minerals. All cases gradually recovered after this treatment and no deaths were reported. The animals had zero epg counts on subsequent faecal sampling on day 14 and 28 post-treatment.

4.6 *Discussion*

In the tropics and subtropics, where climatic conditions are characterised by wet and dry seasons, outbreaks of haemonchosis have been reported only during and immediately following the wet season (Lee *et al.*, 1959; Reinecke, 1960). This is when the infective

Table 4.1: Number and percentage of nematode species recovered at post mortem of calf No. 1625

Date of necropsy	Number (%) of nematode species						
	<i>Haemonchus placei</i>	<i>Trichostrongylus axei</i>	¹ <i>Cooperia</i> sp.	<i>Oesophagostomum radiatum</i>	<i>Nematodirus helvetianus</i>	<i>Trichuris ovis</i>	<i>Strongyloides papillosus</i>
2-8-92	11751 (58.4)	3474 (17.3)	2467 (12.2)	1974 (9.8)	281 (1.4)	125 (0.6)	57 (0.3)

¹*Cooperia pectinata* or *C. punctata*

larvae (L_3) are on pasture (Sprent, 1946), and result from recently acquired infection as demonstrated in the present study. However, a late dry season outbreak of clinical haemonchosis and cooperiosis in cattle was reported by Fabiyi and others (1979) in Northern Nigeria, and was attributed to the simultaneous maturation of a large number of arrested worms acquired during the previous wet season period.

In the outbreak reported in this study, the history of intact appetite in spite of chronic intermittent diarrhoea and progressive emaciation displayed by young weaner calves at pasture, and the favourable response to albendazole treatment suggested that the herd problem was one of primary GI strongylosis (Georgi *et al.*, 1972). This outbreak was probably due to high stocking rate for the available grazing area that led to inadequate nutrition. The stress of inadequate nutrition can predispose any animal to disease (Hotson, 1973). The subsequent improvement of the health of the herd was partially attributed to effective anthelmintic medication, and partially to supplementation of the diet with grain. Another important aspect of this outbreak is that the greater the number of infected animals in a given area, the more L_3 there were to infect or reinfect the animals (Wade *et al.*, 1979). This is seen especially when young animals graze with adult animals or when susceptible animals are put into areas such as dairy calf lots as in the present case (Hotson, 1973). The farmer was advised to raise future replacement stock on larger pasture areas which have not been grazed for at least 12 weeks.

-Chapter 5-

GASTROINTESTINAL NEMATODE INFECTIONS : AN ABATTOIR SURVEY

5.1 Introduction

Gastrointestinal nematode (GI) infections of cattle have been investigated in different climatic environments of Kenya (Omara-Opyene, 1985; Gatongi *et al.*, 1987; Maingi and Gichigi, 1992), and much of the data on epidemiology is based on faecal egg count to estimate the corresponding worm burdens. However, in cattle the egg production of nematodes was found to depend heavily on the season (Kaufmann and Pfister, 1990). During the dry season, when conditions are unfavourable for the development of infective larvae, faecal egg production is reduced. It is therefore more reliable to quantify the worm burden by post mortem examinations, particularly during the dry season (Fritsche *et al.*, 1993).

The present study describes the results obtained from 672 post mortem analyses of cattle, with special emphasis on the seasonal dynamics of GI nematode infections.

5.2 Materials and methods

5.2.1 *Experimental design*

The investigation was conducted on crossbred cattle slaughtered at various abattoirs and slaughter-slabs located throughout Kiambu District. The animals examined were procured locally (animals raised within the abattoir location) and it was envisaged that their worm burdens would reflect the general pattern of worm population in animals of the area surveyed. A total of 672 GI tracts were analysed from freshly slaughtered animals between August 1992 and July 1993. Fourteen GI tracts were collected each week. The samples were taken at random from the animals presented for slaughter during the visits. Age (by number

of teeth) and origin (with the help of cattle owners and butchers) were determined prior to slaughter. Ages of the animals ranged from 13 months to more than 4 years (average age 57 months). Three hundred and ninety (390) animals were females and 282 males. The meteorological data for each month accorded with the average values over the previous 10 years (Anonymous, 1994).

At slaughter, GI nematodes were recovered as described by MAFF (1986). Rectal faecal samples were collected from all animals for strongylid worm egg counts (epg) using a modified McMaster technique (MAFF, 1986). The faecal material from each collection was pooled and cultured at 27 °C for 14 days to harvest the third stage (L₃) larvae which were identified to generic level (MAFF, 1986; Keith, 1953).

5.2.2 Statistical analysis

One way analysis of variance (ANOVA) was used to examine for differences in worm burdens and faecal strongyle egg counts between age classes based on logarithmic transformation similar to that of Field *et al.* (1960).

5.3 Results

5.3.1 Parasitological findings

Of the 672 animals investigated, 583 (86.8%) were found to be infected with one or more species of nematode parasites. The prevalence and mean worm burdens of 8 species encountered in the present study are listed in Table 5.1. *H. placei*, *C. pectinata*, *C. punctata* and *O. radiatum* were the most common species, followed by *T. axei*, *Nematodirus helvetianus*, *Trichuris globulosa* and *Strongyloides papillosus* which, were generally only

found in moderate or low numbers.

The intensity of nematode infections was moderate in most animals, the overall mean nematode burden being 3,353 (range 260-18,300) and the overall mean faecal strongyle egg count being 400 epg. *Haemonchus placei*, *Cooperia* spp. and *O. radiatum* accounted, on average, for 52.3%, 28.5% and 6.9% of the total worm burden, respectively.

The seasonal dynamics of worm burdens and faecal egg output followed a similar pattern (Fig. 5.1). Worm burdens increased with the onset of the short rains in October and reached a peak in November/December. A second peak was observed in May/June, during the long rains, after which time worm numbers steadily decreased. Faecal egg output was at its lowest during the dry seasons. However, it increased gradually through the rainy seasons to reach peaks in December and May. The relative abundance of *H. placei*, *Cooperia* spp. *O. radiatum* and *T. axei* followed the same trend as that of the total worm burden during the different seasons of the year. *N. helvetianus*, *S. papillosus* and *T. globulosa* occurred only occasionally in very low numbers and their populations were apparently not affected by seasonal fluctuations.

Digestion of abomasal mucosae revealed negligible numbers of developing stages of *H. placei* with the proportion of EL₄ ranging between 0 and 5.8% throughout the year.

The mean and range values of the proportion of the larval population of *H. placei*, *Cooperia* spp. *Oesophagostomum* spp. and *Trichostrongylus* spp. were 56.7 (43-78), 29.3 (18-39), 8.4 (6-21) and 5.6 (1-20) %, respectively. *H. placei* (>65%) and *Cooperia* spp. (>30%) were prevalent in high proportions during the rainy seasons.

5.3.3 Age/worm burden relationship

There were 49 animals under 1.5 years, 140 (1.5-3 years), 209 (3-4 years) and 274

Table 5.1: Spectrum, prevalence and mean burdens of nematodes found in cattle in Kiambu District in 672 post mortem examinations between August 1992 and July 1993

Location/species	Prevalence (%)	Worm burden	
		Mean	Range ^a
ABOMASUM			
<i>Haemonchus placei</i>	67.0	3378	125-10375
<i>Trichostrongylus axei</i>	24.3	305	75-1785
SMALL INTESTINE			
<i>Cooperia pectinata</i>	53.0	1050	150-5515
<i>Cooperia punctata</i>	41.7	779	40-4350
<i>Nematodirus helvetianus</i>	19.6	210	15-1675
<i>Strongyloides papillosus</i>	3.6	81	15-420
LARGE INTESTINES			
<i>Oesophagostomum radiatum</i>	38.4	445	25-2275
<i>Trichuris globulosa</i>	9.7	175	30-426

^aRange of positive cases

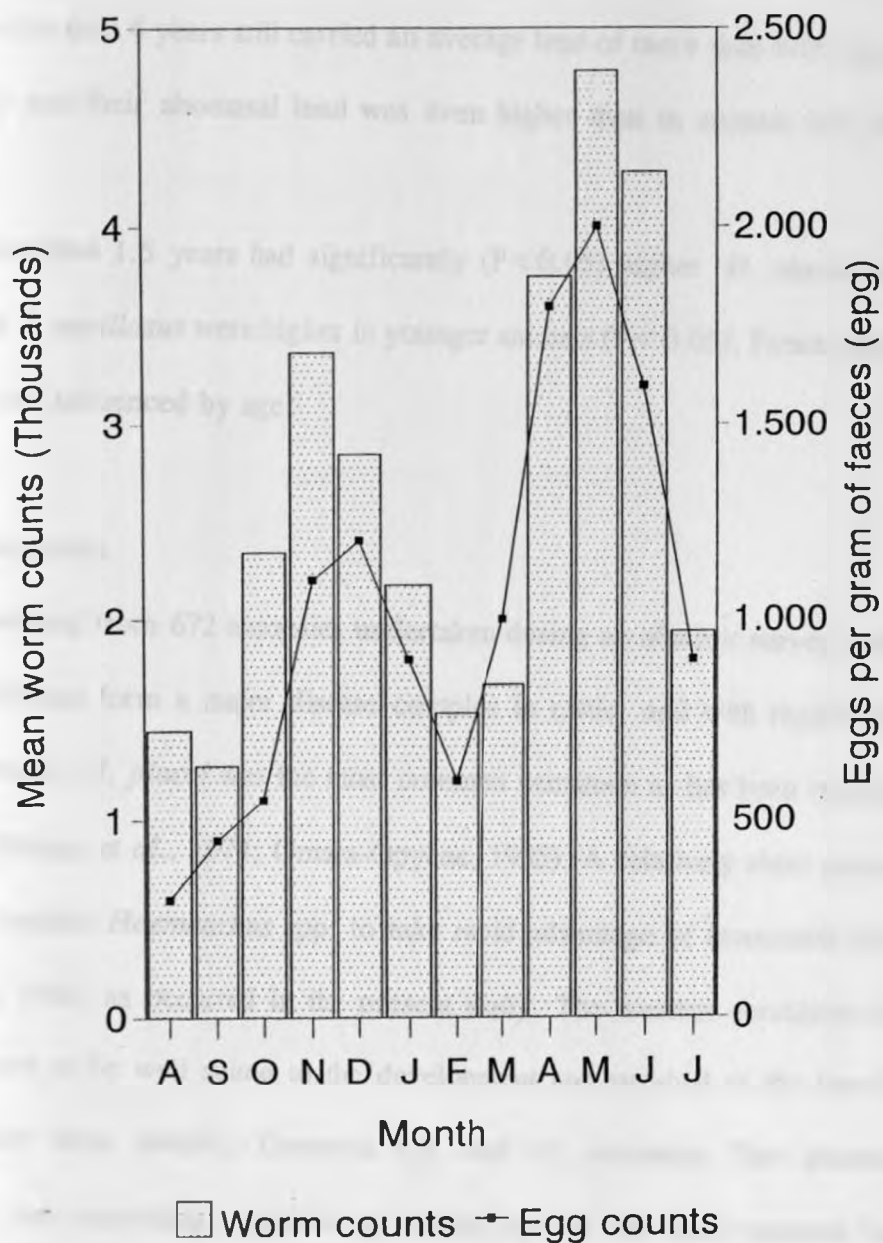


Fig. 5.1: Seasonal pattern of nematode burdens and faecal egg output of cattle slaughtered during the abattoir survey

over 4 years examined. Animals younger than 1.5 years had significantly ($P < 0.05$) lower burdens than older animals (Fig. 5.2). The highest burdens of nematodes (> 5000) were found in animals of 1.5-3 years of age, but the total number decreased only slightly in older animals. Cattle older than 4 years still carried an average load of more than 4700 nematodes (range 446-6831) and their abomasal load was even higher than in animals less than 1.5 years.

Cattle older than 1.5 years had significantly ($P < 0.05$) higher *H. placei* burdens, whereas counts of *S. papillosus* were higher in younger animals ($P < 0.05$). Faecal strongylid egg counts were not influenced by age.

5.4 Discussion

Results obtained from 672 autopsies undertaken during an abattoir survey indicated that helminth infections form a major disease complex in cattle, and with regard to both prevalence and burden, *H. placei* was the most common nematode as has been reported by others in Kenya (Mango *et al.*, 1974; Omara-Opyene, 1985). A relatively short generation interval probably enables *Haemonchus* spp. to take rapid advantage of favourable climatic conditions (Grant, 1981) as occurred in the present study. The weather conditions in the study area also seem to be well suited to the development and survival of the free-living stages of the other three species, *Cooperia* spp. and *O. radiatum*. The absence of *Bunostomum* spp. was surprising, considering that the species has been reported in the highlands of Kenya (Round, 1962). Probably the distribution of this parasite is focal affecting only a few farms and localities.

Of the nematode species encountered, *H. placei* and *O. radiatum* are among those which are serious pathogenic parasites of cattle and are therefore of considerable economic

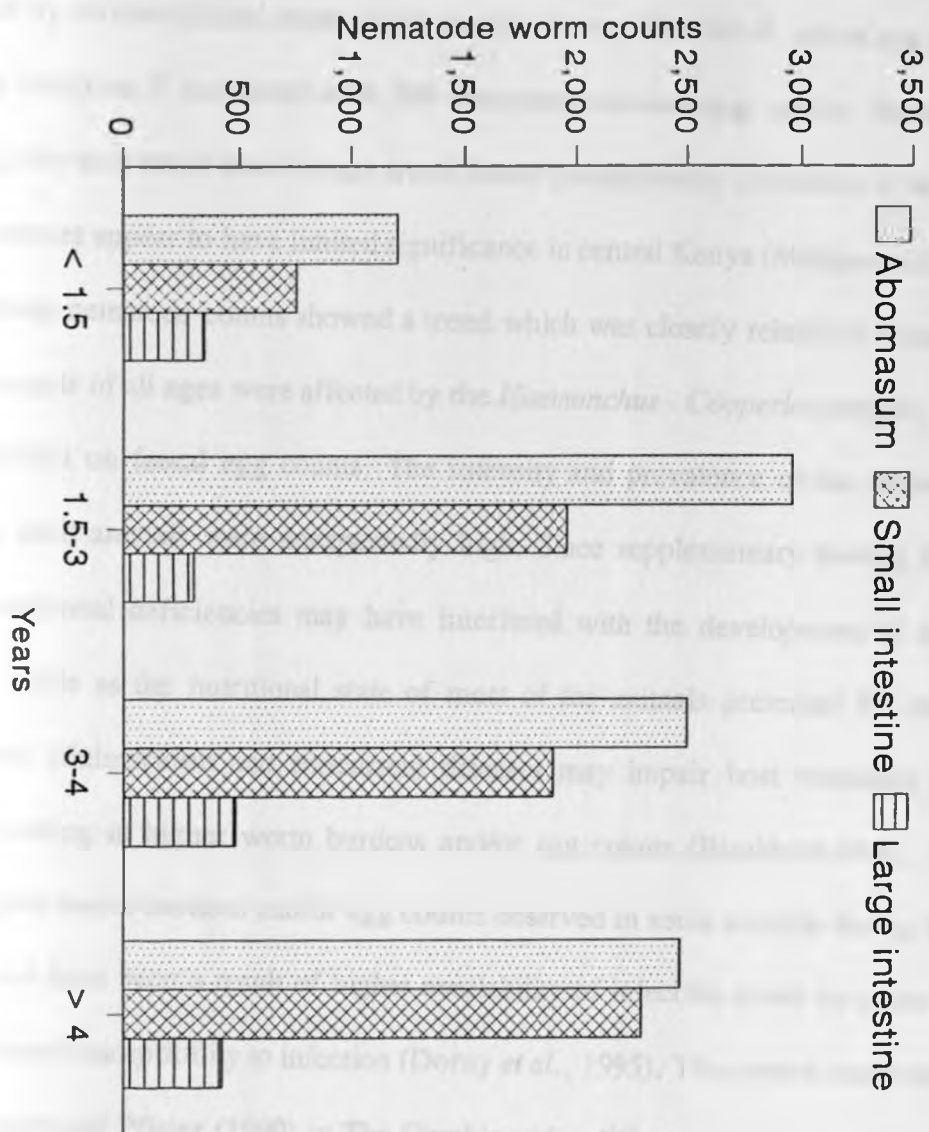


Fig. 5.2: The relationship between gastrointestinal worm burdens and the age of cattle examined during the abattoir survey

importance. The significance of *Haemonchus* spp. and *O. radiatum* is due to the severe trauma and blood loss caused by their migrating and feeding stages (Hutchinson *et al.*, 1980). Roberts and colleagues (1951) observed that egg counts of up to 1000 *H. placei* epg were accompanied by serious clinical signs, while counts of over 500-700 *H. placei* epg reflected a dangerous infection if combined with 300 *Oesophagostomum* epg and/or *Bunostomum*. *Trichostrongylus axei* and *Cooperia* spp are of lower pathogenicity (Anderson *et al.*, 1965) and these parasites appear to have limited significance in central Kenya (Mango *et al.*, 1974).

The total nematode counts showed a trend which was closely related to rainfall, and generally, animals of all ages were affected by the *Haemonchus* - *Cooperia* complex, age not having any effect on faecal egg counts. The intensity and prevalence of the *Haemonchus* infections in adult animals were unexpectedly high. Since supplementary feeding is rarely practiced, nutritional deficiencies may have interfered with the development of acquired immunity in cattle as the nutritional state of most of the animals presented for slaughter appeared poor. Malnutrition and concurrent diseases may impair host resistance against helminths, resulting in higher worm burdens and/or egg counts (Blackburn *et al.*, 1991). Relatively higher worm burdens and/or egg counts observed in some animals during the wet months may not have been a result of higher availability of infective larvae on pasture, but rather of increased susceptibility to infection (Dorny *et al.*, 1995). The present study supports that of Kaufmann and Pfister (1990) in The Gambia under different climatic conditions and with N'dama cattle. Older animals may be a major source of infection for young stock and furthermore detailed studies should be undertaken in other areas of Eastern Africa with different climatic conditions. Although clinical helminthosis occurs more often in calves, older animals should also be included in future control strategies.

The post mortem and faecal examination results showed that the adults of the various

GI nematodes were present throughout the year. The numbers of EL₄ of *H. placei* were very low throughout the year. Thus, it appears that, in the area of study, inhibition of *H. placei* L₄ in cattle does not play a significant role in the biology of this nematode. There are conflicting reports on the actual stimuli for hypobiosis in the field. For example, some studies have indicated that where conditions are favourable for development of the free-living stages, the faculty of hypobiosis is discarded by the parasites (Gupta *et al.*, 1987). By contrast, Ikeme and colleagues (1987) observed that in spite of the year-round tropical rainfall in Malaysia there were still significant numbers of hypobiotic larvae of *H. contortus* in goats. Gatongi (1995) observed high levels of inhibition of *Haemonchus* spp. in sheep and goats in a semi-arid area of Kenya during the dry season. It seems therefore that the degree of environmental adversity and probably other suggested stimuli for hypobiosis may not be important in certain strains of nematodes that are not hypobiotic-prone because, it seems likely, that the prevailing climatic conditions (medium altitude bimodal rainfall) in central Kenya are not severe enough to promote selection for seasonally arrested development, as described elsewhere in Africa (Kaufmann and Pfister, 1990; Ndao *et al.*, 1995).

-Chapter 6-

EPIDEMIOLOGY OF GASTROINTESTINAL NEMATODES : A ONE-YEAR STUDY

6.1 Introduction

For any form of rational control of nematode parasites to be devised, it is essential to understand interactions between worm populations, weather conditions and animal and pasture management. Such basic information is required for the design of control measures to reduce pasture infectivity. The transmission pattern of GI nematodes is dependent on geographic location in which they occur (Rickard and Zimmerman, 1992) and epidemiological data should be developed for different areas. Such data do not exist for most of Kenya which has a variety of agroclimatic zones, each with definite environmental conditions. Consequently, a study was initiated to investigate the seasonal prevalence and importance of the various GI nematodes in weaner-yearling dairy cattle and to further substantiate epidemiological events of *H. placei*.

6.2 Materials and methods

6.2.1 *Exeperimental design*

This investigation was conducted from April 1993 through April 1994, starting at the onset of the long rains. In each of the two farms (Iganjo and Kambaa), 32 Friesian/ Ayrshire crossbred weaner calves of mixed sexes and about 9-10 months of age were used. They had previously been exposed to nematode contaminated pastures and the mean strongyle worm egg counts prior to the beginning of the experiments (March) was 150 (range 0-400). All the calves were given a single dose of albendazole (Valbazen^R, Smith Kline Beecham Animal Health Products) at 7.5 mg kg⁻¹ and were also given triclabendazole (Fasinex^R, Ciba-Geigy)

orally at a dose rate of 12 mg kg⁻¹ at the start of the study in case of *Fasciola gigantica* infections. No further anthelmintic treatment was given except in cases where individual calves appeared to be affected by severe clinical parasitism. The commencing average weight of calves was 120 kg (range 110-138 kg) and 114 (range 98-126 kg) at Iganjo and Kambaa farms, respectively.

The basic design in each farm was to graze all calves together over a series of four 2.6 ha pastures. They were rotated from one pasture to another at varying intervals depending upon forage availability and the timing of the moves depended on the farmer's judgement. Before initiation of the experiment, the 32 calves in each farm were assigned to each of two groups based on equal distribution of body weights: (1) from the first group of 12 animals, 2 calves were randomly selected bi-monthly for slaughter and analysis of worm burdens; (2) the second group of 20 resident animals were weighed and individually sampled for blood and faeces every month. Three of the resident calves from each farm were available for slaughter at the end of the study for analysis of worm populations. The primary forage present on pastures was Kikuyu grass (*Pennisetum clandestinum*), and the red oat grass (*Themeda triandra*) also made up a considerable part of herbage, especially at Iganjo farm. All the animals were subjected to normal farm routines including continuous free access to water and mineral-salt.

The calves selected randomly for slaughter were removed from pasture and confined in concrete-floored stalls for 3 weeks prior to slaughter. In addition, groups of 3 tracer Friesian/Ayrshire crossbred bull calves, 5-7 months of age and raised worm-free since birth, were grazed alongside the resident herd in each farm for 1 month periods during each month of the study period. The tracer calves were also held in confinement for 3 weeks before slaughter.

Faecal egg counts were made using the modified McMaster technique (MAFF, 1986). A sedimentation technique was used to detect the presence of liver fluke eggs in the samples (Hansen and Perry, 1994). Faeces were pooled and cultured (MAFF, 1986) and the first 100 third stage infective (L₃) larvae were differentiated into their genera (Keith, 1953). Herbage samples were collected from all pastures each month for determination of numbers of L₃ available/kg of dry matter based on techniques described by Hansen and Perry (1994). Larval counts and differentiation at genus level were carried out using the morphological characteristics described by Keith (1953). Jugular blood samples were also taken for estimation of packed cell volume (PCV), haemoglobin (Hb), total protein (TP), and serum pepsinogen levels according to techniques described by Ross *et al.*, (1967) (see Appendix 6.1) and Coles (1974).

All necropsies, adult worm recovery procedures, counting and identification techniques were performed as previously described (MAFF, 1986). In addition, mucosal scrapings of each abomasum were digested as described by Herlich (1956) to retrieve immature stages (including arrested larvae) which were identified according to Blitz and Gibbs (1971) and counted.

6.3 Results

Following a single, initial anthelmintic treatment of the weaner calves prior to beginning of the grazing experiments (March), they were not subsequently treated except for occasional treatment of individual sick animals in Kambaa farm. However, due to clinical signs of haemonchosis at Iganjo farm, one anthelmintic treatment with albendazole was given to all 20 resident weaner calves on 26th August 1993.

6.3.1 *Meteorological data*

The monthly rainfall, relative humidity and the mean monthly minimum and maximum temperatures recorded at the study farms are shown in Figures 6.1a and 6.1b. The rainfall has a bimodal pattern and amounts of about 822 mm (Iganjo farm), and 1102 mm (Kambaa farm) per annum divided into long and short rains, were well below a 10-year average for the two farms. The long rains usually peak in May with the short rains peaking in December. On both farms, mean relative humidity varied from 73.5-100% in the wet months, and 69.1-98.2% in the dry months (Fig. 6.1b). Minimum temperatures fluctuated between 9.7-17.5°C (Iganjo farm) and 7.0-10.1°C (Kambaa farm) while maximum temperature range was 21.7 to 28.6 °C, and 18.3 to 25.6°C for Iganjo and Kambaa farms, respectively.

6.3.2 *Faecal egg and herbage larval counts*

The mean number of faecal strongylid eggs and pasture larval counts for the two farms are shown in Fig. 6.2. Initial faecal egg counts were low in both farms since cattle had previously been treated with albendazole 21 days earlier. Peak egg counts were observed in July and August in Kambaa and Iganjo farms, respectively. A smaller, secondary egg count peak occurred during January 1994, but larval counts remained consistently low except for a small increase in December following the rains of October and November. Increased egg counts and high rainfall (Fig. 6.1a) paralleled increased number of herbage larval counts in March and April 1994. *Fasciola* eggs were (epg range 0-300) sporadically observed from 6 animals at Kambaa farm.

The larval distribution in faecal cultures of the yearling cattle are shown in Fig. 6.3. The genera were identified as *Haemonchus*, *Trichostrongylus*, *Cooperia* and

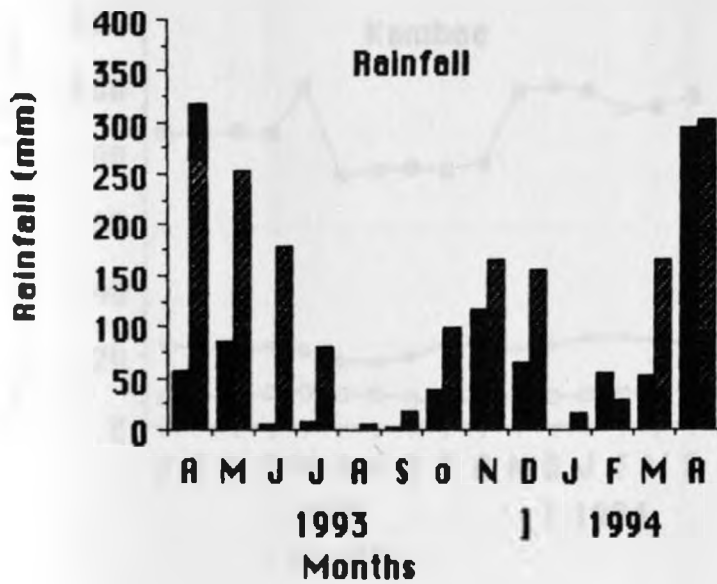
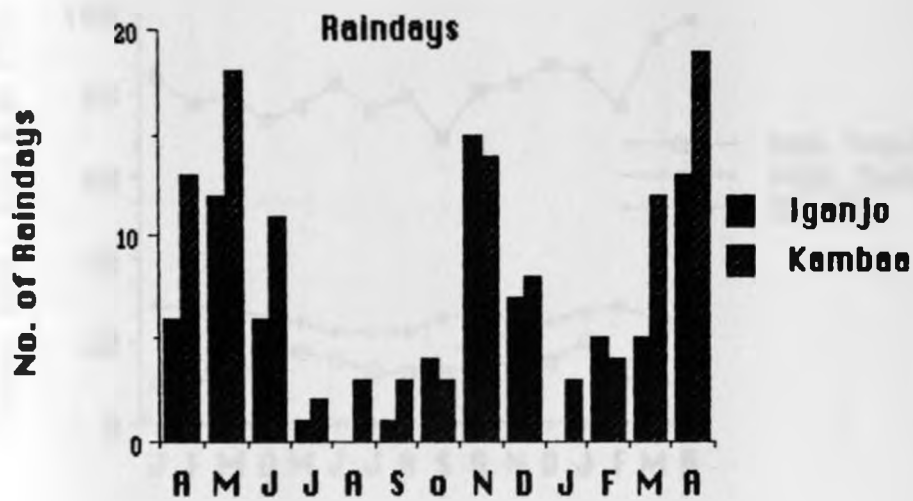


Fig. 6.1a: Monthly number of raindays and monthly pattern of rainfall for Igonjo and Kamboo farms from April, 1993 to April, 1994

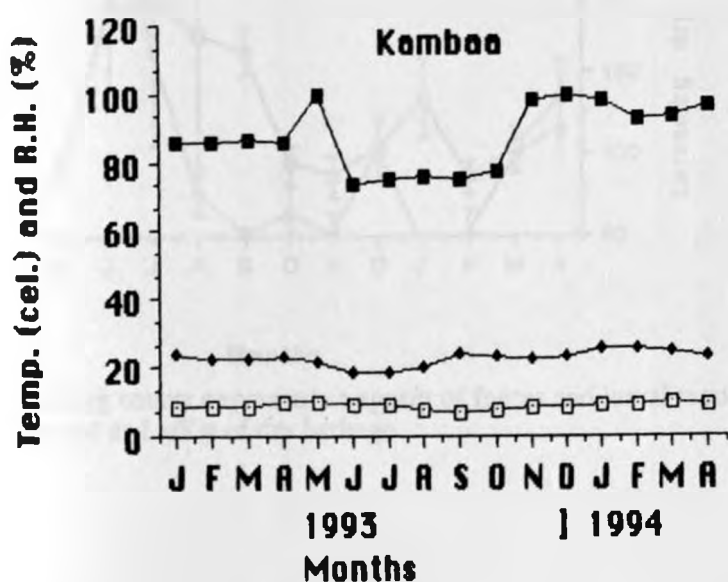
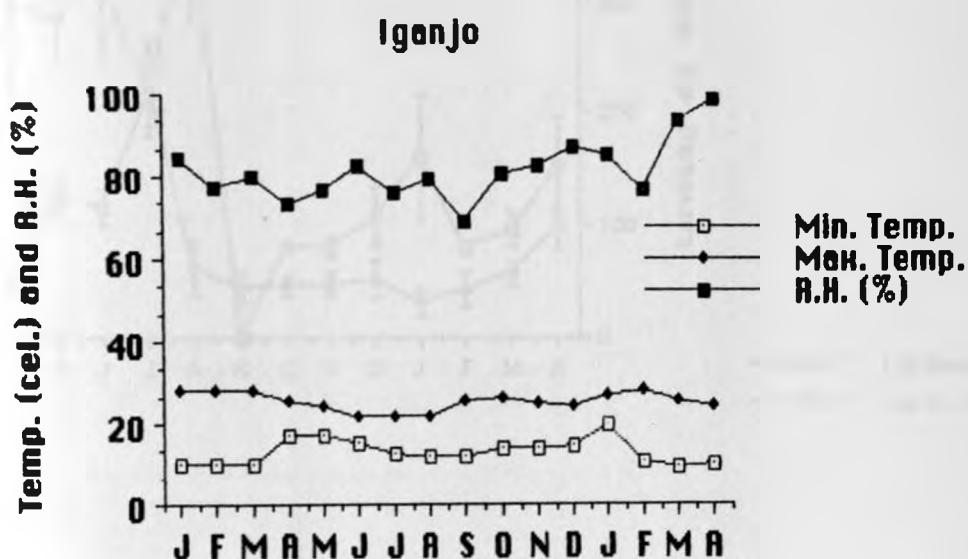


Fig. 6.1b: Mean relative humidity (R.H.), mean monthly maximum and minimum temperatures for Iganjo and Kambaa farms from April, 1993 to April, 1994

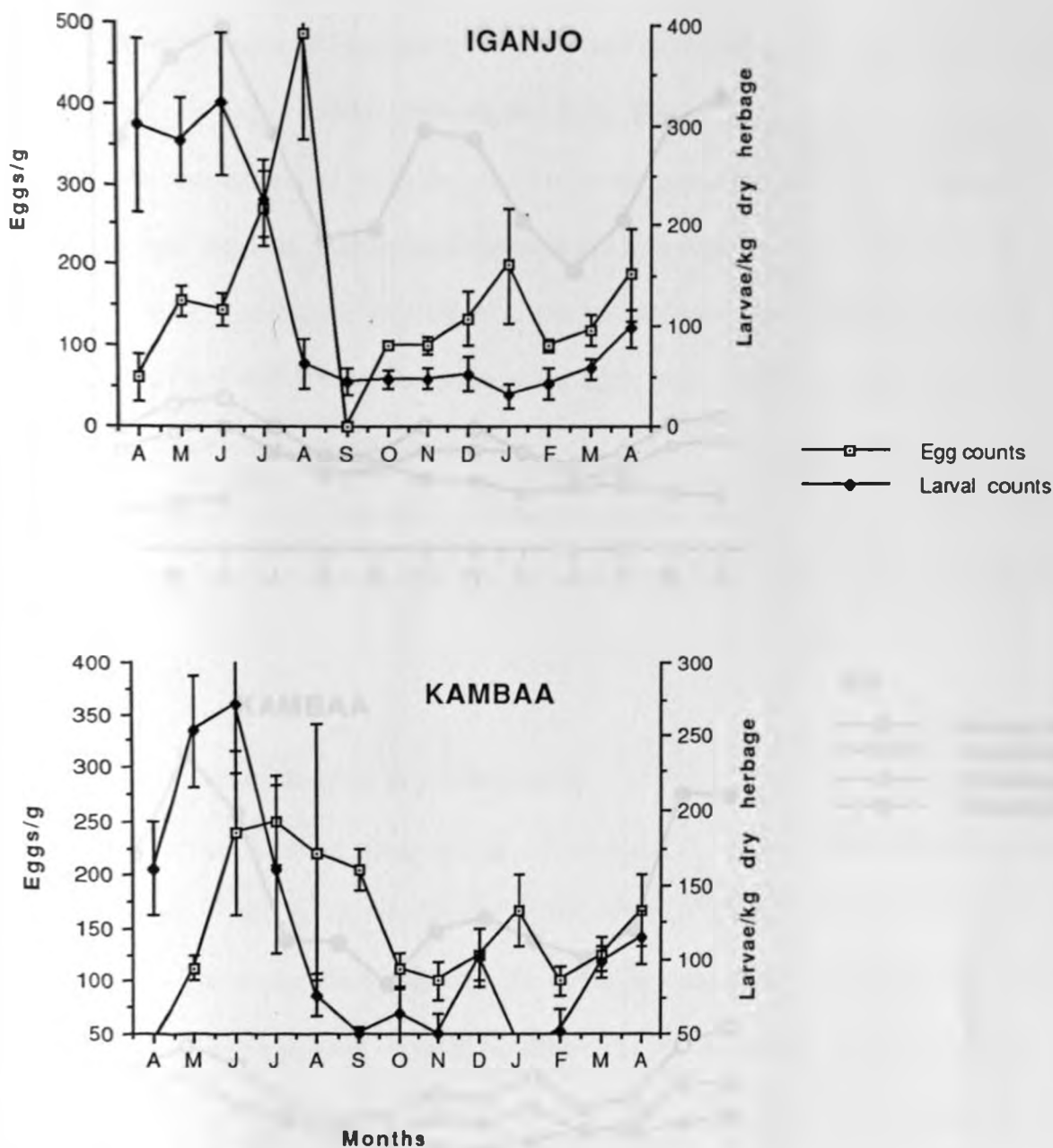


Fig. 6.2: Faecal egg counts expressed as eggs/g of faeces and larval recovery from herbage expressed as L₃/Kg of dry herbage

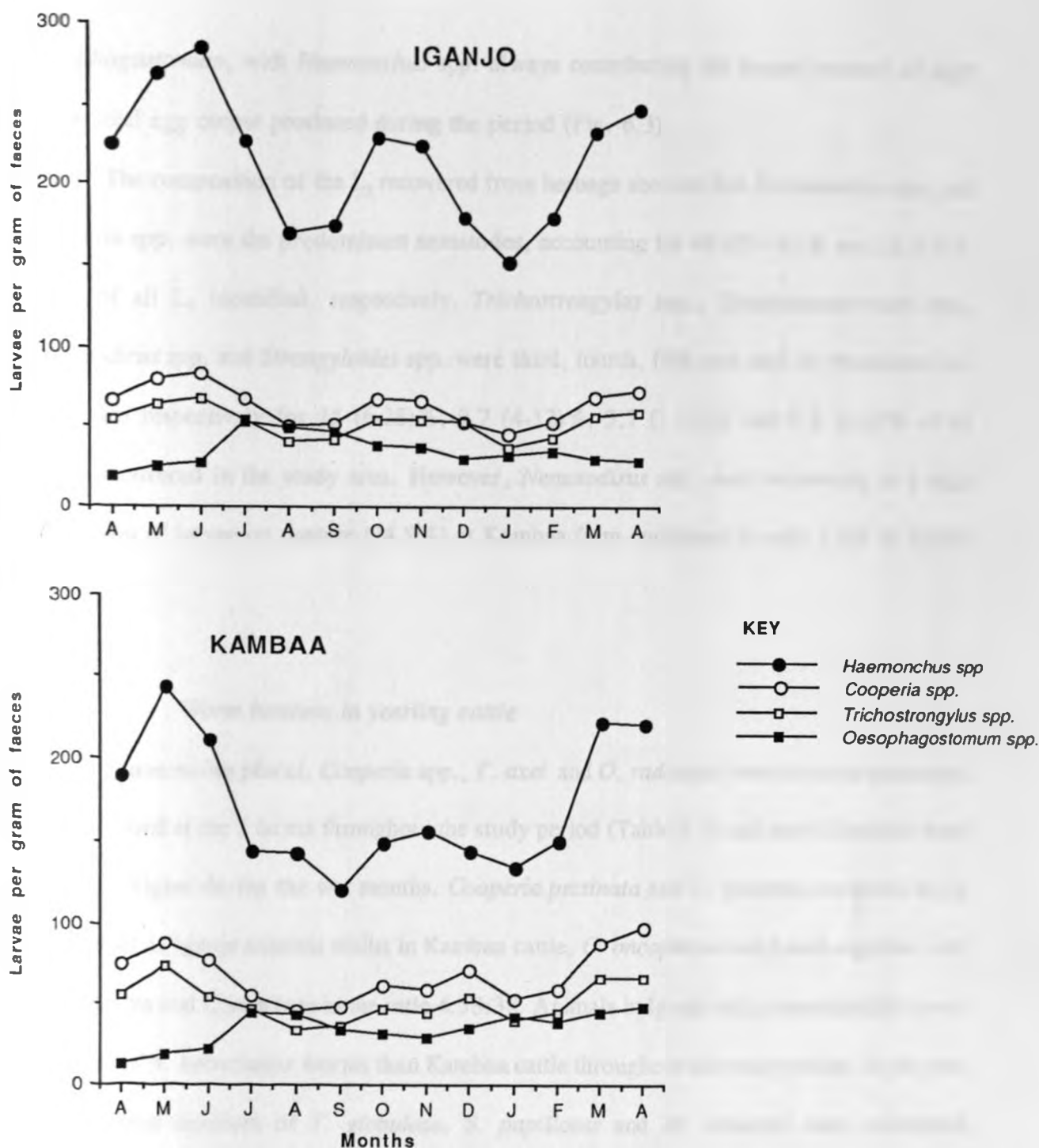


Fig. 6.3: Strongylate nematode infective larvae recovered from faecal egg output of resident cattle

Oesophagostomum, with *Haemonchus* spp. always contributing the largest number of eggs to the total egg output produced during the period (Fig. 6.3).

The composition of the L₃ recovered from herbage showed that *Haemonchus* spp. and *Cooperia* spp. were the predominant nematodes, accounting for 46 (32-74) % and 21.6 (12-39)% of all L₃ identified, respectively. *Trichostrongylus* spp., *Oesophagostomum* spp., *Nematodirus* spp. and *Strongyloides* spp. were third, fourth, fifth and sixth in abundance and accounted respectively for 15 (6-28)%, 9.7 (4-12)%, 3.7 (1-11)% and 0.8 (0-5)% of all larvae recovered in the study area. However, *Nematodirus* spp. were recovered in a high proportion of larvae on pasture (14.9%) at Kambaa farm compared to only 1.4% at Iganjo farm.

6.3.3 Worm burdens in yearling cattle

Haemonchus placei, *Cooperia* spp., *T. axei* and *O. radiatum* were the most numerous species found at the 2 farms throughout the study period (Table 6.1) and worm burdens were generally higher during the wet months. *Cooperia pectinata* and *C. punctata* occurred in the ratio 59:41 in Iganjo animals whilst in Kambaa cattle, *C. oncophora* was found together with *C. pectinata* and *C. punctata* in the ratio 4:58:38. Animals in Iganjo had proportionally lower numbers of *N. helvetianus* worms than Kambaa cattle throughout the study period. In the two farms, small numbers of *T. globulosa*, *S. papillosus* and *M. benedeni* were recovered sporadically. The mean ($\pm$ S.E) number of adult liver flukes in infected livers of 4 yearlings at Kambaa farm was 16.0 ± 7.1 (range 3-39).

6.3.4 Worm burdens in tracer calves and resident animals

Worm counts from individual tracer calves ranged from 800 to 4700, with mean

Table 6.1: Recovery of gastrointestinal nematodes from yearling dairy cattle necropsied bi-monthly (n=2) during the study period

Farm	Genera	MONTHS					
		June 1993	August	October	December	February 1994	April
Iganjo	<i>Haemonchus</i> adults	5518	1352	2075	3210	1133	4774
	<i>Haemonchus</i> ^a EL ₄	0 (0)	20 (1.5)	47 (2.3)	0 (0)	33 (2.9)	69 (1.4)
	<i>Trichostrongylus</i>	1887	1170	828	1123	528	1312
	<i>Cooperia</i>	2110	1493	1188	1210	647	3900
	<i>Nematodirus</i>	37	21	219	137	26	210
	<i>Oesophagostomum</i>	1551	894	628	801	510	1113
	<i>Trichuris</i>	0	47	53	13	45	160
	Total	11183	4997	5038	6494	2922	11538
Kambaa	<i>Haemonchus</i> adults	4248	1453	2551	2400	2055	5715
	<i>Haemonchus</i> ^a EL ₄	81 (1.9)	38 (2.6)	0 (0)	55 (2.3)	36 (1.8)	69 (1.2)
	<i>Trichostrongylus</i>	2193	927	1350	1180	555	1668
	<i>Cooperia</i>	3213	1124	1933	1467	647	1680
	<i>Nematodirus</i>	2075	607	910	893	529	2568
	<i>Oesophagostomum</i>	575	165	143	80	227	627
	<i>Trichuris</i>	33	27	0	0	72	105
	Total	12418	4341	6887	6075	4121	12432

^aThe percentage of EL₄ (early fourth stage larvae) to monthly worm burden are enclosed in brackets.

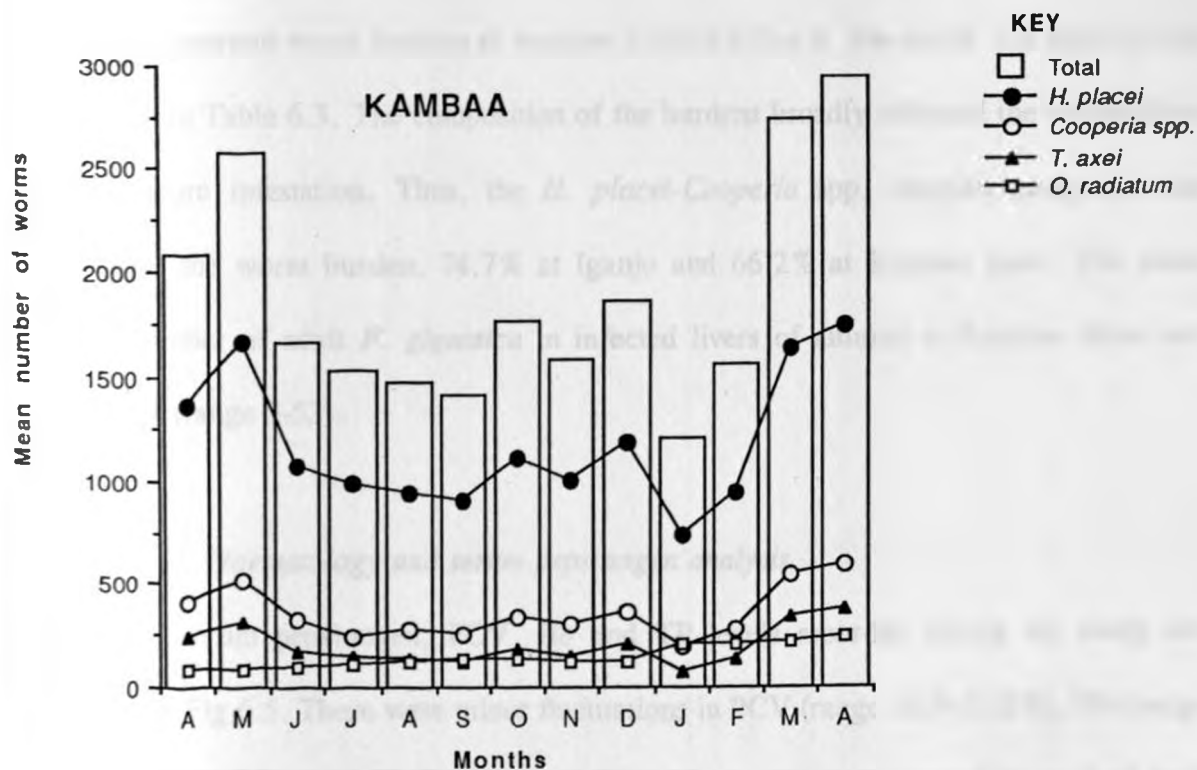
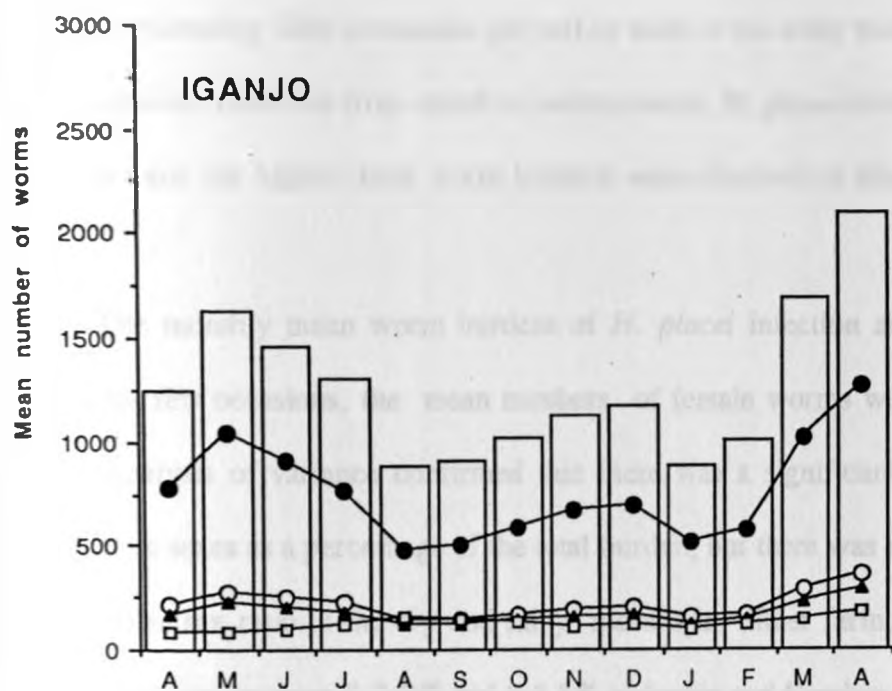


Fig-6.4: Mean monthly worm burden and frequency of occurrence of the dominant gastrointestinal nematode species in tracer calves

numbers exceeding 1000 nematodes per calf in most of the study months. While the number of each species recovered from month to month varied, *H. placei* dominated in the two farms (Fig. 6.4) and the highest total worm burdens were observed in May in 1993 and April in 1994.

The monthly mean worm burdens of *H. placei* infection are shown in Table 6.2. Except for few occasions, the mean numbers of female worms were higher than those of males. Analysis of variance confirmed that there was a significant difference ($P < 0.001$) between the sexes as a percentage of the total burden, but there was no significant difference ($P > 0.05$) in sex ratio in the dry and rainy months in either farm. The proportion of EL₄ of *H. placei* was between 0-3.9% and 0-6.8% at Iganjo and Kambaa farms, respectively and there was no indication of accumulation of arrested *Haemonchus* EL₄ at any sampling period in either farm (Table 6.2).

Post mortem worm burdens of resident animals killed at the end of the study period are shown in Table 6.3. The composition of the burdens broadly reflected the composition of the pasture infestation. Thus, the *H. placei-Cooperia* spp. complex comprised the majority of the worm burden, 74.7% at Iganjo and 66.2% at Kambaa farm. The mean ($\pm$ S.E) number of adult *F. gigantica* in infected livers of animals at Kambaa farm was 19.0 ± 10.4 (range 3-52).

6.3.5 *Haematology and serum pepsinogen analysis*

The serum pepsinogen, PCV, Hb and TP levels recorded during the study are presented in Fig 6.5. There were minor fluctuations in PCV (range 26.9-33.6%), Hb (range 83-111 g/l) and TP (range 59-85 g/l) levels during the experimental period in cattle of both farms. The values of each of these indices were within the normal limits of uninfected

Table 6.2: Monthly changes in the composition of *Haemonchus placei* in tracer calves in Iganjo and Kambaa farms

Month/Farm	IGANJO			KAMBAA		
	Mean worm burden		^a EL ₄	Mean worm burden		^a EL ₄
	Total	Female (%)		Total	Female (%)	
April 1993	724	50	0 (0)	1285	62	0 (0)
May	945	75	0 (0)	1545	51	82 (5.3)
June	849	50	0 (0)	1059	59	0 (0)
July	774	60	0 (0)	985	63	0 (0)
August	478	63	19 (3.9)	942	50	14 (1.5)
September	502	63	6 (1.2)	901	43	0 (0)
October	584	70	9 (1.5)	1104	50	29 (2.6)
November	655	75	25 (3.8)	1001	75	0 (0)
December	690	70	13 (1.9)	1164	57	79 (6.8)
January 1994	516	60	7 (1.4)	736	40	21 (2.9)
February	579	40	6 (1.0)	942	53	10 (1.1)
March	978	50	0 (0)	1625	58	60 (3.7)
April	1215	57	0 (0)	1732	53	0 (0)

^aThe percentage of EL₄ (early fourth stage larvae) to monthly mean worm burden are enclosed in brackets

Table 6.3: Mean and range of worm burdens of resident yearling animals necropsied at study termination

Location/Farm	IGANJO (n=3)		KAMBAA (n=3)	
	Number of worms		Number of worms	
	Mean	Range	Mean	Range
Abomasum				
<i>Haemonchus placei</i>	3819	830-4768	3292	520-5760
<i>Trichostrongylus axei</i>	910	550-1270	887	33-1405
Small intestine				
<i>Cooperia</i> spp.	1130	61-2351	1805	161-2760
<i>Nematodirus helvetianus</i>	85	0-170	1023	68-1550
Large intestine				
<i>Oesophagostomum radiatum</i>	640	320-1360	610	110-1720
<i>Trichuris globulosa</i>	43	9-104	86	12-148
Total worm burden	6627	1928-8698	7703	1865-9675

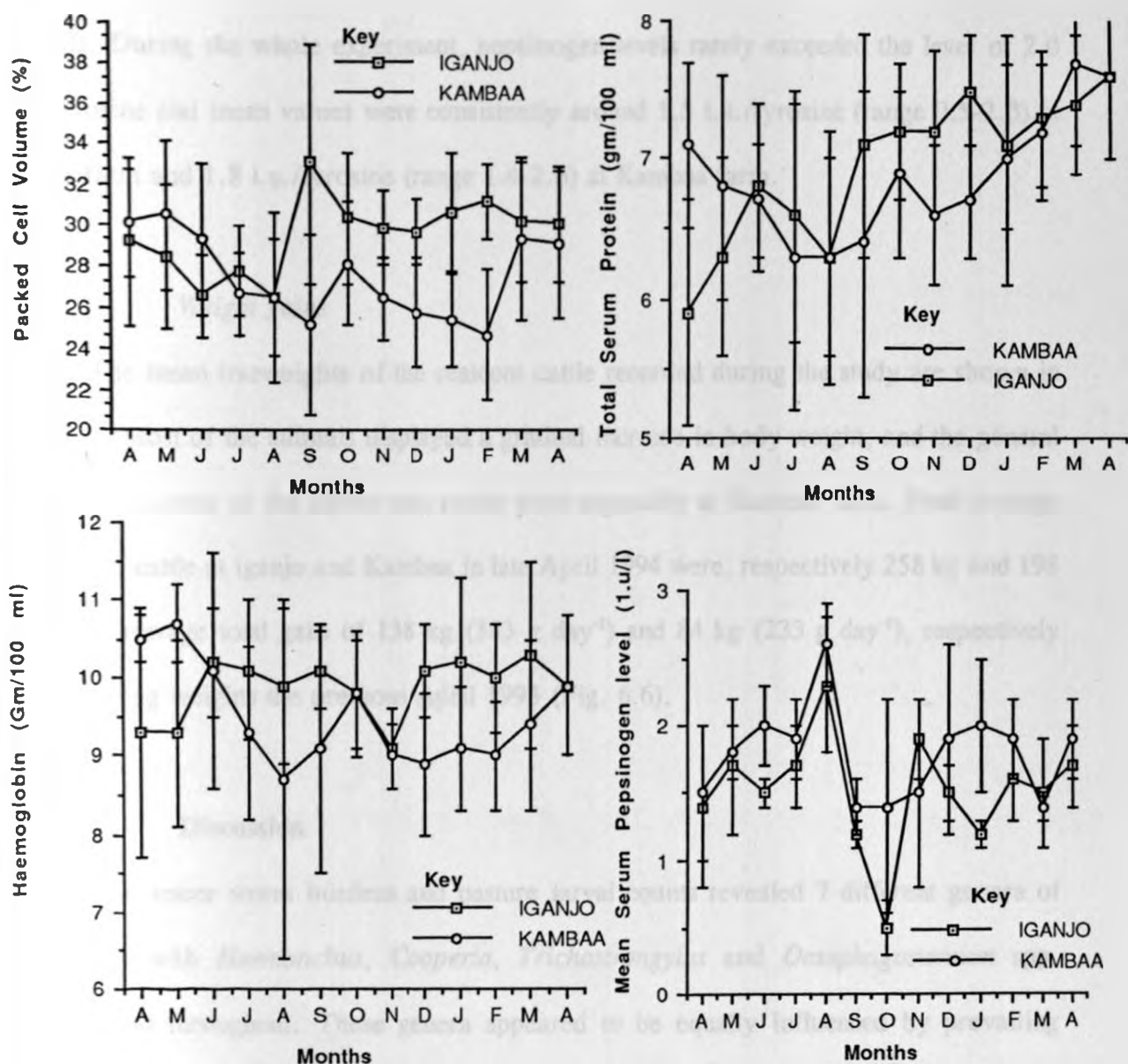


Fig. 6.5: Mean (±S.D) haematological, serum protein and plasma pepsinogen values for resident cattle at Iganjo and Kambaa farms

animals. During the whole experiment, pepsinogen levels rarely exceeded the level of 2.0 i.u./tyrosine and mean values were consistently around 1.5 i.u./tyrosine (range 0.5-2.3) at Iganjo farm and 1.8 i.u./tyrosine (range 1.4-2.6) at Kambaa farm.

6.3.6 *Weight gains*

The mean liveweights of the resident cattle recorded during the study are shown in Fig. 6.6. Most of the animals displayed a gradual increase in body weight, and the general condition of some of the calves was rather poor especially at Kambaa farm. Final average weights of cattle at Iganjo and Kambaa in late April 1994 were, respectively 258 kg and 198 kg, with average total gain of 138 kg (383 g day⁻¹) and 84 kg (233 g day⁻¹), respectively from starting weights the previous April 1993 (Fig. 6.6).

6.4 *Discussion*

The tracer worm burdens and pasture larval counts revealed 7 different genera of nematodes with *Haemonchus*, *Cooperia*, *Trichostrongylus* and *Oesophagostomum* spp. predominant throughout. These genera appeared to be equally influenced by prevailing weather conditions, although *C. oncophora* was only found at Kambaa farm which had cooler temperatures than Iganjo farm.

Haemonchus spp. larvae were the most numerous on pastures and in faecal cultures and infective L₃, although available at all times, were most numerous during the rainy months. The consistent high prevalence of *H. placei* infection indicate that the parasite is ubiquitous in cattle in these farms; it is generally considered to be the most prevalent and pathogenic nematode species of cattle in Kenya (Mango *et al.*, 1974; Waruiru *et al.*, 1993a). The next most numerous species, *Cooperia* and *Trichostrongylus*, were also most prevalent

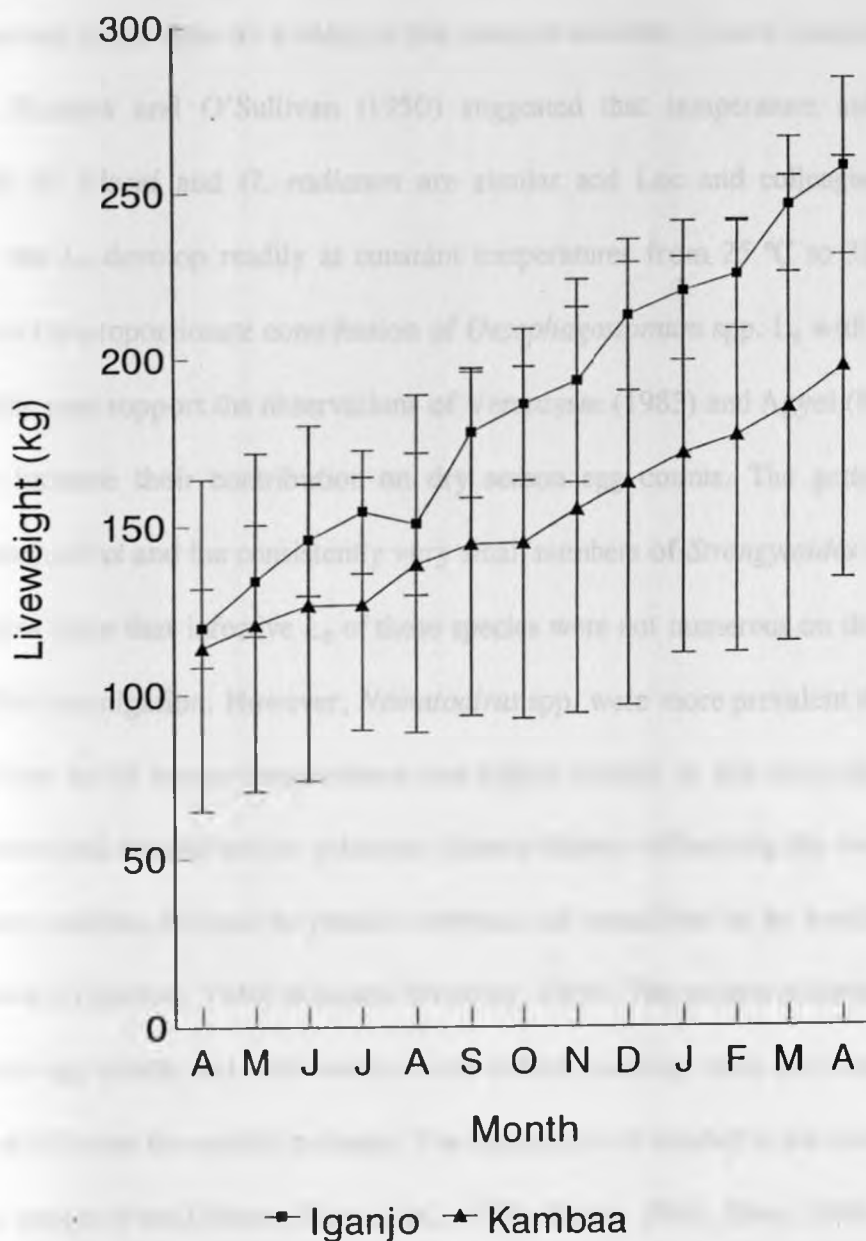


Fig. 6.6: Mean ($\pm$ S.D) liveweight changes in resident cattle at Iganjo and Kambaa farms

on the pastures during the wet months. The main feature of *O. radiatum* L₃ was the gradual rise during the early months of the rains, compared to the L₃ of *Haemonchus*, *Trichostrongylus* and *Cooperia* species. This can be explained by the longer pre-patent period of oesophagostomes rather than by a delay in the onset of suitable climatic conditions (Lee *et al.*, 1960). Roberts and O'Sullivan (1950) suggested that temperature and rainfall requirements of *H. placei* and *O. radiatum* are similar and Lee and colleagues (1960) confirmed that the L₃ develop readily at constant temperatures from 25 °C to 33 °C. The slight increase in the proportionate contribution of *Oesophagostomum* spp. L₃ with the onset of the dry months may support the observations of Vercruysse (1983) and Agyei (1991) who found them to increase their contribution on dry season egg counts. The generally low numbers of *Nematodirus* and the consistently very small numbers of *Strongyloides* recovered from tracer calves show that infective L₃ of these species were not numerous on the pastures at any time of the investigation. However, *Nematodirus* spp. were more prevalent at Kambaa farm, possibly due to its cooler temperatures and higher rainfall in this farm than Iganjo farm. Temperature and rainfall are the principal climatic factors influencing the incidence of internal parasites and can be used to predict outbreaks of parasitism to be predicted with reasonable accuracy (Gordon, 1948; Ross and Woodley, 1968). The pattern of herbage larval counts, strongyle egg counts and total worm counts in both yearling cattle and tracer calves in the two farms reflected the rainfall patterns. The importance of rainfall in the development of pre-parasitic stages is well known (Lee *et al.*, 1960; Durie, 1961; Rose, 1964) as is its effect in facilitating the migration of L₃ larvae from faecal pats onto pasture (Rose, 1962; Chiejina and Fakae, 1984).

Total worm counts in both yearling and tracer animals increased during the wet periods. The counts made from tracer calves demonstrated that this increase was associated

not only with a possible lack of resistance but also with a greatly increased level of contamination of the pastures with infective L₃ during the wetter months (March-June and October-December). The low counts over the drier months indicated a reduced larval availability on pastures and a close relationship between level of pasture contamination and rainfall, as temperatures rarely fall below 10 °C which is the minimum limit for development (Dinnik and Dinnik, 1958). The smaller nematode populations in yearling cattle in the drier months (August and February) could be attributed, therefore, not only to increasing resistance but also to a lower challenge from reduced infective L₃ population on the pastures.

Persistence of a parasitic nematode infection may be due to the successful survival of the pre-parasitic stages on the pasture and/or of the adults or hypobiotic larvae in the host. The post mortem and faecal examination results showed that adult worms of various GI nematodes and the digestion of abomasal mucosa did not reveal the presence of significant numbers of hypobiotic *H. placei* worms in the digesta in the present study. The absence of arrested *H. placei* larvae in cattle probably reflects the rainfall distribution and/or relative mild climatic conditions in the study area. The dry hot (January-March) and the cold dry (July-September) seasons, with occasional rainfall are short in comparison with the severe dry seasons in West or Central Africa (Kaufmann and Pfister, 1990; Pandey *et al.*, 1993). Hypobiosis therefore, does not seem to play any important role in the epidemiology of *H. placei* in the study area. Owing to the high fecundity of *H. placei*, residual female worms in the total population as shown in Table 6.2 could favour the transmission of the parasite from one rainy season to the other, and for the successful repopulation of the pastures during the favourable rainy season (Fakae, 1990).

Serum pepsinogen values were low and were not correlated with faecal worm egg counts even though the abomasal nematodes (*H. placei* and *T. axei*) were among the

predominant species. However, it is evident from other studies that a rise in plasma pepsinogen is not necessarily an important feature in the pathophysiology of *H. placei* or *T. axei* infections in calves (Ross *et al.*, 1967; Gennari *et al.*, 1991).

The lower haematological indices and weight gains of yearling cattle at Kambaa farm might be attributable to differences in farm management, other farm-level factors (Hassan Wan *et al.*, 1989) and concurrent infection with *F. gigantica* as liver fluke eggs and adult liver flukes were detected sporadically in their faeces and livers, respectively. Earlier studies by others have shown that there is enhanced pathogenicity of simultaneous exposure to *F. hepatica* and *H. contortus* in sheep (Presidente *et al.*, 1973), and *O. ostertagi* in cattle (Burden *et al.*, 1978). Other workers have shown that bovine fasciolosis results in considerable economic loss attributable to impaired feed conversion efficiency at the low level of (subclinical) infections, while at the higher (clinical) infection levels, inappetence also contribute to the loss (Hope Cawdery *et al.*, 1977; Chick *et al.*, 1980).

-Chapter 7-

DEVELOPMENT AND SURVIVAL (PLOT STUDIES) OF INFECTIVE LARVAE ON PASTURE

7.1 Introduction

The size of the populations of infective third-stage larvae (L_3) of ruminant nematode parasites on pasture are the result of the number of eggs spread with the faeces by the animals, their development rate into larvae and the survival and traslation of these larvae onto the grass (Rossanigo and Gruner, 1994).

The relationships between climatic factors and strongylid populations have been established for several different objectives: determination of areas and season favourable to the development of the different species (Levine, 1980; Fabiyi *et al.*, 1988; Gatongi *et al.*, 1988), determination of factors of parasitic risk between different herds (Suarez and Cabaret, 1991), mathematical modelling of the parasitic populations on pasture and/or in the hosts so as to forecast periods with higher risk of infection for the herd and the most efficient times for deworming (Thomas and Starr, 1978; Callinan *et al.*, 1982). For determining factors such as the developmental rate of larval populations on pasture, a primary feature is the use of the best climatological parameter (for example, rainfall in the present study) that is related to the parasite population level and which can be easily measured (Rossanigo and Gruner, 1994). For development of ruminant nematodes, the main constraint is availability of moisture inside the faeces (Berbigier *et al.*, 1990) and herbage (Gruner *et al.*, 1989; Besier and Dunsmore, 1983).

Knowledge of the origin of larvae of the major GI nematodes of cattle and the time course of their availability on pasture is useful. A control system involving the movement

of calves at set times of the year to avoid exposure to high larval infestation has been based on such information (Michael, 1969b). Various alternative procedures designed to prevent the build-up of dangerous numbers of larvae on the pasture (Michael *et al.*, 1981) also depend on the knowledge of seasonal pattern of larval infestation on herbage.

The present study was aimed at providing such information relevant to central Kenya, to guide in the formulation of sound control strategies.

7.2 Material and methods

7.2.1 Experimental design

Within a fenced area of pasture measuring about 50 m X 100 m a series of 72 plots of 2 m² each were demarcated. Ditches were dug between the plots to avoid cross-contamination during heavy rains, and the array of plots was fenced to prevent the entry of animals. Observations commenced in June 1995, when no further larvae from previous grazing could be recovered from the pasture. The grass on each plot was periodically clipped to maintain the herbage at a height and density similar to that found in adjacent paddocks which were permanently grazed by cattle. The clippings remained on the plot.

At the beginning of each month from July 1995 to June 1996, faecal pats, each weighing 2 kg and with a known number of eggs per gram of faeces (epg), as determined by a modified McMaster technique (MAFF, 1986), were deposited at the centre of each of 5 plots. The faeces were obtained from 4 donor calves each previously artificially infected with mixed cultures of between 10,000 and 20,000 infective larvae (L₃) of strongylid nematodes of cattle. The proportion of the eggs of each nematode genera ranged from 35 to 65 % for *Haemonchus* spp., 30-50 % for *Cooperia* spp., 10-25 % for *Oesophagostomum* spp. and 5-15 % for *Trichostrongylus* spp., as judged by differential larval counts from the

cultures. The potential production of L_3 from the pats in each series was estimated by culturing 4 separate 10 g samples and was found to be invariably high. Faecal cultures were prepared by the method of Hansen and Perry (1994) and identification of L_3 was based on the characteristics described by Keith (1953). A sixth plot was not contaminated, but was sampled each fortnight to detect any extraneous infection.

The plots were sampled fortnightly, after contamination, by collecting 60 uniformly distributed plucks of herbage per plot, taken at ground level. Sampling was continued until no larvae were recovered from the herbage on 2 consecutive occasions (Gibson and Everett, 1967). Infective larvae were recovered from pasture samples using the technique of Hansen and Perry (1994) and larval abundance was expressed as L_3 per kg of herbage dry matter ($L_3 \text{ kg}^{-1}$).

Meteorological measurements were supplied from the Keriita Forest Station, 1 km from the experimental plots. The records were limited to the maximum and minimum air temperatures, relative humidity and rainfall.

7.3 Results

No infective, third-stage larvae (L_3) were recovered from any of the control plots on any sampling occasion throughout the experiment. Third-stage larvae of *Haemonchus*, *Cooperia*, *Oesophagostomum* and *Trichostrongylus* were consistently recovered from the second week after contamination in each month of the year, with substantial numbers being recovered between 2 and 6 weeks after contamination. Larval counts were substantially higher during the rainy seasons but moderate levels of pasture infestation were obtained throughout the rest of the year. The concentrations on pasture of L_3 of the four genera, corrected for the numbers of eggs used to contaminate the plots, are shown for each month in Fig. 7.1.

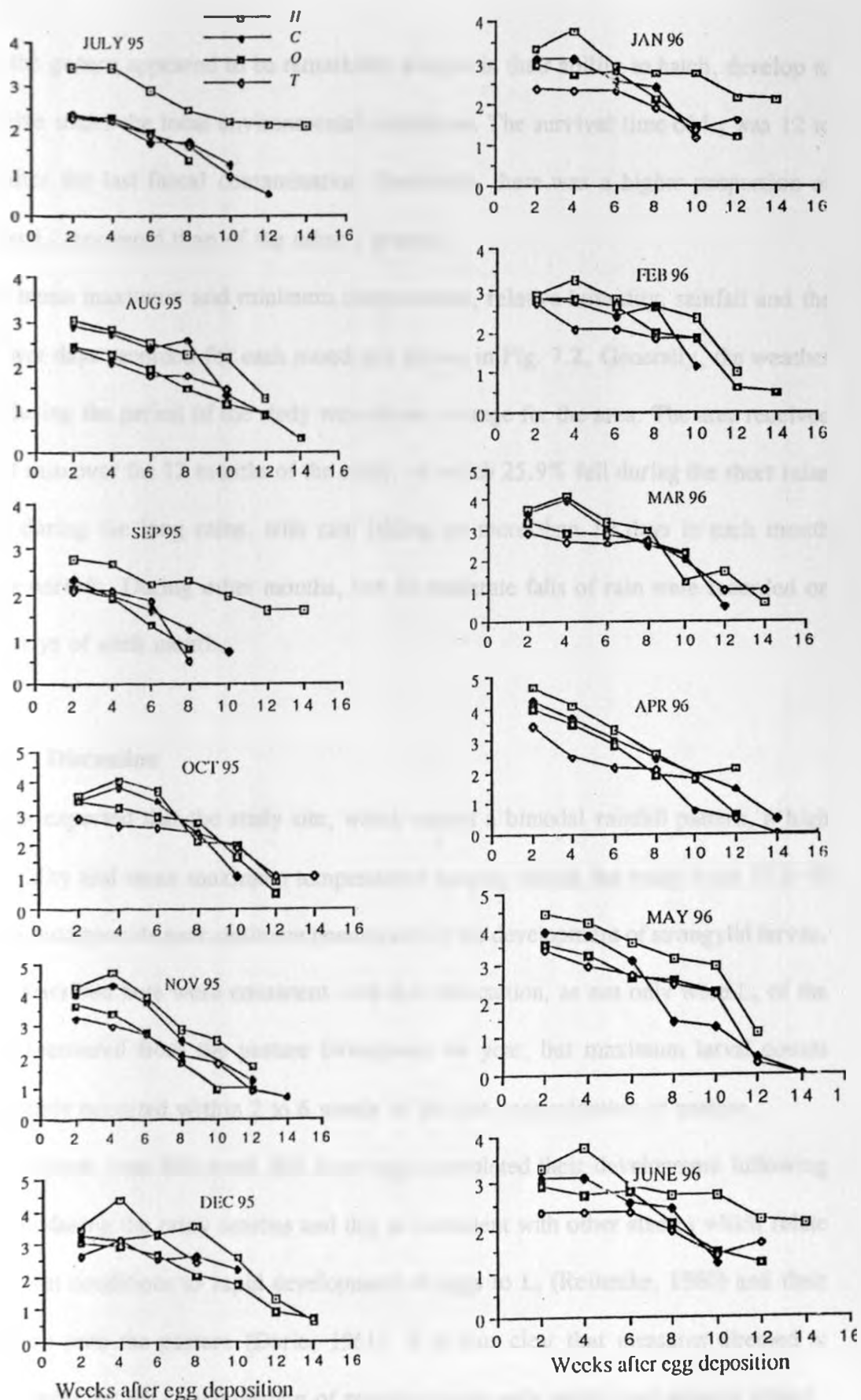


Fig. 7.1: Recoveries (average of 5 plots) of infective larvae per kilogram of pasture dry matter, log transformed, ($\log_{10}(X+1)$) and corrected for the numbers of eggs deposited, on plots contaminated sequentially from July 1995 to June 1996. H, *Haemonchus*; C, *Cooperia*; O, *Oesophagostomum*; T, *Trichostrongylus*

All the genera appeared to be remarkably similar in their ability to hatch, develop to L_3 and survive under the local environmental conditions. The survival time of L_3 was 12 to 16 weeks after the last faecal contamination. Generally, there was a higher proportion of *Haemonchus* L_3 recovered than of the other 3 genera.

The mean maximum and minimum temperatures, relative humidity, rainfall and the number of wet days recorded for each month are shown in Fig. 7.2. Generally, the weather conditions during the period of the study were about average for the area. The area received 1395 mm of rain over the 12 months of the study, of which 25.9% fell during the short rains and 52.3% during the long rains, with rain falling on more than 10 days in each month during these periods. During other months, low to moderate falls of rain were recorded on only a few days of each month.

7.4 Discussion

It was expected that the study site, which enjoys a bimodal rainfall pattern, a high relative humidity and mean maximum temperatures ranging during the study from 17.5 °C to 21.6 °C, would provide near-optimum conditions for the development of strongylid larvae. The results described here were consistent with this expectation, as not only were L_3 of the four genera recovered from the pasture throughout the year, but maximum larval counts almost invariably occurred within 2 to 6 weeks of the last contamination of pasture.

It is evident from this work that more eggs completed their development following contamination during the rainy seasons and this is consistent with other studies which relate such warm, wet conditions to rapid development of eggs to L_3 (Reinecke, 1960) and their mass migration onto the pasture (Durie, 1961). It is thus clear that measures directed at substantially reducing the contamination of pasture during such warm, wet seasons would

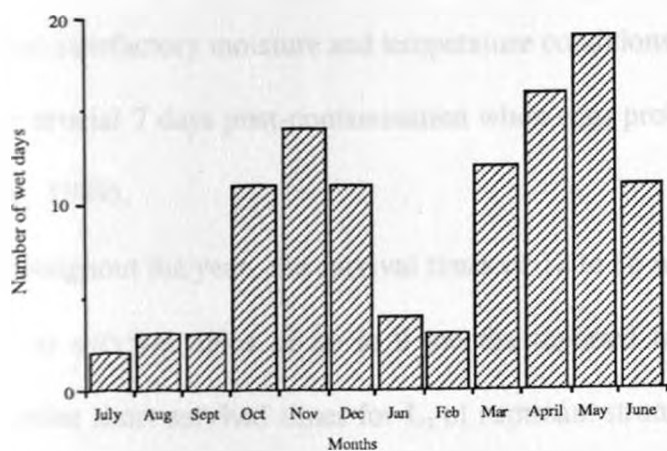
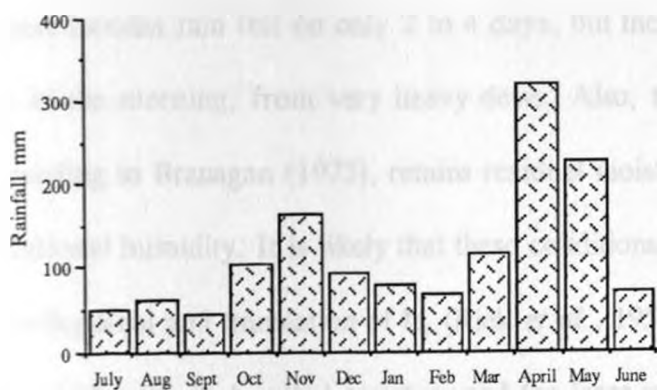
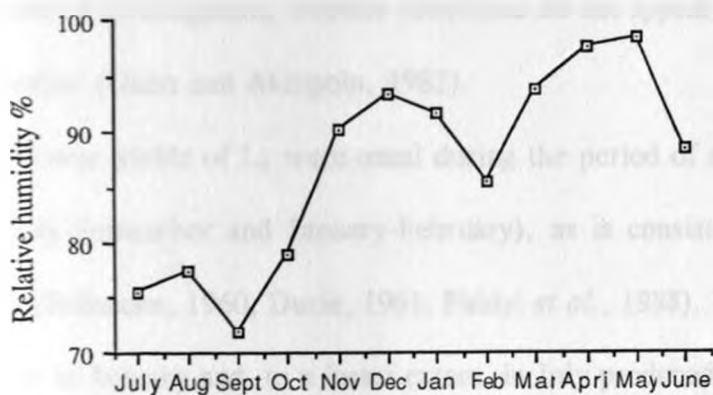
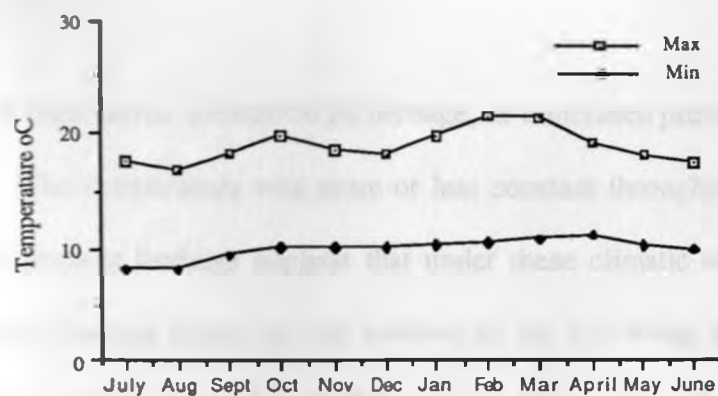


Fig. 7.2 : Meteorological data for Kimende, Kiambu District (Study site) from July 1995 to June 1996

forestall high larval infestation on herbage, as concluded previously by Fabiyi and Copeman (1986). The temperature was more or less constant throughout the study period (Fig. 7.2) and the present findings suggest that under these climatic conditions, rainfall is the most important limiting factor for the survival of the free-living larvae of the GI nematodes of cattle. In addition, since L_3 of all the genera were recovered from the herbage throughout the period of investigation, weather conditions do not appear to have a greater effect on one than another (Okon and Akinpelu, 1982).

Lower yields of L_3 were usual during the period of scarce, erratic and non-soaking rains (July-September and January-February), as is consistent with the findings of other workers (Reinecke, 1960; Durie, 1961; Fabiyi *et al.*, 1988). It is notable, however, that pats deposited in January and, to a lesser extent, in July produced relatively high numbers of L_3 . During these months rain fell on only 2 to 4 days, but the pasture was nevertheless moist, especially in the morning, from very heavy dews. Also, the grass was particularly dense, which according to Branagan (1973), retains residual moisture and contributes substantially to transpirational humidity. It is likely that these conditions provided the necessary moisture for the development and translation of L_3 (Riek *et al.*, 1953; Krecek *et al.*, 1995). Also, it would appear that the high initial moisture and the large size of the pats used in this study ensured that satisfactory moisture and temperature conditions were maintained inside the pats during the crucial 7 days post-contamination when eggs probably developed to L_3 (Chiejina and Fakae, 1989).

Throughout the year, the survival times of 12 to 16 weeks were relatively short when compared to survival times of up to 9 months reported from temperate countries (Rose, 1962). Similar short survival times for L_3 of ruminant strongylids were recorded during the wet season in Nigeria by Okon and Enyenihi (1977), in the wet tropical climate of Northern

Queensland (Fabiyi *et al.*, 1988) , in the wet and dry zones of Fiji (Banks *et al.*, 1990) and in the wet tropical environment of the Pacific island of Tongatapu, Tonga (Barger *et al.*, 1994).

Several workers have correctly cast doubt on the practice of pasture spelling and rotational grazing as a means of controlling internal parasites of ruminants in temperate regions, mainly because the time needed to ensure a significant reduction in larval numbers is too long for economic management of the pastures (Gibson and Everett, 1967; Michel, 1969b; Donald, 1974). The rapid decline in larval populations noted here, however, may well make rotational control methods based on changing pasture practicable in this type of climate, with no real dry season. In addition, if pasture could be maintained free from new egg deposition for longer than the survival time of L_3 , the use of novel forms of chemotherapeutic control like the sustained release devices (Waruiru *et al.*, 1997) may also be feasible. Both these possibilities are being explored in continued studies in Kenya.

-Chapter 8-

COMPARATIVE EFFICACY OF MORANTEL SUSTAINED RELEASE TRILAMINATE BOLUS AND CONVENTIONAL ANTHELMINTIC TREATMENT AGAINST GASTROINTESTINAL NEMATODES

8.1 Introduction

Anthelmintics have been widely used for the treatment of clinical parasitic gastroenteritis (PGE) in cattle, but in recent years the importance of subclinical infection has increasingly been recognized and attention has been more directed to the prevention of infection. Although conventional single anthelmintic treatment effectively eliminates the majority of GI worms, the already infested herbage to which grazing cattle are exposed is altered little by such treatment and re-exposure to new infections continues if the cattle remain in the same environment after treatment (Prosl *et al.*, 1983). A combination of strategic treatment and movement to safe pasture as proposed by Michael (1969a) has since been widely used together with other strategies (Nansen, 1993). However, in many places pasture and management limitations have raised a need for preventive measures based on continuous medication or repeated treatments with anthelmintics, in order to eliminate existing worm burdens and reduce pasture contamination and reinfection (Thomas and Bell, 1988).

The use of intraruminal sustained release bolus containing morantel tartrate (MSRB; Paratect[®] Bolus, Pfizer) has provided a successful method to remove existing worm burdens and prevent reinfection over a period of 60 to 90 days (Jones, 1983). The efficacy of the MSRB in controlling PGE in cattle under a variety of field grazing conditions has been confirmed by a number of workers (Guldenhaupt and Burger, 1983; Hawkins *et al.*, 1985;

Ciordia *et al.*, 1987). The release profile of the morantel bolus now marketed (MSRT; Paratect Flex^R Bolus, Pfizer) has been shown to confer protection of first year calves throughout a 90 day grazing period (Vercruysee *et al.*, 1989).

The strategic use of the MSRT bolus has been well documented under temperate field conditions with distinct grazing season (Rickard *et al.*, 1989; Grimshaw *et al.*, 1989; Vercruysee *et al.*, 1992; Hertzberg *et al.*, 1994). However, there is little information on the use of MSRT in tropical environments and the aim of this study was to determine the efficacy of MSRT in controlling GI nematode parasitism in naturally infected grazing calves in a Kenyan tropical environment.

8.2 Materials and methods

8.2.1 Experimental design

The experiment was conducted at Iganjo farm from 4 March to 30 December 1993, starting at the onset of the long rains. Forty Friesian/Ayrshire crossbred calves (24 male and 16 female) originating from the farm and previously grazed on infected pastures were selected for the trial. Fourteen days prior to the start of the trial the calves were introduced to the experimental pasture plots of 10 acres, grazed previously by infected young cattle, to balance nematode levels in the calves and on the pasture. Prior to the trial, all calves were sprayed with an acaricide once a week for ticks and were tested for haemoparasites.

At the start of the trial, the calves were treated with oral triclabendazole (Fasinex^R, Ciba-Geigy; 12 mg kg⁻¹) for *Fasciola gigantica* infection. The calves were divided into 4 equal groups of 6 male and 4 female calves on the basis of age and weight; the mean ($\pm$ S.D) weight and age of the calves was 159.2 $\pm$ 9.3 kg and 9.0 $\pm$ 0.5 months, respectively. The experimental plot was divided into four 1 ha paddocks of similar configuration and the

four groups of calves allocated to them. Throughout the trial the calves grazed Kikuyu grass (*Pennisetum clandestinum*) but due to grass shortage caused by drought, they were fed equal amounts of supplementary hay and wheat bran from late August to the end of the trial. Water and mineral blocks were available at all times. Calves in group 1 (T-1) were non-medicated controls and those in group 2 (T-2) each received one MSRT bolus on day zero. Calves in group 3 (T-3) were drenched with albendazole on day zero while, those in group 4 (T-4) were drenched with albendazole on day zero and day 14 post turnout. After MSRT administration the calves in T-2 group were confined for 24 h to a pen for observations to confirm retention of the bolus and to monitor any adverse reactions. Thereafter the experimental calves were placed in their respective paddocks for the entire experimental period. The anthelmintic bolus was administered by a specially designed balling gun according to the manufacturers recommendations. Albendazole (Valbazen^R, SmithKline Beecham Animal Health Products) was administered orally using a calibrated syringe at a dose of 7.5 mg kg⁻¹. The MSRT cost to farmer/> 100 kg animal during the study period was US\$ 15 and the handling cost of animals for set up, round up and treatment (average Kenyan wage/day) US\$ 2 giving a total of US\$ 17, while the basic costs per animal for conventional anthelmintics (excluding veterinary costs) ranged from US\$ 6.5, for ivermectin; 5.9, for albendazole and fenbendazole and, 2 for levamisole, respectively.

Three calves from each of the 4 groups were selected at random for slaughter at the end of the trial for residual worm recovery. Pairs of tracer calves, 5-6 months of age and raised worm-free since birth, were introduced into each paddock to evaluate the initial (March), interim (May and September) and final (December) levels of pasture infectivity by nematode larvae. These tracers were allowed to graze for 28 days after which they were housed on concrete floor for 21 days prior to necropsy and worm recovery.

Herbage samples for strongylid larval counts and rectal faecal samples were collected at the start of the trial and monthly thereafter. Individual body weights were recorded for all experimental calves before the trial commenced and then at monthly intervals until trial termination. Jugular blood samples were taken monthly from all the calves for estimation of packed cell volume (PCV), haemoglobin (Hb), total protein (TP), and serum pepsinogen levels (Ross *et al.*, 1967; Coles, 1974). Animals were observed daily as part of the normal management practices in the experimental farm and were examined thoroughly at each sampling.

Herbage and faecal samples were processed as described by Hansen and Perry (1994) and MAFF (1986), respectively, while all necropsy, adult worm recovery procedures, counting and identification techniques were performed as previously described (MAFF, 1986).

8.2.2 *Statistical analysis*

A one way analysis of variance was performed on the data for individual calves for each sampling period to assess the effect of treatments on nematode egg excretion, worm counts, and liveweight gains. To normalize distribution of the data of various parameters to fulfill parametric test requirements, the data were transformed in the form of $\ln(x + 10)$. Treatment comparisons were made by using Tukey's test (Fowler and Cohen, 1990).

8.3 **Results**

8.3.1 *Pasture larval counts*

The pasture larval patterns for the 4 paddocks are shown in Fig. 8.1. Herbage samples taken at the start of the study (day 0) showed that the 4 paddocks were contaminated

with similar numbers of strongylid infective larvae (L_3). There was a gradual, parallel decrease of pasture larval concentration from May to August in the 4 paddocks followed by an increase to a peak mean count of 1600, 1100 and 1300 $L_3 \text{ Kg}^{-1}$ dry matter in September on paddocks T-1, T-3, and T-4, respectively. In the T-2 paddock only 495 $L_3 \text{ kg}^{-1}$ were recovered in September and over the rest of the experiment the larval counts remained lower than those of control T-1 paddock (Fig. 8.1). There were no statistical significant differences ($P > 0.05$) between counts of paddocks T-1, T-3 and T-4 during this period.

8.3.2 *Faecal egg counts*

The mean number of strongylid egg counts from the four groups of calves throughout the trial period are presented in Fig. 8.2. There were no significant differences between the groups at the start of the trial. However, counts from the T-2 calves were reduced by 100% ($P < 0.001$) following treatment and remained significantly lower ($P < 0.05$) than counts from control T-1 calves up to the end of the trial. Faecal egg counts in the albendazole treated calves were significantly lower after treatment up to May for T-3 calves ($P < 0.01$) and July for T-4 calves ($P < 0.01$), compared to control T-1 calves. Thereafter, egg counts rose steadily in both the T-3 and T-4 groups and were indistinguishable from those of the T-1 control calves at the termination of the study (Fig. 8.2).

8.3.3 *Worm counts in tracer calves*

Worm numbers in tracer calves are presented in Table 8.1. Tracer calves grazing the

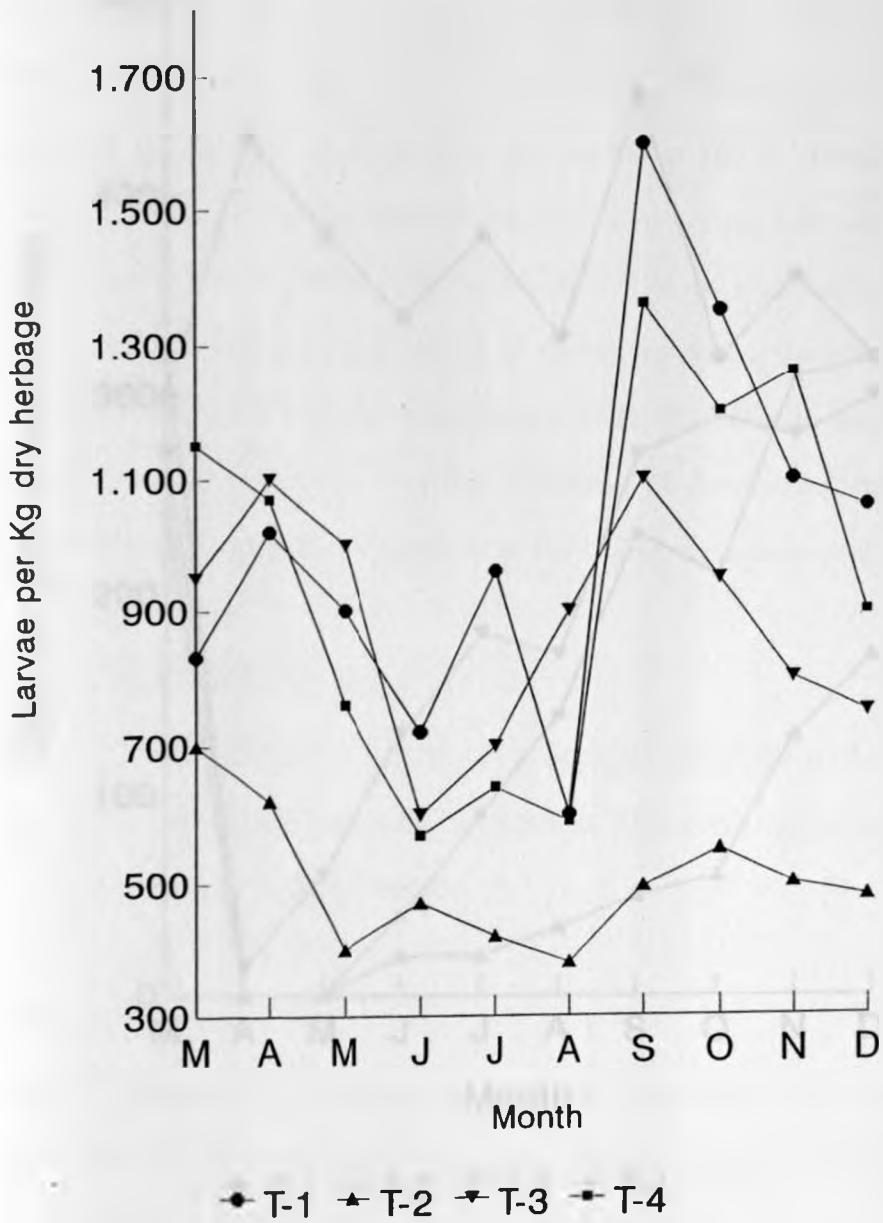


Fig. 8.1: Pasture contamination with strongylid Infective larvae

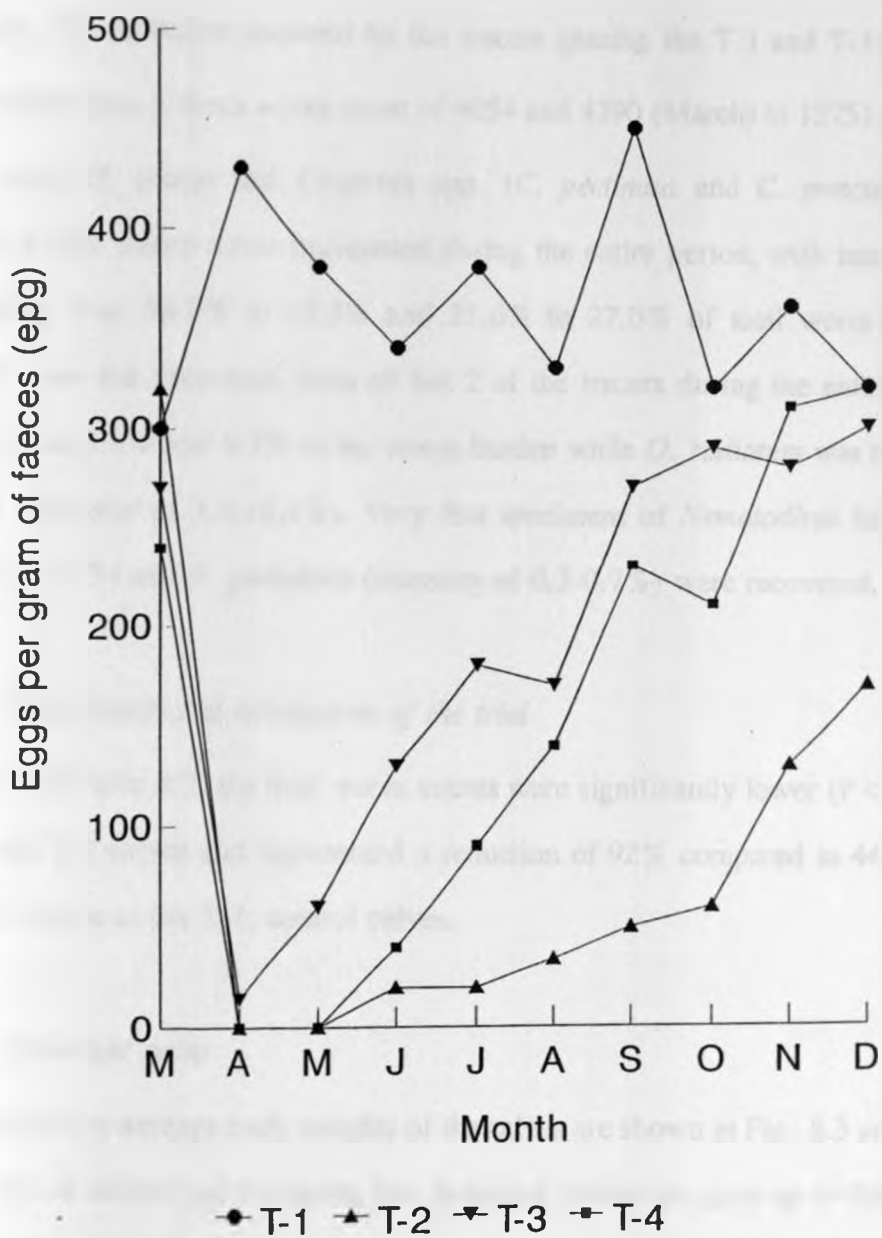


Fig. 8.2: Mean strongylid eggs per gram of faeces

first month (March) became infected with similar worm burdens. The number of nematodes recovered from subsequent T-2 and T-4 tracer calves decreased and remained at a low level for the rest of the trial, with a 55-85.7 % reduction in the worm counts of the tracers grazing the T-2 paddock. The infection acquired by the tracers grazing the T-1 and T-3 paddocks after turnout ranged from a mean worm count of 4054 and 4390 (March) to 12751 and 7801 (September). Adult *H. placei* and *Cooperia* spp. (*C. pectinata* and *C. punctata*) were recovered from all the tracer calves necropsied during the entire period, with intensities of infections ranging from 56.9% to 65.3% and 21.6% to 27.0% of total worm burdens, respectively. *T. axei* was recovered from all but 2 of the tracers during the entire period, constituting between 3.7% and 9.2% of the worm burden while *O. radiatum* was recovered from all calves (intensity of 3.5-10.4%). Very few specimens of *Nematodirus helvetianus* (intensity of 0.3-1.9 %) and *T. globulosa* (intensity of 0.2-0.7%) were recovered.

8.3.4 *Worm burdens at termination of the trial*

As shown in Table 8.2, the total worm counts were significantly lower ($P < 0.05$) in the MSRT-treated T-2 calves and represented a reduction of 92% compared to 44% (T-3) and 58% (T-4) relative to the T-1, control calves.

8.3.5 *Liveweight gains*

The comparative average body weights of the calves are shown in Fig. 8.3 and Table 8.3. The 4 groups of calves had increasing but diverging liveweight gain up to September after which the weight curves levelled off presumably due to lowering of grass quality and quantity because of lack of rain. At the end of the trial, the T-2 calves had a highly significant ($P < 0.001$) mean liveweight gain advantage of 69.2 kg over the T-1 control calves, while the T-4 calves had an advantage of 23.2 kg ($P < 0.05$) over the controls (Table 8.3).

Table 8.1: Worm counts recovered from tracer calves (2 calves per group) and in parenthesis the percentage worm reduction on paddocks T-2, T-3, and T-4 compared to T-1

Month	Paddock	<i>H. placei</i>	<i>T. axei</i>	<i>Cooperia</i> spp.	<i>N. helvetianus</i>	<i>O. radiatum</i>	<i>T. globulosa</i>	Total
March	T-1	2710	359	578	11	695	6	4359
		1668	607	864	7	592	11	3749
	T-2	2679	744	912	0	611	4	4950
		2147	324	694	0	819	2	3986
	T-3	2567	516	678	19	588	7	4375
		2175	538	902	9	816	0	4440
	T-4	2100	539	929	40	713	0	4321
		2540	493	617	75	663	0	4388
May	T-1	3600	810	1338	75	910	33	6766
		3326	730	774	121	1091	18	6060
	T-2	1579	595	422	14	392	7	3009
		1237 (59.3)	397 (35.6)	573 (52.8)	29 (75.0)	509 (54.9)	16 (53.8)	2761 (55.0)
	T-3	2917	487	713	43	736	18	4914
		1901 (30.4)	583 (30.5)	893 (23.9)	26 (64.8)	692 (28.6)	7 (50.9)	4102 (29.7)
	T-4	2681	251	409	14	306	3	3664
		1560 (38.8)	189 (28.6)	319 (65.5)	8 (88.8)	363 (66.6)	5 (84.3)	2444 (52.4)
September	T-1	7824	761	1439	19	2182	16	12241
		8838	353	2151	37	1854	28	13261
	T-2	1119	0	451	0	518	11	2099
		853 (59.3)	0 (100)	305 (78.8)	0 (100)	374 (77.9)	19 (31.8)	1553 (85.7)
	T-3	4767	970	1617	22	1254	16	8646
		4566 (43.9)	891 (-)	1172 (22.3)	50 (-)	1365 (35.1)	12 (36.4)	8056 (34.5)
	T-4	4118	471	1139	12	572	6	6318
		2049 (62.9)	818 (-)	835 (53.5)	20 (42.9)	796 (66.1)	4 (77.3)	4522 (57.5)
December	T-1	6355	338	2010	109	1299	64	10175
		5165	552	1364	179	1715	90	9065
	T-2	1285	0	340	18	275	22	1940
		1421 (76.5)	0 (100)	494 (75.3)	8 (90.9)	339 (79.6)	12 (77.9)	2274 (78.1)
	T-3	4100	770	1113	104	913	14	7014
		3602 (33.1)	676 (-)	1055 (35.7)	68 (40.3)	1013 (36.1)	22 (76.6)	6436 (30.1)
	T-4	2584	695	722	83	719	30	4833
		2320 (57.4)	798 (-)	1463 (35.2)	59 (50.7)	669 (53.9)	46 (50.6)	5355 (47.0)

Table 8.2: Mean number and, in parenthesis, the percentage worm reduction after anthelmintic treatment of adult and immature worms from 3 calves per group necropsied at trial termination

Parasite/group	T-1	T-2	T-3	T-4
<i>Haemonchus placei</i>	2773 ^a	233 ^b (91)	1432 ^a (44)	1154 ^a (55)
<i>Trichostrongylus axei</i>	572 ^a	25 ^b (96)	418 ^a (27)	189 ^a (70)
<i>Cooperia</i> spp.	858 ^a	65 ^b (92)	425 ^a (50)	319 ^a (63)
<i>Nematodirus helvetianus</i>	95 ^a	0 ^b (100)	53 ^a (44)	0 ^b (100)
<i>Oesophagostomum radiatum</i>	667 ^a	53 ^b (92)	32 ^a (51)	329 ^a (51)
<i>Trichuris globulosa</i>	0	3	0	4
All worms	4765 ^a	379 ^b (92)	2652 ^a (44)	1995 ^a (58)

Worm counts in the same row with different superscripts differ significantly ($p < 0.05$).

Table 8.3: Mean bodyweight and weight gains of control (T-1) and treated (T-2, T-3 and T-4) calves

Groups	No. of animals	Mean initial weights (kg)	Mean final weights (kg)	Mean gain (kg)	Mean daily gain (g day ⁻¹)
T-1	10	159.8±6.1	219.2±5.8	59.4±4.8	200.0±7.4
T-2	10	158.5±8.7	287.1±6.2	^a 128.6±7.5	530.0±13.1
T-3	10	161.2±9.7	213.7±4.3	52.5±5.7	170.0±6.9
T-4	10	162.2±9.1	244.8±9.1	^a 82.6±6.3	270.0±9.1

^aSignificantly different from group T-1 ($P < 0.05$).

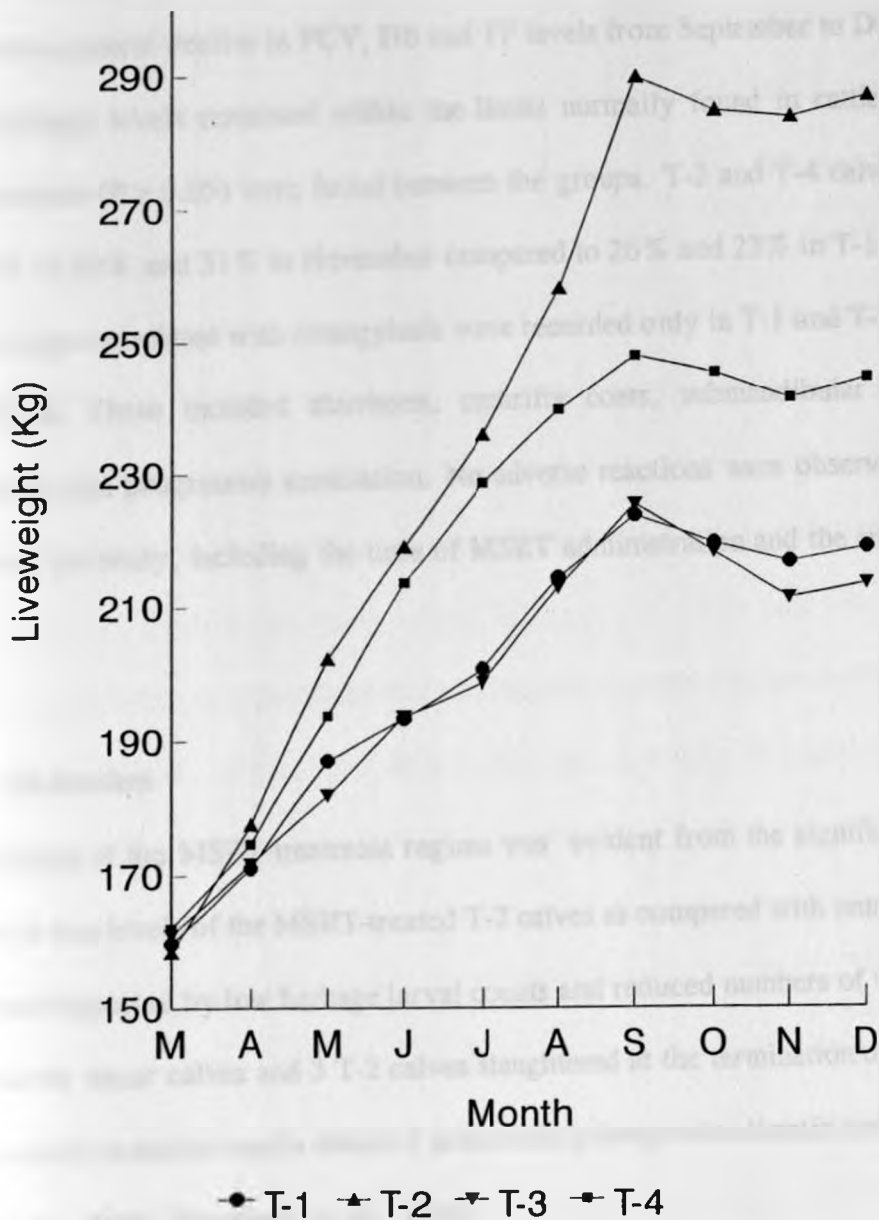


Fig. 8.3: Mean liveweights of treated and control calves

8.3.6 *Blood parameters and clinical observations*

Mean serum pepsinogen levels were within the range of 1-2 i.u. tyrosine per litre with no significant differences ($P > 0.05$) between the 4 groups, and did not correlate with epg counts. There was a general decline in PCV, Hb and TP levels from September to December in all groups although levels remained within the limits normally found in cattle and no significant differences ($P > 0.05$) were found between the groups. T-2 and T-4 calves had a group mean PCV of 30% and 31% in November compared to 26% and 23% in T-1 and T-3 calves. Clinical signs associated with strongylosis were recorded only in T-1 and T-3 groups during this period. These included diarrhoea, unthrifty coats, submandibular oedema, anaemia, weakness and progressive emaciation. No adverse reactions were observed in the T-2 calves during the study, including the time of MSRT administration and the subsequent 24-h period.

8.4 **Discussion**

The efficacy of the MSRT-treatment regime was evident from the significant long-term reduction in epg levels of the MSRT-treated T-2 calves as compared with untreated T-1 calves. This was supported by low herbage larval counts and reduced numbers of nematodes recovered from the tracer calves and 3 T-2 calves slaughtered at the termination of the trial. These findings confirm earlier results obtained in the field in temperate climatic environments (Vercruysse *et al.*, 1992; Hertzberg *et al.*, 1994).

The MSRT-treated T-2 calves gained significantly more weight than the control T-1 and albendazole (T-3 and T-4) treated calves. In addition to the pathophysiological changes brought about by GI nematodes, parasite induced inappetence may also contribute to poor performance (Entrocasso *et al.*, 1986a; Thomas and Bell, 1988; Bell *et al.*, 1988). It is

therefore highly probable that GI nematodes caused a significant reduction in feed intake of T-1 and T-3 calves contributing to their poorer performance in liveweight gain compared with the other groups (Holmes and Coop, 1994).

Although single conventional anthelmintic treatment leads to an immediate removal of parasite burdens in the gut, the parasite load of the grazing pastures is only altered little by this treatment (Prosl *et al.*, 1983). Thus, unless animals are moved to 'safe pastures' they will quickly become reinfected with subsequent further damage and depression of performance as observed in the albendazole-treated (T-3 and T-4) calves in the present study. However, had the albendazole treatment been given at regular intervals (i.e. 3 months) over the study period, one could expect a long-term preventive effect of this drug as well.

The 92% reduction in mean total numbers of parasites at the end of the trial and the improved weight gain of the MSRT-treated (T-2) calves in this study was similar to the results of previous studies using the older morantel sustained release bolus (MSRB) (Jones and Bliss, 1983; Sykes *et al.*, 1987). These data indicate that in the Kenya highlands, MSRT was as effective as in the temperate environments in reducing GI nematode infections and pasture infectivity as well as providing a significant weight gain advantage over non-medicated control calves (Vercruysse *et al.*, 1992; Hertzberg *et al.*, 1994).

There has been concern that use of controlled release devices might promote development of drug resistant populations of parasites (Donald, 1985). Administration of anthelmintics in sustained release formulations, could cause an intense selection pressure resulting in anthelmintic resistance. Little empirical evidence is currently available to assess these potential effects (Anderson, 1985; Zimmerman and Hoberg, 1988). The MSRT bolus, field tested and in use for more than 10 years, has yet to be directly associated with drug resistance in helminths (Coles *et al.*, 1994) although selection of a morantel resistant strain

of *O. ostertagi* has been reported where the sustained release bolus was experimentally used (Borgsteede, 1988).

The advent of highly effective chemoprophylaxis has raised the question of whether or not animals so protected receive sufficient antigenic stimulation to induce immunity (Vercruysse et al., 1994; Bell *et al.*, 1996). Control systems in temperate environments based on sustained release boli have a long active life, with expected duration of efficacy of 90 to 135 days. This raises the question of whether these systems allow sufficient larval challenge for development of immunity (Armour *et al.*, 1988; Vercruysse *et al.*, 1992; 1994). The development of immunity might not be affected to the same extent by use of sustained release boluses in those tropical areas where the animals graze all year round and trickle infections occur continuously. However, the acquired immunity may be compromised during prolonged dry spells leading to outbreaks of parasitic gastroenteritis (PGE).

For the control of endoparasitic nematodes of ruminants, the development of sustained-release bolus technology for the administration of anthelmintics is viewed as an important advance (Probert, 1994). The use of a bolus allows a measured quantity of drug to be delivered directly to the rumeno-reticulum over an extended time period. This allows for a reduction in labour costs to the farmer and handling stress to the animal while giving sustained anthelmintic cover. However, for all classes of anthelmintics, a proportion of the administered drug is excreted either in the faeces or urine, often in largely unmetabolised form (Strong and Wall, 1990). Concern has been expressed by many researchers about the impact of excreted anthelmintics on insects of the dung community and the potential effects on dung degradation and nutrient recycling (Herd *et al.*, 1993; Strong *et al.*, 1996).

The success of controlled release systems in the control of PGE in ruminants in developing countries will depend upon the demonstration of cost effectiveness. The

anthelmintic compound will have to be released and be effective at a time when the susceptible population of target parasites is present. In many tropical and subtropical regions however, the actual patterns of infection have yet to be elucidated. Until it is known when effective levels of the anthelmintics should be delivered to the host, the true value of such treatment in these environments cannot be fully recognized or evaluated. Moreover, although controlled release systems should prove to be among the most significant improvements in the control of helminth parasites, they will not necessarily preclude the need for conventional administration of anthelmintics. As the epidemiology and transmission patterns are being clarified, control programmes can incorporate the use of boluses at one time of the year with conventional dosing at other times. In cases where grazing on contaminated pastures following conventional single-dose therapy would result in immediate reinfection, the bolus approach would be preferable. But if continual exposure to infection is not likely, the cost of a bolus may not justify its use (Zimmerman and Hoberg, 1988; Waruiru *et al.*, 1997).

-Chapter 9-

OVERWINTERING RESIDUAL GRASS INFECTIVITY IN PASTURES PREVIOUSLY GRAZED BY *DUDDINGTONIA FLAGRANS* FED CALVES

9.1 Introduction

Field studies conducted in Denmark on the control of gastrointestinal nematodes of cattle have demonstrated that feeding of calves during the first two months of the grazing season with the microfungus, *D. flagrans* grown on barley grain reduced herbage infectivity and subsequently ingestion of trichostrongylid larvae, mainly *O. ostertagi*, later in the season (Wolstrup *et al.*, 1994). An experiment conducted in 1993, showed that the strategic feeding of first season calves with *D. flagrans* over the first three months of the grazing season was able to prevent severe clinical trichostrongylosis in the late summer. These results also demonstrated that larval populations of *Ostertagia* and *Cooperia* were significantly reduced on the pasture grazed by fungus-treated calves. In comparison, numbers of *Nematodirus* larvae seemed less affected (Nansen *et al.*, 1995).

The present investigation, conducted in 1994 was designed to determine the overwintering residual grass infectivity in a pasture previously grazed by *D. flagrans* treated and untreated calves.

9.2 Materials and methods

9.2.1 Study area

The experiment was conducted at the field station of the Royal Veterinary and Agricultural University, 20 km West of Copenhagen, Denmark, on a permanent low-lying pasture known to have a mixed parasite fauna of the following genera: *Ostertagia*, *Cooperia*,

Nematodirus, *Strongyloides* and *Trichuris*. This pasture was grazed by two experimental groups of calves the previous year and group 1 calves had been fed fungal material (*D. flagrans* grown on barley grain) once daily over a three-month period starting from turnout (25th May 1993). The fungal-barley amount given corresponded to 200 g dry weight per animal per day, and the approximate number of chlamydospores was 10^6 per g fungal-barley (d. w). The control, group 2 calves received barley grains only, in the same amount as group 1, over the same period.

9.2.2 *Experimental design*

Ten parasite-naïve male Jersey calves, 6 months old were used. On May 2nd 1994 the animals were divided into two comparable groups, A and B, on the basis of body weight. The average weight in group A was 188.4 ± 10.7 kg, and in group B, 188.0 ± 22.9 kg. The total area available to the experiment was 2.24 hectares and was divided by a fence into two comparable plots, experimental plot B and control plot A, which had been grazed by group 1 and 2 calves of the previous experiment, respectively. In the present study, these plots were allocated to groups B and A calves which were set-stocked for 4 weeks, before being housed for 3 weeks prior to slaughter on week (wk) 7 post-turnout. Herbage samples were collected weekly, for the first 4 weeks, while faecal samples were obtained at weekly intervals until termination of the study. The calves were weighed on three occasions, at turnout (week 1), at week 4 and during termination of the experiment (wk 7).

9.2.3 *Herbage larval counts*

Three grass samples were collected randomly from each of the two plots weekly, for the first 4 weeks. Each grass sample consisted of 300-500 g of grass collected by hand

following a W-shaped route across each plot. Grass in close proximity to dung pats was avoided. Third-stage (L₃) trichostrongylid infective larvae were isolated by a technique established by Jørgensen (1975) and Mwegoha and Jørgensen (1977), and subsequently (for detailed description of the methods see Appendix 9.1) enumerated.

9.2.4 *Faecal egg counts*

Numbers of trichostrongylid eggs per gram (epg) of faeces were determined using a modified McMaster technique (Henriksen and Aagaard, 1976) (Appendix 9.2). From each of the two groups of calves three faecal cultures were established to determine the nematodes at genus level. Each faecal culture comprised of a bulk sample of 2 gram of faeces from each animal. Four grams of vermiculite and eight ml of water were added to the (for more details see Appendix 9.3) faeces (Henriksen and Korsholm, 1983). After incubation at 20-22 °C for 14 days, larvae were harvested by a modified Baermann technique (Jørgensen and Madsen, 1982).

9.2.5 *Post mortem worm counts*

At post mortem, the abomasum and the proximal four metres of the small intestines were eviscerated and examined according to the procedures described by Grønvold *et al.* (1989) and Satrija and Nansen (1993). Larvae and adult parasites were enumerated and identified to genus level (MAFF, 1986).

9.3 **Results**

9.3.1 *Herbage larval counts*

The mean larval counts for the two plots remained very low especially in the first two

weeks of sampling and were comparable throughout the sampling period (Fig. 9.1). *Cooperia* spp. dominated numerically over *Ostertagia* spp. L₃, while, *Nematodirus* spp. L₃ counts of the two plots were comparable throughout the study period.

9.3.2 *Faecal egg counts*

The two groups of calves started shedding eggs after week 2 post turnout and mean faecal egg counts between the groups were roughly comparable (Fig. 9.2). There was a predominance of strongylid-type eggs (*Ostertagia* spp., *Cooperia* spp.) and in addition, eggs of *Nematodirus* spp. were encountered but in very small numbers in both groups of calves. *Trichuris* spp. eggs were also recovered on a few occasions.

9.3.3 *Faecal cultures*

Larvae recovered from faecal cultures set from pooled faeces of the two groups of calves were comparable throughout the experimental period as shown in Fig. 9.3. However, recovery of larvae on the last day of sampling was lower in group A compared to group B cultures. There was a predominance of *Cooperia* spp. over *Ostertagia* spp. in both groups of calves over the entire sampling period, while, *Nematodirus* spp. larvae did not appear in the faecal cultures, presumably because they require a longer period of time to permit hatching of eggs and larval development.

9.3.4 *Post mortem worm counts*

Trichostrongylid species of *Ostertagia*, *Cooperia* and *Nematodirus* were found in the alimentary tract of slaughtered calves. Table 9.1 shows that the intestinal worm *Cooperia* spp. was the predominant parasite in the gastrointestinal tract of the two groups of calves.

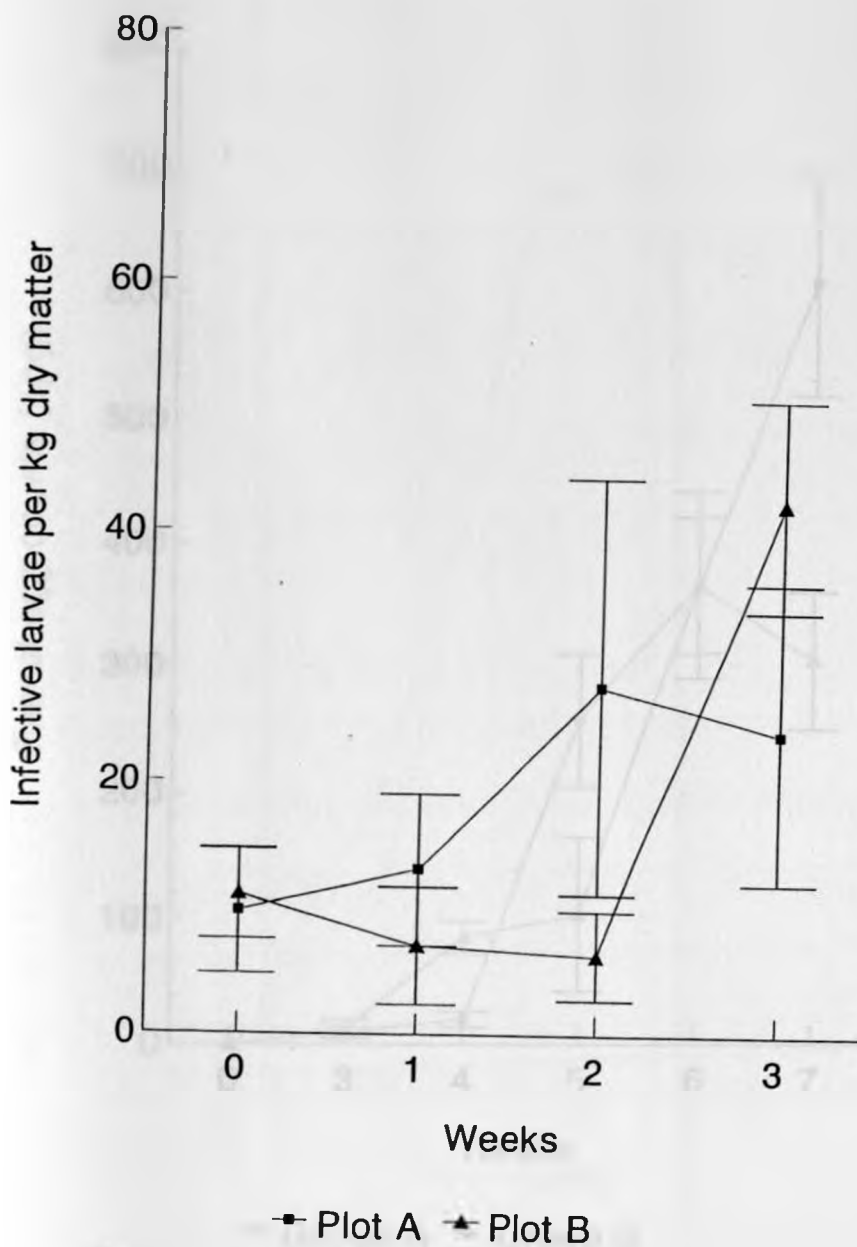


Fig. 9.1: Herbage trichostrongylid larval counts on the plots previously grazed by calves fed with nematode-trapping fungus *D. flagrans* (plot B) and by calves not offered any nematode-trapping fungus (plot A)

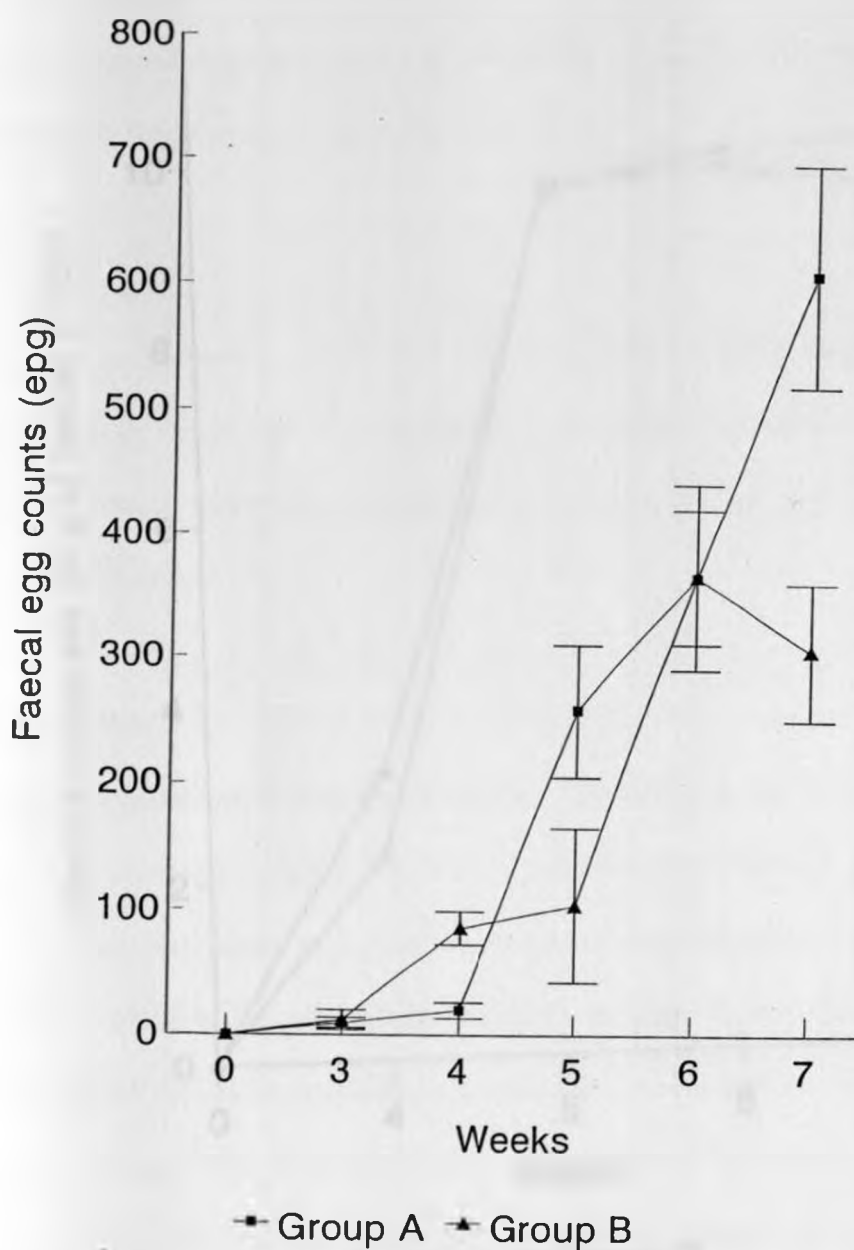


Fig. 9.2: Mean trichostrongylid faecal egg counts from experimental (group B) and control (group A) calves grazed on plots previously grazed by calves fed with the nematode-trapping fungus *D. flagrans* (plot B) and by calves not offered any nematode-trapping fungus (plot A)

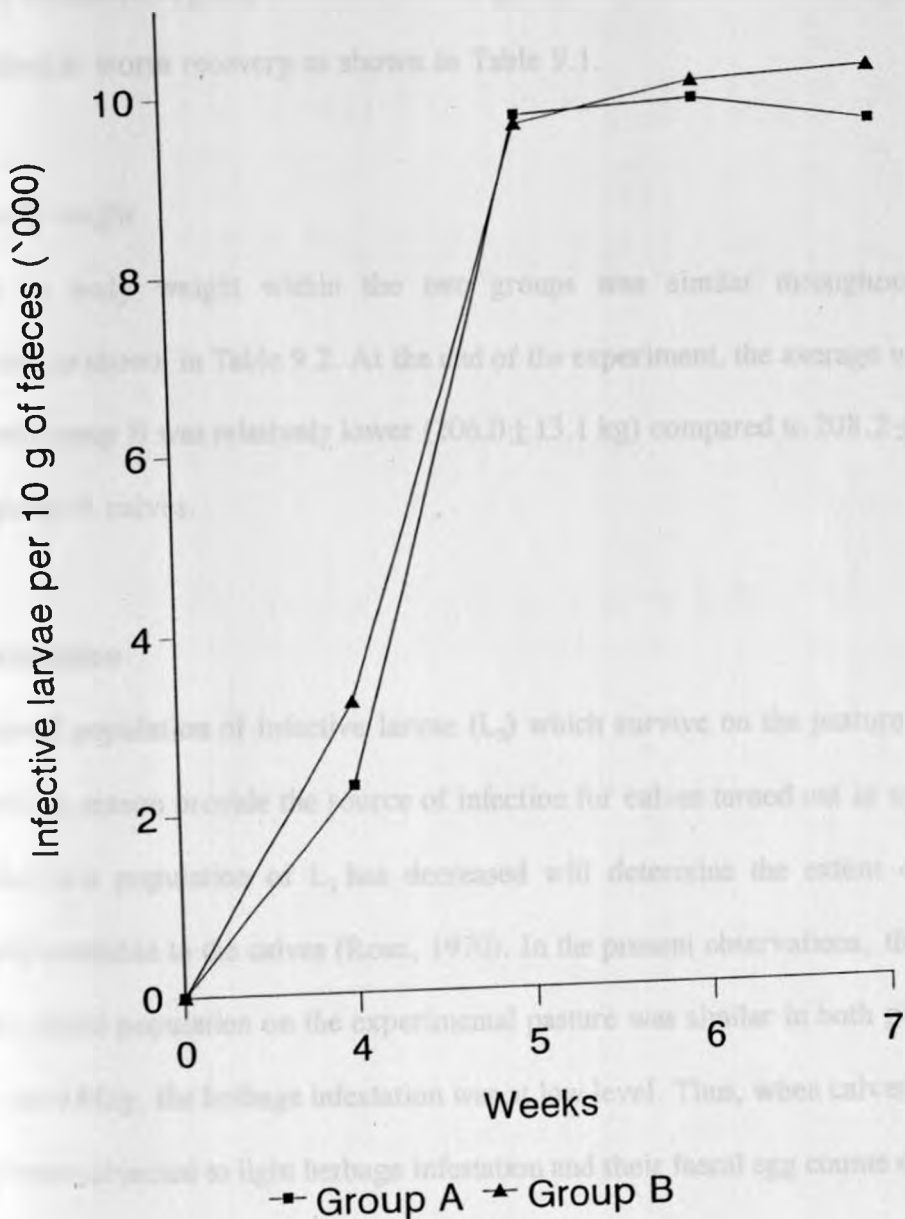


Fig. 9.3: Number of trichostrongylid larvae harvested from faecal cultures of experimental (group B) and control (group A) calves from 30th May (week 4) to 20th June (week 7) 1994

The average abomasal larval and adult worm counts of *Ostertagia* spp. were markedly similar in the two groups. In the small intestine, markedly large numbers of *Cooperia* spp. relative to *Nematodirus* spp. were recovered. The average total number of parasites was relatively higher in group A compared to group B calves. In both groups of calves, there were apparent individual variations in worm recovery as shown in Table 9.1.

9.3.5 *Body weight*

The increase in body weight within the two groups was similar throughout the experimental period as shown in Table 9.2. At the end of the experiment, the average weight of the experimental group B was relatively lower (206.0 ± 13.1 kg) compared to 208.2 ± 19.9 for the control group A calves.

9.4 **Discussion**

Overwintered population of infective larvae (L_3) which survive on the pasture from the preceding grazing season provide the source of infection for calves turned out in spring. The rate at which this population of L_3 has decreased will determine the extent of the infestation initially available to the calves (Rose, 1970). In the present observations, the rate of decrease of the larval population on the experimental pasture was similar in both plots A and B in that by early May, the herbage infestation was at low level. Thus, when calves were turned out, they were subjected to light herbage infestation and their faecal egg counts during early summer were comparatively low.

The present observations confirm the impression gained from previous field experiments that the large numbers of eggs passed by infected calves later in the grazing season (September and October) do not contribute significantly to the herbage infestation

Table 9.1 Mean worm counts of two groups of calves at necropsy on 22nd of June 1994. Five animals were slaughtered from each group of calves. The experimental group (B) of calves grazed on a plot previously grazed by calves fed with the nematode-trapping fungus *D. flagrans*. The control group (A) of calves grazed on a plot previously grazed by calves not offered any nematode-trapping fungus

Group of calves	Animal No.	Abomasum			Small intestines			
		<i>Ostertagia</i> spp.		Total	<i>Cooperia</i> spp.		<i>Nematodirus</i> spp.	Total
		Larvae	Adults		Larvae ^a	Adults	Adults	
Controls (A)	17	176	760	936	186	14420	80	14686
	516	274	700	974	244	4590	90	4924
	524	242	10	252	4	20	0	24
	518	100	2150	2250	9	12640	550	13199
	559	177	0	177	777	7450	770	8997
	Mean	194	724	918	244	7824	298	8366
	(s.e)	(30)	(392)	(372)	(142)	(2629)	(153)	(2694)
Experimental (B)	530	117	510	627	5	810	30	845
	533	20	2090	2110	31	800	60	891
	514	24	0	24	49	8380	1310	9739
	515	49	1740	1789	218	2520	410	3148
	307	151	70	221	0	130	0	130
	Mean	72	882	954	61	2528	362	2951
	(s.e)	(26)	(434)	(421)	(40)	(1516)	(248)	(1771)

^aLarvae = Larvae not differentiated to species level

Table 9.2: The average ($\pm$ S.D) increase in body weight (kg) of experimental (group B) and control (group A) calves from 2nd May (week 0) to 22nd June (week 7)

Group	No. of animals	Mean initial weight (kg) (Week 0)	Mean interim weight (kg) (week 3)	Mean final Weight (kg) (week 7)	Mean gain (kg)	Mean daily gain (g day ⁻¹)
A	5	188.0 $\pm$ 22.9	193.4 $\pm$ 22.1	208.2 $\pm$ 19.9	20.2 $\pm$ 5.8	388.5 $\pm$ 110.8
B	5	188.0 $\pm$ 10.6	190.2 $\pm$ 9.8	206.0 $\pm$ 13.1	17.6 $\pm$ 7.3	338.4 $\pm$ 140.4

(Rose, 1970). However, outbreaks of early-season trichostrongylosis in calves were reported by Nansen *et al.* (1989) in Denmark. It was hypothesized that the preceding extremely dry summer followed by a hard (very cold) winter had indirectly retarded the degradation of dung pats and thereby favoured the overwintering of the larval populations in the dung reservoirs (Nansen *et al.*, 1989).

In an earlier (1993) experiment where grazing calves were fed fungal material during the first three months of the season, a clear effect of the treatment was demonstrated as larval populations of *Ostertagia* spp. and *Cooperia* spp. were significantly reduced on the pasture grazed by fungus-treated calves (Nansen *et al.*, 1995). In the present study (1994 experiment) the parasitological parameters (herbage, faecal egg counts) for the two groups of calves and their respective grazing plots were roughly comparable, indicating that there were no differences in pasture infectivity between plot B previously grazed by calves fed nematode-trapping *D. flagrans* and plot A previously grazed by calves not fed nematode-trapping fungus. This showed that the treatment effect of *D. flagrans* was eventually lost later in the grazing season as tracer calves in plot B had strikingly comparable worm burdens as control calves in plot A, even though there were apparent individual variations in worm recovery within the groups. Body weight gains within the two groups of calves remained relatively similar throughout the experimental period. It is reasonable to speculate that the number of overwintering larvae were most likely influenced by the level of pasture contamination the previous season and weather conditions during winter rather than the effect of nematode-trapping fungus *D. flagrans*. The present results demonstrated that the effectiveness of *D. flagrans* in destroying parasitic nematodes of cattle in dung pats was limited only to the early grazing season. Thus, further studies are indicated to elucidate the effect of the fungus when fed for the entire normal grazing season or while administered in controlled release devices.

-Chapter 10-

THE INFLUENCE OF EGG COUNTS AND FUNGAL DOSE LEVELS ON THE NEMATODE-TRAPPING CAPABILITY OF *DUDDINGTONIA FLAGRANS* AGAINST FREE-LIVING STAGES OF GASTROINTESTINAL NEMATODES OF CATTLE

10.1 Introduction

The control of nematodes in cattle and other domestic animals is presently based on anthelmintic treatments and grazing management. However, the threats of development of anthelmintic resistance, concerns about chemical residues in livestock products and possible ecotoxicity of excreted drugs causes anxiety (Herd *et al.*, 1993; Strong *et al.*, 1996). This has stimulated attempts to develop alternative methods for worm control, including biological control of free-living stages of strongylid nematodes of ruminants by using nematode-trapping microfungi (Grønvold *et al.*, 1993a; Waller and Larsen, 1993; Waller and Faedo, 1996). Recent investigations successfully demonstrated that feeding of calves during the first months of the grazing season with *D. flagrans* grown on barley grain reduced herbage infectivity and a reduced acquisition of *Ostertagia* spp. and *Cooperia* spp. later in the season (Larsen *et al.*, 1995a; Nansen *et al.*, 1995).

The nematode-destroying effect of a fungus may both be determined by the capacity of the nematode larvae to induce traps in the fungus and later to become effectively ensnared in these. In an earlier *in-vitro* experiment (Nansen *et al.*, 1988) with a closely related fungus, *A. oligospora*, it was shown that trap induction was in all cases dependent on the presence of living nematodes, and all categories of nematodes, parasitic as well as free-living, were able to stimulate trap morphogenesis. However, there seems to be a correlation between the

locomotive behaviour and the ability to induce traps, e.g. the rapidly moving intestinal trichostrongylids of cattle and sheep were potent trap inducers, while the slow-moving *D. viviparus* had a poor capability in this respect (Nansen *et al.*, 1988).

One important objective of the on-going research on nematophagous fungi as an adjunct to conventional anthelmintic treatment for the control of parasitic gastroenteritis in cattle, is to determine the effect of *D. flagrans* against the various important nematode species and to evaluate the impact of different fungal dose levels. The present experiment was specifically designed to quantify the effects of varying fungal concentrations of *D. flagrans* on free-living stages of *H. placei*, *T. axei*, *C. oncophora* and *O. radiatum* of cattle in faecal cultures and, to define the minimum amounts of fungal material needed for a significant reduction of the number of larvae of the individual species. In general, irrespective of species of nematode, it may be anticipated that the extent of trap formation, and hence nematode-killing potential of *D. flagrans*, may be enhanced at higher nematode larval densities. Therefore, the effect of increasing doses of nematode eggs admixed to the faecal cultures, was examined as well.

10.2 Materials and methods

10.2.1 Parasite material

Faeces, containing eggs of *H. placei*, *T. axei*, *C. oncophora* and *O. radiatum*, were obtained from calves experimentally infected with monocultures of these parasites. Faeces, without eggs, were obtained from two uninfected calves. These animals were originally parasite-free, and were kept in experimental housing facilities (National Veterinary Laboratory, Copenhagen, Denmark) where parasite transmission was not possible.

10.2.2 *Fungal material*

A *D. flagrans* (CI3) isolate previously selected by Larsen *et al.* (1991; 1992), was used. The fungus was cultivated on barley grains as described by Grønvold *et al.* (1993b). One-litre Ehrlenmeyer flasks containing 200 g barley grains plus 200 ml tapwater were autoclaved and subsequently inoculated with agar pieces from a stock culture of *D. flagrans* grown on 1:10 diluted corn meal agar (Difco). After incubation for 6 weeks at 24°C the fungal material was washed off the grains by thorough shaking and mixing with sterile water. The material was poured successively through a kitchen sieve, a layer of fine gauze, and finally a 38 μ nylon net. Fungal material in the suspension contained less than 5% conidia, the rest were chlamydospores. The concentration of fungal units in the suspension was determined by using a haemocytometer and were added at the desired concentration in small volumes (1ml) of fluid to standard 10 g faecal cultures. Fungal concentrations used were 0 (controls), 1000, 5000 and 25000 units g⁻¹ faeces.

10.2.3 *Experimental design*

Faeces were collected from the calves to avoid any contamination with soil nematodes. After thorough mixing, the number of parasite eggs gram⁻¹ of faeces (epg) was determined by a modified McMaster method (Henriksen and Aagaard, 1976). For each nematode species other than *T. axei*, faecal portions with three to four epg levels were made using nematode free faeces as a diluting medium. These were low (40-50 epg), medium (200-280 epg), high (600-680 epg) and very high (1288-4800 epg), respectively.

10.2.4 *Faecal culture assay*

Faecal cultures (10 g faecal material mixed with 4 g vermiculite, plus 8 ml tap water for

control, and 7 ml H₂O plus 1 ml fungal material for the fungal treated cultures) were set according to a procedure described by Henriksen and Korsholm (1983). Five replicate cultures at each fungal concentration were set for each egg level per parasite species and incubated for 14 days in darkness, and under constant temperature (25°C) and 95 % relative humidity. After incubation, the larvae were harvested using a modified Baermann technique (Jørgensen and Madsen, 1982), and the number of third stage larvae (L₃) determined. After 14 days incubation only L₃ larvae harvested were recoverable. At low and medium egg levels all harvested L₃ were counted, while at high and very high egg levels subsamples were counted under a stereo microscope.

10.2.5 *Trapping efficacy and statistical analysis*

The trapping efficacy of *D. flagrans* was expressed as the percentage reduction in numbers of larvae recovered from fungal treated relative to numbers in fungal free cultures and was calculated thus: $[(N \text{ in controls} - N \text{ in fungal treated cultures}) / N \text{ in controls}] \times 100$, where N represents the mean number of infective larvae in fungus free and fungus treated replicates, respectively. The data for L₃ recoveries did not meet assumptions of normality and equality of treatment variance necessary for valid statistical analysis, using parametric methods. Consequently, the one-tailed Mann-Whitney U test (Siegel, 1956) (non parametric analogue of the unpaired t-test) was used to test the differences between median larval counts between the fungal treated and fungal free cultures (ultimately reported in terms of percentage efficacy).

The influence of the inoculation level of *D. flagrans* on the number of infective nematode L₃ recovered from faecal cultures per epg level is shown in Tables 10.1 to 10.4b. In the control cultures, recovery of L₃ of *H. placei* and *O. radiatum* (batch A and B) was significantly ($P < 0.05$) higher at low epg level compared to the high and very high epg levels and, overall yield of L₃ ranged from 16.7% (*C. oncophora*, batch A) to 95.5% (*O. radiatum*, batch A). Recovery of L₃ of all four nematodes decreased significantly ($P < 0.05$) at each epg level with increasing fungal spore concentration. At each level of fungal spore concentration, percent recovery of larvae decreased significantly ($P < 0.05$) for each increase in epg level. As shown in Table 10.3a, there were no significant differences ($P > 0.05$) in percent larval recovery between the high and very high epg levels at 1000, 5000 and 25000 fungal spore concentrations. At the 1000 and 5000 fungal spore concentrations, the range of percent reduction of larvae at the medium epg level for the 4 species was 53.5% for *O. radiatum*, 78.8% for *C. oncophora*, 80.4% for *T. axei* and 83.8% for *H. placei*.

The percent yields of larvae from faecal cultures of the 4 species are presented in Figs. 10.1 to 10.4b. In the control cultures of *H. placei* and *O. radiatum* (batch B), percent yields decreased with each increase in epg level. However, there was much variability in percent yields for other species, especially *C. oncophora* (Figs. 10.3a and 10.3b). For the fungus inoculated cultures in all species, there were definite reductions of percent yield of larvae for each fungal spore concentration and at each epg level. At low and medium epg levels, average percent reductions for all nematode species at 1000 and 5000 fungal spore concentrations were 52.2% and 71.9%, respectively. At high epg levels, percent yields were significantly ($P < 0.05$) reduced for *H. placei*, *C. oncophora* and *O. radiatum*. However, percent reductions were generally lower for *O. radiatum* compared to the other 2 species.

Table 10.1: Mean number of infective *H. placei* larvae (L_3) recovered from 10g of cattle faeces mixed with 1000, 5000 and 25000 *D. flagrans* chlamydo spores per gram of faeces

*Egg levels	0 (Controls)	Mean $\pm$ S.D larval counts (% reduction)		
		**1000	5000	25000
50	^a 376.6 $\pm$ 17.2	^b 219.6 $\pm$ 16.0 (41.7)	^b 166.4 $\pm$ 15.4 (55.8)	^b 75.4 $\pm$ 12.7 (79.9)
200	^a 594.8 $\pm$ 23.0	^b 136.2 $\pm$ 13.6 (77.1)	^b 56.4 $\pm$ 8.7 (90.5)	^b 28.6 $\pm$ 4.2 (95.2)
680	^a 1300.4 $\pm$ 130.2	^b 147.8 $\pm$ 21.3 (88.6)	^b 62.0 $\pm$ 6.2 (95.2)	^b 23.0 $\pm$ 4.7 (98.2)

*Faecal egg counts g⁻¹ faeces. **Clamydospore units g⁻¹ faeces. Larval counts in the same row differ significantly (P<0.05).

Table 10.2: Mean number of infective *T. axei* larvae (L_3) recovered from 10g of cattle faeces mixed with 1000, 5000 and 25000 *D. flagrans* chlamydo spores per gram of faeces

*Egg levels	0 (Controls)	Mean $\pm$ S.D larval counts (% reduction)		
		**1000	5000	25000
50	^a 162.0 $\pm$ 8.8	^b 76.6 $\pm$ 14.1 (52.7)	^b 47.2 $\pm$ 14.0 (70.8)	^b 29.2 $\pm$ 7.8 (81.9)
236	^a 1189.4 $\pm$ 20.0	^b 351.0 $\pm$ 147.0 (70.5)	^b 114.2 $\pm$ 26.3 (90.4)	^b 61.6 $\pm$ 16.3 (94.8)

*Faecal egg counts g⁻¹ faeces. Clamydospore units g⁻¹ faeces. Larval counts in the same row differ significantly (P<0.05).

Table 10.3a: Mean number of infective *C. oncophora* larvae (L₃) recovered from 10g of cattle faeces mixed with 1000, 5000 and 25000 *D. flagrans* chlamydospores per gram of faeces (Batch A)

*Egg levels	0 (Controls)	Mean ± S.D larval counts (% reduction)		
		**1000	5000	25000
40	^a 66.6 ± 25.8	^b 26.8 ± 11.9 (59.8)	^b 17.2 ± 6.2 (74.2)	^b 10.2 ± 6.9 (84.7)
280	^a 468.0 ± 92.3	^b 155.8 ± 47.5 (66.7)	^b 35.2 ± 12.9 (92.5)	^b 22.0 ± 5.5 (95.2)
1640	^a 3525.0 ± 653.5	^b 234.8 ± 64.3 (93.3)	^b 43.8 ± 22.2 (95.2)	^b 9.8 ± 5.3 (99.9)
4800	^a 7967.2 ± 908.2	^b 376.0 ± 116.5 (95.3)	^b 60.6 ± 45.8 (99.2)	^b 7.2 ± 1.3 (99.9)

*Faecal egg counts g⁻¹ faeces. Chlamydospore units g⁻¹ faeces. Larval counts in the same row differ significantly (p<0.05).

Table 10.3b: Mean number of infective *C. oncophora* larvae (L₃) recovered from 10g of cattle faeces mixed with 1000, 5000 and 25000 *D. flagrans* chlamydospores per gram of faeces (Batch B)

*Egg levels	0 (controls)	Mean ± S.D larval counts (% reduction)		
		**1000	5000	25000
40	^a 109.2 ± 6.7	^b 53.2 ± 6.3 (51.3)	^b 33.2 ± 4.5 (69.6)	^b 16.8 ± 2.8 (84.6)
240	^a 940.0 ± 44.6	^b 324.4 ± 11.5 (65.5)	^b 91.0 ± 6.6 (90.3)	^b 59.0 ± 3.8 (95.2)
620	^a 2805.6 ± 126.6	^b 240.4 ± 7.8 (91.4)	^b 112.0 ± 6.3 (96.0)	^b 31.2 ± 3. (98.9)

*Faecal egg counts g⁻¹ faeces. Chlamydospore units g⁻¹ faeces. Larval counts in the same row differ significantly (p<0.05).

Table 10.4a: Mean number of infective *O. radiatum* larvae (L_3) recovered from 10g of cattle faeces mixed with 1000, 5000 and 25000 *D. flagrans* chlamydo spores per gram of faeces (Batch A)

*Epg levels	Mean $\pm$ S.D larval counts (% reduction)			
	0 (Controls)	**1000	5000	25000
50	^a 477.4 $\pm$ 33.1	^b 351.4 $\pm$ 35.8 (26.4)	^b 244.6 $\pm$ 48.4 (48.8)	^b 199.6 $\pm$ 6.3 (58.2)
250	^a 1125.0 $\pm$ 162.2	^b 689.0 $\pm$ 29.6 (38.7)	^b 476.2 $\pm$ 80.1 (57.7)	^b 390.6 $\pm$ 70.9 (65.3)
644	^a 3820.0 $\pm$ 74.2	^b 1916.6 $\pm$ 56.4 (49.8)	^b 1304.8 $\pm$ 68.4 (65.9)	^b 881.0 $\pm$ 104.7 (76.9)
1288	^a 4510.0 $\pm$ 353.7	^b 1752.6 $\pm$ 43.9 (61.1)	^b 978.4 $\pm$ 46.1 (78.3)	^b 317.4 $\pm$ 46.6 (92.9)

*Faecal egg counts g^{-1} faeces. Chlamydo spore units g^{-1} faeces. Larval counts in the same row differ significantly ($p < 0.05$).

Table 10.4b: Mean number of infective *O. radiatum* larvae (L_3) recovered from 10g of cattle faeces mixed with 1000, 5000 and 25000 *D. flagrans* chlamydo spores per gram of faeces (Batch B)

*Epg levels	Mean $\pm$ S.D larval counts (% reduction)			
	0 (Controls)	**1000	5000	25000
40	^a 190.0 $\pm$ 11.2	^b 136.2 $\pm$ 12.7 (28.3)	^b 93.2 $\pm$ 8.2 (50.9)	^b 61.6 $\pm$ 5.1 (67.6)
230	^a 656.6 $\pm$ 29.3	^b 341.8 $\pm$ 12.9 (47.9)	^b 199.2 $\pm$ 10.8 (69.7)	^b 30.6 $\pm$ 4.4 (86.1)
600	^a 1270.8 $\pm$ 121.3	^b 349.0 $\pm$ 6.4 (72.5)	^b 13.2 $\pm$ 4.5 (89.4)	^b 53.8 $\pm$ 3.7 (95.8)

*Faecal egg counts g^{-1} faeces. Chlamydo spore units g^{-1} faeces. Larval counts in the same row differ significantly ($p < 0.05$).

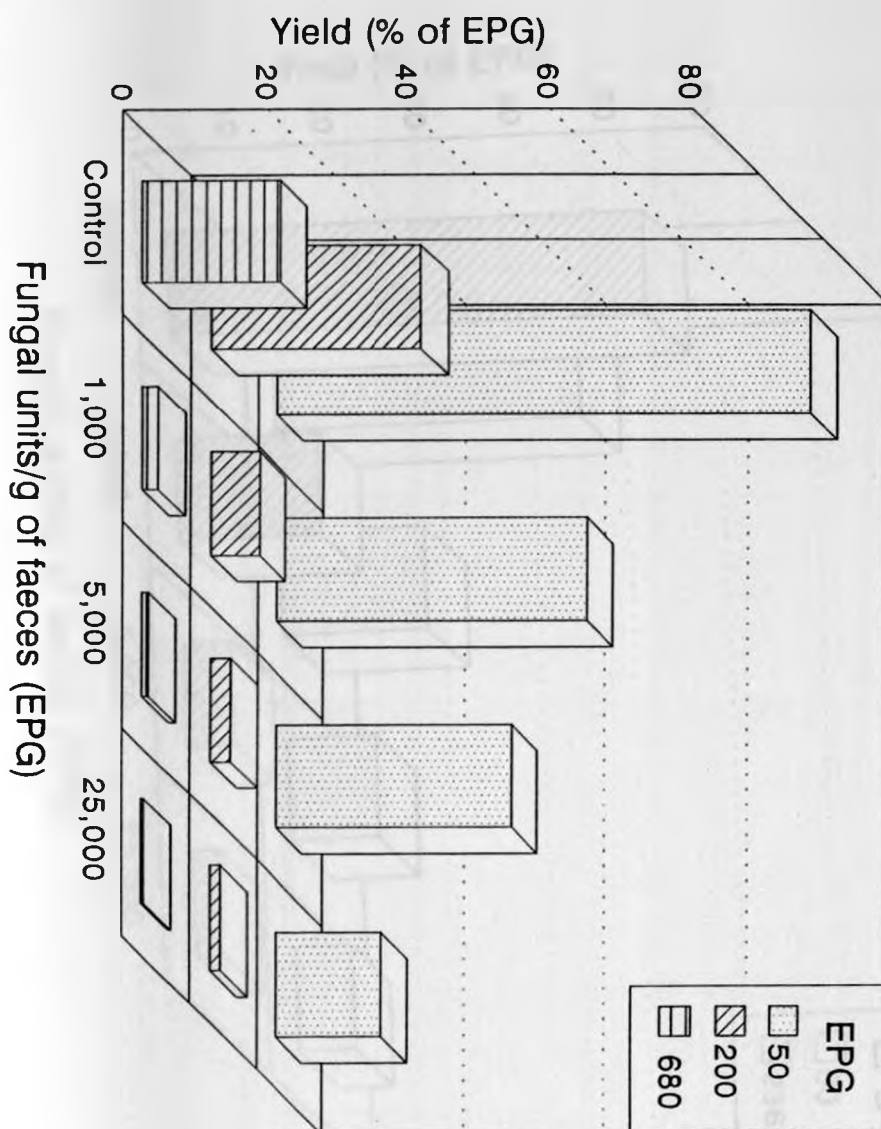


Fig. 10.1: Effects of *D. flagrans* on the recovery of *H. placei* infective larvae

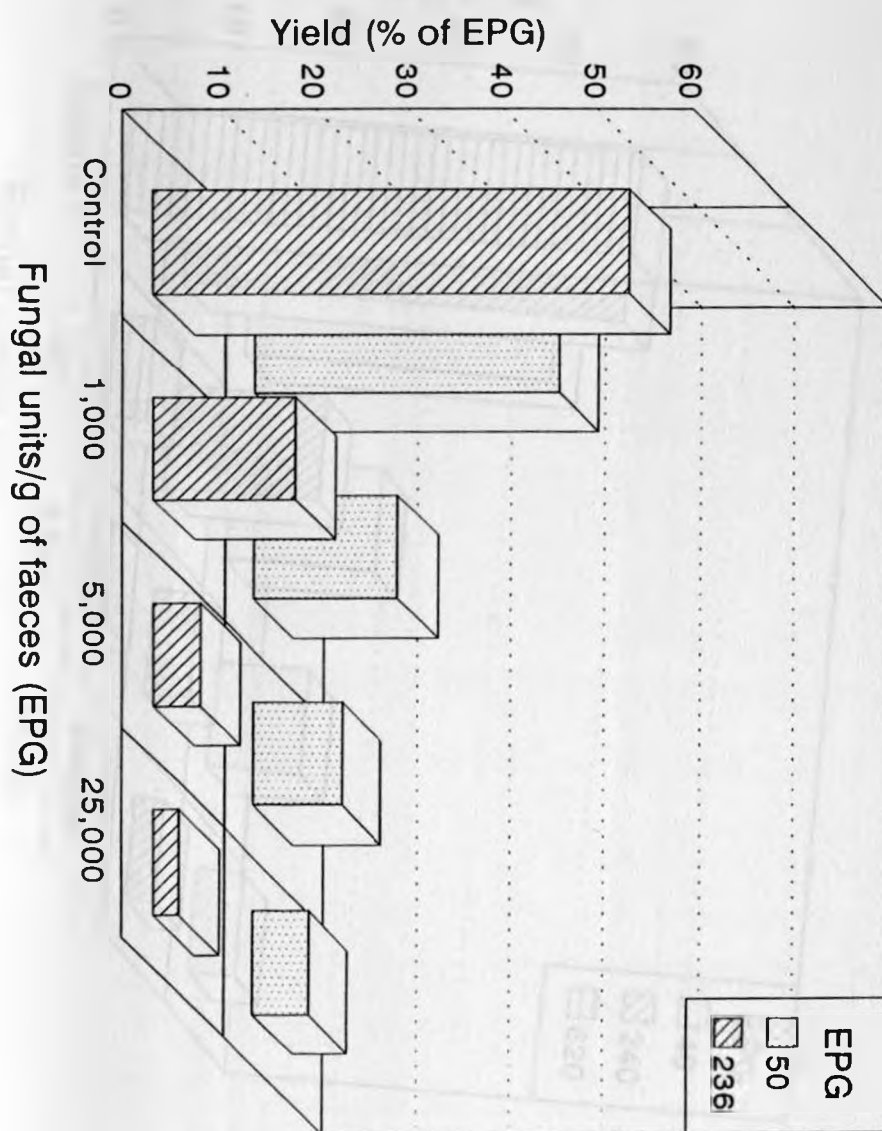


Fig. 10.2: Effects of *D. flagrans* on the recovery of *T. axei* infective larvae

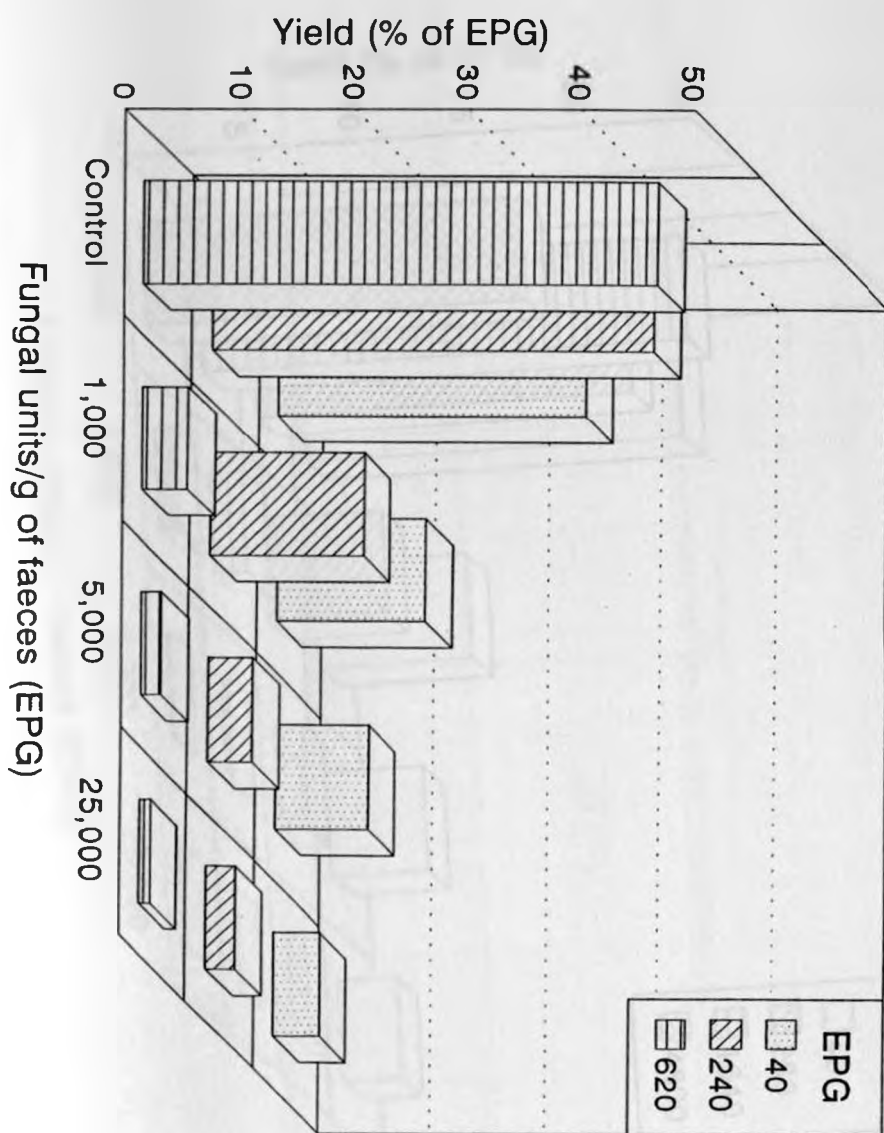


Fig. 10.3a: Effect of *D. flagrans* on the recovery of *C. onchophora* infective larvae (batch A)

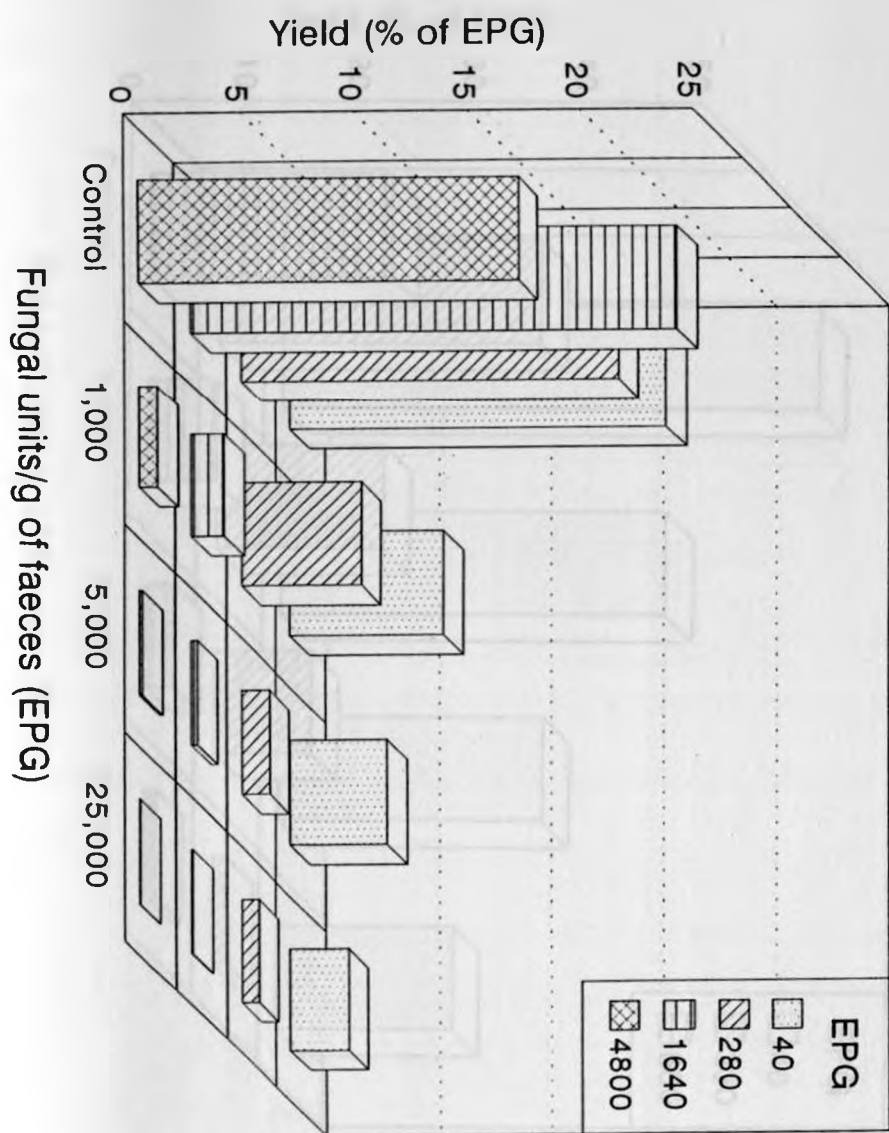


Fig. 10.3b: Effects of *D. flagrans* on the recovery of *C. onchophora* infective larvae (batch B)

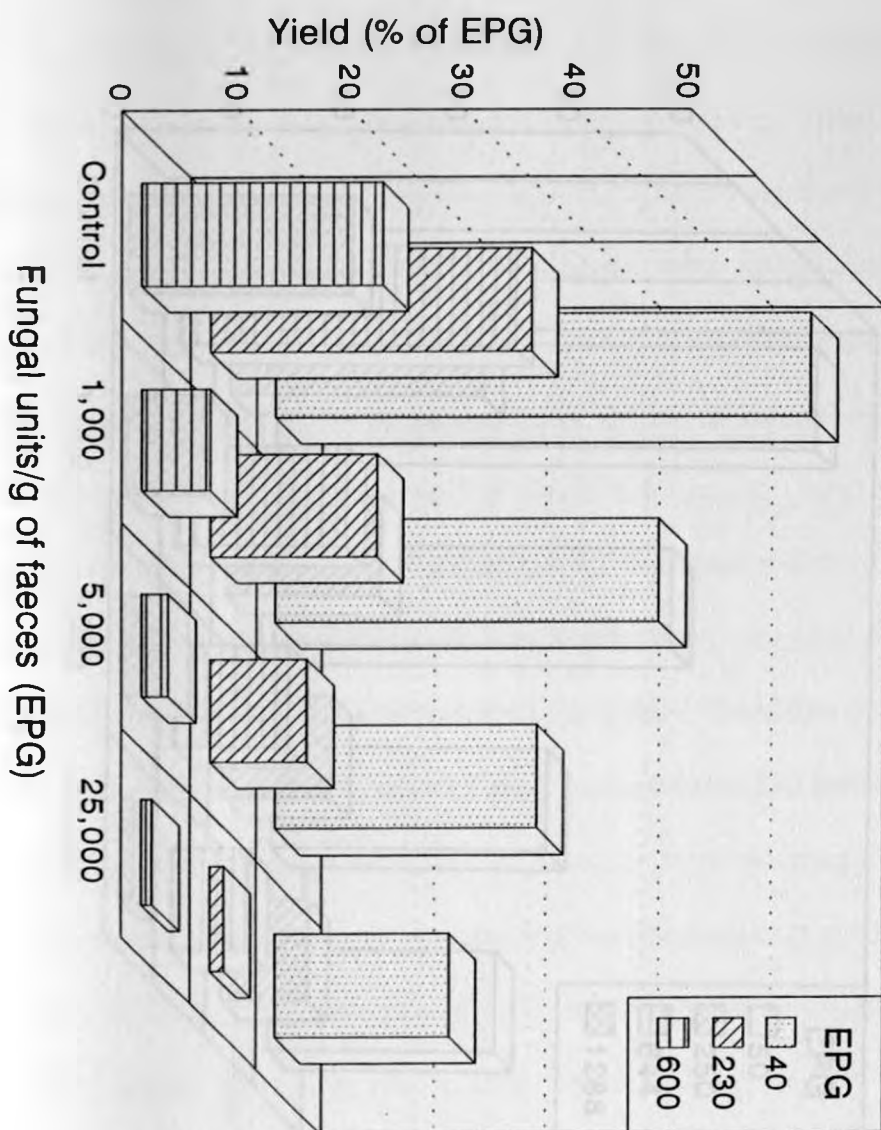


Fig. 10.4a: Effects of *D. flagrans* on the recovery of *Q. radiatum* infective larvae (batch A)

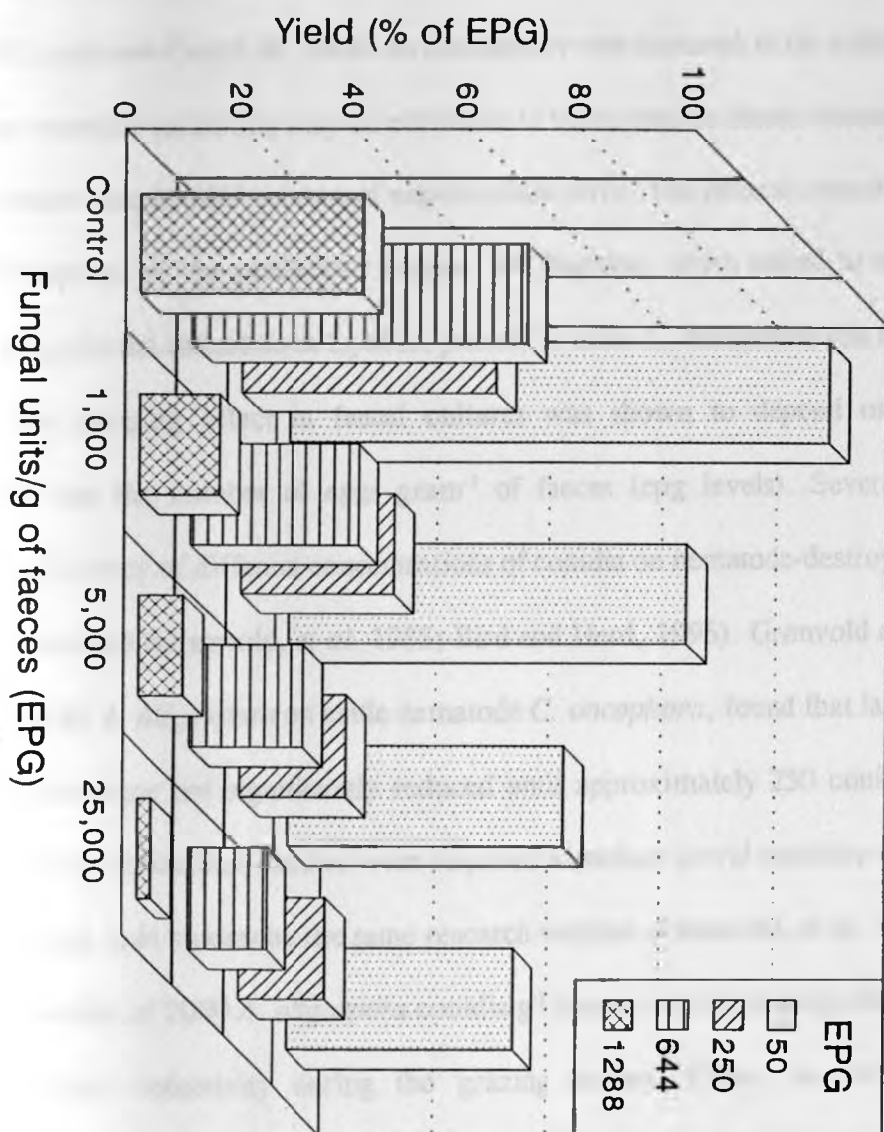


Fig. 10.4b: Effects of *D. flagrans* on the recovery of *Q. radiatum* infective larvae (batch B)

There was appreciable variability in the recovery of L_3 as depicted by the control cultures of most species. Recovery of L_3 decreased with each increase of epg level for *H. placei* and *O. radiatum* (batch B), while no consistency was detected in the cultures of other species. The recorded variability may be attributed to biotic and/or abiotic factors in cultural medium or other uncontrolled sources of experimental error. The present results have shown that chlamydospores of the predacious fungus *D. flagrans*, when added to cattle faeces, resulted in a significant reduction of L_3 of *H. placei*, *T. axei*, *C. oncophora* and *O. radiatum*. Moreover, the trapping effect in faecal cultures was shown to depend on the fungal concentration and the number of eggs gram^{-1} of faeces (epg levels). Several studies to quantify the efficiency of different concentrations of conidia on nematode-destroying capacity have been conducted (Grønvold, *et al.* 1985; Bird and Herd, 1995). Grønvold *et al.* (1985), who worked with *A. oligospora* on cattle nematode *C. oncophora*, found that larval numbers in faecal culture were not significantly reduced until approximately 250 conidia g^{-1} faeces were used and ten times that number were required to reduce larval numbers by more than 99%. Subsequent field studies by the same research workers (Grønvold, *et al.* 1988) showed that a concentration of 2000 *A. oligospora* conidia g^{-1} faeces resulted in a significant lowering of herbage larval infectivity during the grazing season. Other, as yet unpublished observations referred to by Hashmi and Connan (1989), indicated that substantial reductions in larval numbers can result from the addition of 20-50 *A. oligospora* conidia g^{-1} to either cattle or sheep faeces. The present studies show that the 1000 and 5000 fungal spore concentrations (at all epg levels) gave significant reduction (73%) of the number of parasite larvae in the faecal cultures. Further experiments using different chlamydospore densities per egg to determine the minimal number of fungal units to obtain the desired reduction (i.e 75-

80 %) in the larval population is warranted.

The efficacy of nematode capture by *D. flagrans* was dependent on the faecal egg count and thus the number of larvae obtained from cultures, as percent yield of L_3 decreased with each increase of epg level. This was as reported for *A. oligospora* that increasing numbers of nematode-trapping hyphal nets were produced with increasing numbers of *O. ostertagi* larvae (Grønvold, 1989). In the present study, no significant species variation in entrapment was detected at the various fungal and/or epg levels, respectively, as all species were readily entrapped as earlier reported by Nansen *et al.* (1988) and Waller and Faedo (1993). The design of the experiment allowed exposure of all the three free-living stages (L_1 , L_2 and L_3) to *D. flagrans*, but it was not possible to determine whether a certain stage experienced a greater predation. Under natural conditions, 2 or more nematode species co-exist in a host and it seems they can be destroyed readily by the fungus in the dung pats as predacious fungi trap and digest nematodes to a large extent independently of their species or genus position (Drechsler, 1941). The investigated strain of *D. flagrans* forms capturing traps in response to soil organisms, especially nematodes, in their environment. It may therefore be expected that not only the parasitic nematode larvae already present, but also soil nematodes invading the dung pats, may be responsible for the induction of capturing traps and thereby enhance the entrapment of parasitic nematodes. It may also be anticipated that free-living nematodes in the dung have an indirect influence on eventual fungal predation on animal parasitic nematodes in the dung, e.g. through their role as baits and disseminators of fungi (Linford, 1937).

As illustrated by *C. oncophora*, percent larval recoveries from 1000 to 25000 fungal concentrations were not significantly different between the high and very high epg levels. This indicates that the epg threshold which is needed for a significant reduction of L_3 in

faecal cultures would be much lower. Indeed, at the medium egg level, the range of percent reduction of L_3 at 5000 fungal spore concentration was $>60\%$ for all the 4 nematode species. Under normal circumstances, worm egg counts in naturally infected cattle are on average <300 as the medium egg levels used in the present study. Thus, as entrapment of $>60\%$ was noted, the fungi can be of value in the field if given orally to cattle and entrap the free-living stages in dung pats, with subsequent reduction of pasture infectivity and economic losses attributable to GI nematode parasitism. In Australia, worm control in sheep was improved over the standard (wormkill) program for fungal controlled-release devices with efficacy of at least 75% and duration of at least 60 days (Barnes *et al.*, 1995).

In the present study, percent yield at low egg level and at 1000 and 5000 chlamydospores g^{-1} faeces was markedly higher than at higher and very high egg levels. Working with cyathostomes of horses, Bird and Herd (1995) argued that there appeared to be a threshold below which the number of spores per egg failed to effect sufficient mortality as low mean egg counts may fail to produce enough larvae to effectively induce traps, thus explaining the marked difference in percent yield between low and high egg levels for the nematode species examined. Further experiments should be undertaken to determine the minimum number of larvae required to induce trap formation and subsequent capture of parasitic nematodes in faecal cultures.

-Chapter 11-

GENERAL DISCUSSION

The main objective of this thesis was to determine the seasonal prevalence, intensity and importance of GI nematodes in dairy cattle in Kiambu District, central Kenya. Other objectives were to evaluate the efficacy of a sustained release anthelmintic bolus in controlling GI nematode parasitism in naturally infected grazing yearlings, and to quantify the nematode-trapping effects of the fungus *D. flagrans* on free-living larval stages of the four main GI nematodes of cattle in the tropics.

In an effort to achieve the above objectives, a number of studies (8) were conducted as summarized herebelow:

Results in chapters 3, 5, and 6 demonstrate a generally high prevalence of GI nematode infections of cattle in the study area, even outside the two rainy seasons. However, the degree of infection or the level of helminth-egg load was generally low to moderate in most cases; the infections therefore being subclinical (Soulsby, 1965). Subclinical or economic parasitism is the level of infection that prevents the host from reaching its genetic potential in the production of meat, milk or other measureable criteria (Craig, 1988). Economic parasitism is widespread, seasonal and often affected by other factors including quality and abundance of feed, stocking rate, age, sex, breed or acquired resistance. Compared to clinical parasitism, economic parasitism is the most difficult to assess because of the many factors that may be involved. One common way of assessing economic parasitism is by administration of an anthelmintic to cattle and then, after a predetermined period of time, comparing weight gains between treated and untreated animals (see Chapter 8). This is a valid method of determining anthelmintic effects, but it may not always assess

the true effects of parasitism (Craig, 1988).

A survey of gastrointestinal parasites (GIP) of cattle (Chapter 3) showed that strongylosis (due mainly to *H. placei*) was the most prevalent GIP, followed by liver fluke, coccidial and tapeworm infections. The seasonal, management system, and age of the animals influenced occurrence and intensity of infection. A higher intensity of strongylid eggs was found in the wet season compared to the dry season ($P < 0.05$). The age-specific intensity was in the following order: yearlings had the highest egg counts, followed by calves and adult cattle.

The strongylid egg counts followed the negative binomial pattern of distribution at each sampling, which suggests highly overdispersed worm burdens. Thus, by eliminating "wormy" individuals of the herd (i.e. by selective anthelmintic treatment), effective control of parasite transmission can be achieved (Sreter *et al.*, 1994).

An outbreak of haemonchosis caused by *H. placei* which occurred in weaner calves in late June 1992 is reported in Chapter 4. Post mortem examination of an animal which died during the outbreak confirmed *H. placei* as the primary pathogen. The number of other nematode species found was very small.

Seven nematode genera were found during an abattoir survey (Chapter 5), with the predominant genus being *Haemonchus* while the genus *Strongyloides* was the least predominant. Other genera present were *Cooperia*, *Trichostrongylus*, *Oesophagostomum*, *Nematodirus* and *Trichuris* in that order of prevalence.

As reported by Gatongi (1984), all the genera found during this study appeared to be equally influenced by prevailing weather conditions (Chapter 6) and there was no indication that a particular genus was more favoured or more adversely affected by a particular season than others.

It is important to note that the digestion of the abomasal mucosae of slaughtered cattle did not reveal any significant numbers of immature (histotropic) *H. placei* worms in the digesta. Hypobiosis is now widely recognized as a form of adaptation, whereby the parasite arrests at a particular stage in the host when environmental conditions are adverse for its free-living development, for example, in winter in Europe or in the dry season in parts of Africa (Benitez-Usher *et al.*, 1984; Gatongi, 1995). Benitez-Usher *et al.* (1984) emphasized that in areas with weather conditions favourable for development of stages, the faculty of hypobiosis was discarded by the parasites (Gupta *et al.*, 1987). Accordingly, it is significant that *H. placei* encountered in the present study showed no hypobiosis.

In Chapter 7, observations were made on the abundance and survival of *H. placei*, *Cooperia* spp., *T. axei* and *O. radiatum* infective larvae from cattle faecal pats exposed at various times of the year (July 1995 to June 1996) in the study area. Rainfall rather than temperature played the major role in the development and survival of the larvae on pasture during the study period. These results show that the hatching of the eggs and the development of the larvae can take place at any time of the year, and suggest that similar results would be obtained in other tropical regions provided the rainfall throughout the year was sufficient to allow development of eggs to infective larvae (L₃). Similar observations were made elsewhere under tropical conditions (Okon and Akinpelu, 1982; Fabiyi *et al.*, 1988; Banks *et al.*, 1990; Barger *et al.*, 1994). It can be deduced that L₃ would be expected to have died off on pasture after 12 to 16 weeks of resting the pasture. Thus, it is suggested that safe pasture can be produced by spelling contaminated pasture for a minimum of 3 months (Ndamukong and Ngone, 1996).

Results presented in Chapter 8 demonstrated that subclinical infection by GI nematodes has adverse effects on weight gains in calves. MSRT bolus administered to weaner calves

prior to their turnout onto pasture produced adequate control of nematode infection dominated by *H. placei*. Benefit of the bolus was reflected in the improvement of liveweight gain and a reduction of infectivity of herbage with larvae, faecal egg counts, post mortem worm counts of tracer calves that grazed on the pasture where treated animals were maintained, and post mortem worm counts of principal (treated) calves slaughtered at termination of the trial.

In this experiment, average liveweight gain benefit was 69.2 kg per animal over the experimental period. This is the most important parameter in production efficiency (Block *et al.*, 1985). The weight gain is achieved with a single treatment without a concomitant increase in the amount of herbage available or the size of the grazing area. The MSRT bolus also has an advantage over conventional helminth-prophylactic programmes in areas where land and/or labour are scarce or expensive, which either involves treatment of calves and a subsequent move to a safe pasture or strategic anthelmintic treatment. Both systems require extra labour, land, and money (Jones and Bliss, 1983).

Albendazole, one of the more recently developed benzimidazole anthelmintics was used as a single drench in group T-3 calves and as two dosings, 14 days apart for group T-4 calves. Faecal egg counts were significantly reduced in both groups T-3 and T-4, 2 weeks after single treatment (Fig. 8.2) and the gradual increase in egg counts recorded in group T-3 may be attributed to reinfection. With the second treatment (group T-4) faecal egg output was significantly reduced up to July. This prolonged effect of two treatments with albendazole indicates that the deposition of eggs in the early rainy season is very important for buildup of pasture infectivity. Treatment during this period must be regarded as strategic as they resulted in a relatively reduced buildup of pasture contamination followed by a favorable production response (weight gains). Not until the next rainy season (short rains) did the egg counts reach the levels of the other groups (i.e. T-1 and T-3).

This study offers good indications for the use of few effective strategic treatments in the control of cattle nematode infections. However, this benefit can be enhanced under practical field conditions when anthelmintic treatment is combined with grazing management, like placing animals on relatively clean pastures (Block *et al.*, 1985). This unfortunately is difficult to practice in Kenya, particularly in areas where animals graze on communal land. However, other approaches like zero grazing in certain periods of the year may be considered as adjunct control strategies (Nansen, 1991). The anthelmintic activity of plants like papaya latex (*Carica papaya* Linn) against gastrointestinal nematodes of cattle should be investigated as it has been found to be effective against intestinal nematodes of monogastric animals (Satrija *et al.*, 1994) and against *H. contortus* in sheep (Murdiati and Beriajaya, 1997).

An attempt was made in Chapter 9 to determine the overwintering residual grass infectivity in a pasture previously grazed by *D. flagrans* fed calves using tracer calves over a period of 7 weeks. Parasitological parameters were generally comparable indicating that there were no differences in pasture infectivity between the plot (B) previously grazed by calves fed the fungus *D. flagrans* and the plot (A) previously grazed by calves not fed the microfungus. Body weight gains within the two groups of calves remained relatively similar throughout the experimental period and on slaughter, tracer calves in plot B had comparable worm burdens as control calves in plot A.

The effects of different fungal concentrations and faecal epg levels on nematode-trapping ability of *D. flagrans* against the four predominant genera of cattle nematodes in the tropics are discussed in Chapter 10. Results show that the nematode-trapping capacity of *D. flagrans* was dependent on the fungal concentration and number of eggs in the faecal cultures. Thus, percent reduction increased with corresponding increase in fungal concentration and epg levels in all the four genera of nematodes examined.

The following observations and conclusions were made from this study:

- a) That GI nematode infections are major constraints to the health of young dairy cattle of the study area.
- b) Moisture, which is influenced by rainfall, was the major epidemiological factor affecting the development and survival of the strongylid nematode larvae, and the prevalence and intensity of infection with nematode parasites.
- c) Hypobiosis does not seem to play any important role in the epidemiology of *H. placei* as adult worms persisted in the host throughout the year and there was no indication of inhibition of EL₄.
- d) That control of established subclinical nematode infections is indicated, this can be accomplished using sustained release boluses where economically justifiable.
- e) That strategic application of conventional (drenches) anthelmintics could be of value in the farming systems studied. Three strategic treatments of young cattle may be attempted before (mid-March) and after (July) the long rains, and before the short rains (mid-October).
- f) That the trapping efficiency of *D. flagrans* against *H. placei*, *T. axei*, *C. oncophora* and *O. radiatum* increased with the corresponding increase in fungal concentration and epg levels.

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APPENDICES

Appendix 3.1 The recovery and identification of gastrointestinal nematodes post mortem

3.1.1 *Recovery of nematodes from the abomasum*

- : Immediately after removing the alimentary tract from the body cavity, the abomasum was ligated twice just below the pylorus and at the omasal-abomasal junction. It was separated from the small intestine and omasum by cutting the portion between the two ligatures by means of a knife. This was done immediately after slaughter.
- : The abomasum was placed in a bucket and opened along the greater curvature and the contents washed out.
- : The abomasum was rinsed 3-5 times with water with special attention being paid between folds and mucous membranes.
- : The abomasum was put in a small plastic bag with proper identification number until processing in the laboratory.
- : The contents of the abomasum were adjusted to 10 litres by tapwater.
- : Using a ladle the contents were carefully mixed until all food materials, mucus and water were thoroughly mixed.
- : Quickly a subsample of 1 litre (10%) was recovered.
- : The subsample was processed through a 200 μ m sieve which retains adult nematode parasites.
- : The residue on the sieve was rinsed with a jet of tap water and the volume made up to 200 ml and put in a plastic container.

- : 10-15 ml of concentrated Lugol's iodine solution was added into the contents to preserve retained parasites until identification and enumeration.
- : The sample was adjusted to 300ml and was constantly stirred by the use of a magnetic stirrer.
- : 30 ml (10% of the total volume) of the sample was poured into one or more small petri dishes with ruled bottom. Using a stereo microscope at X 12-16 magnification, all the worms in the sub-sample were counted. Further sub-samples were taken until 10% of the total volume was examined.
- : Mature males and females were picked and put into small plastic cup with glycerol ethanol.
- : Mature male worms were identified to species level if possible, using light microscope at a magnification of 40-400 according to MAFF (1986).

3.1.2 *Processing of the abomasal mucosa by pepsin/HCl digestion*

- : The entire abomasal mucosa was removed by scraping with a knife.
- : For every 100 g of mucosa a preheated (37°C) 500 ml of digestion fluid was added into a plastic bag, and blended in the stomacher at 39°C for 30 minutes.
- : The larvae were being isolated from the fluid by straining them through a 37 μ m sieve.
- : All the materials on top of the sieve were transferred by rinsing with tap water into a beaker; the total volume was made up to 30 ml.
- : The larvae were stained using 2-3 ml of iodine solution; and then counted in a petri dish under a stereo microscope 30-40 X magnification.

3.1.2.1

Chemicals and equipment

A: Digestive fluid

Water	1,000 ml
HCl, 1N (86 ml conc. HCl + 914 ml water)	150 ml
Pepsin (1: 3,000)	8 g

(The solution was stable for one day when kept at 37°C).

B: Stomacher 3,500 (thermo model)

C: Strainer 37 μ m aperture

D: Iodine solution

Potassium iodide (KI)	250 g
Iodine crystals	50 g
Water	500 ml

3.1.3

Recovery of nematodes from the small intestines

- : The small intestine was ligated below the pyloric pore and between small and large intestine, and removed from the gastrointestinal tract.
- : The small intestine was freed from the mesentery by use of a pair of scissors or by stripping and divided into 3 equal parts which were processed separately.
- : Each part was transferred into a plastic tray, then opened along the entire length and washed carefully.
- : The contents were adjusted to 10 litres by tap water, and a sub-sample of 1 litre was washed through 53 μ m sieve to retain adult parasites as well as larval stages.
- : Only the first 3 metres of the front part was digested following the ordinary digestion procedure as described for the abomasal mucosa.
- : The procedure for staining and counting was the same as previous described for worms of the abomasum.

3.1.4 *Recovery of nematodes from the large intestine*

- : The colon was ligated twice at the point where it joins the caecum.
- : It was separated from the caecum by cutting with a pair of scissors in between the two ligatures.
- : The two organs were processed separately.
- : For both of them, the contents were washed into a bucket and adjusted to 10 litres by tap water.
- : A sub-sample of 1 litre was passed through a coarse mesh sieve (500 μm) and any worms retained were picked and put into small plastic bottles with glycerol ethanol until use.
- : Mature male worms were identified to species level if possible using a light microscope at a magnification of 40-400 according to MAFF (1986).

Reference : MAFF (1986).

Appendix 6.1 The serum pepsinogen test

6.1.1 *Principle*

The principle of the test is that the sample of serum is acidified to pH 2.0, thus activating the inactive zymogen, pepsinogen, to the active proteolytic enzyme pepsin. The activated pepsin is then allowed to react with the protein substrate (serum albumine) and the enzyme concentration is calculated in international units (I.U: μ mols tyrosine released 1000 ml serum minute⁻¹ at 37°C). The tyrosine (a phenolic compound) liberated from the protein substrate by the pepsin is estimated by the blue colour which is formed when it react with

Folin-Ciocalteu's reagent.

6.1.2 Procedure

- : To 0.25 ml of serum were added 1.25 ml of 0.6 N hydrochloric acid and mixed well.
- : The mixture was incubated at 37°C for 3 hrs.
- : The reaction was then stopped by adding 1 ml of 10% trichloroacetic acid.
- : A parallel control was prepared for each test by precipitating the mixture as above without incubation.
- : The tubes were allowed to stand for 10 min and, then centrifuged (at 2,000 r.p.m.) for 10 min.
- : The tubes were allowed to stand for another 10 min.
- : The supernatant (1ml) was placed in a clean test tube and made alkaline with 2 ml of 0.5 N sodium hydroxide and 0.5 ml of freshly diluted (1 in 3) Folin and Ciocalteu's reagent was added and mixed well.
- : Colours of test and control were read within 20 min. at 560 ml and tyrosine-like products estimated by reference to standard tyrosine (0.2 μ mole) and blank (water) treated similarly.

Reference : Ross *et al.* (1967).

Appendix 9.1

Technique for recovery of gastrointestinal nematode larvae from herbage

9.1.1 *Procedure for collecting herbage samples*

- : Three small samples of grass per plot/paddock were collected following a "W" shaped route. The sampler walked along the route and after every ten steps he/she stopped and a small wisp of grass was picked to the right, to the left and in front of the picker and put into a plastic bag.
- : Areas of heavy faecal contamination and soil collection were avoided.
- : The grass was collected by hand and sampling was undertaken at the same time of the day (9-10 am) in order to avoid diurnal variations in pasture infectivity.
- : The grass collection of approximately 300-600 g was placed in a plastic bag.
- : In the laboratory, the collected samples were weighed to get the wet weight.

9.1.2 *Isolation of debris and larvae form herbage*

- : The sample was then soaked overnight in a bucket containing 10 litres of tap water.
- : The sample was placed in a concrete mixer machine and 2 drops of teepol (0.02% teepol) was added to loosen the larvae from grass blades.
- : The water with the herbage was agitated for 5 minutes to further loosen the larvae.
- : Subsequently the washings were filtered through a 70 x 70 cm piece of nylon mesh (25-30 mm apertures) cloth placed in the conical support. The nylon mesh cloth was used to retain debris and larvae.
- : The herbage was caught in a metal grid above the cloth, it was removed, squeezed and put in a cheese cloth bag and dried so as to get the dry weight.
- : Support for the nylon mesh cloth was a 40 cm ring-stand fitted with a conical shaped 30 cm deep bag made of plastic fly screen material.
- : Occasionally, the flow rate through the nylon mesh was increased by clearing the

mesh with a water jet. The debris collected on the mesh was washed down the sides and concentrated at the bottom.

: A 16 cm diameter filter of the nylon mesh was inserted between the cylindrical part and the funnel of the sieving cylinder. The mesh with the debris was then everted and the debris washed off into the sieving cylinder.

: The water was allowed to drain; when necessary suction pump was attached to the stem of the funnel.

: The filter with debris and larvae was removed and the debris rinsed with water into a beaker where the total volume of the sample was made up to 60 ml.

9.1.3 *Isolation of larvae from debris*

: The mixture of debris and larvae in water was heated to 39 °C in an incubator.

: For each sample to be processed, 60 ml of warm debris, 3% agar without bile at a temperature of 38°C were mixed and kept at 38-40°C in a water bath until used.

: A 20 x 35 cm piece of gauze or non-woven lysine (J. Cloth, Johnson and Johnson, Slough, Great Britain) was fixed in the tray by a fine mist of water.

: Equal volumes of agar and debris at 38°C were poured into the tray containing the cloth to form a layer of even thickness.

: 15 to 30 min later the gauze with the solid agar slab was lifted out of the tray, kept in a vertical position and wound into a roll.

: The agar roll was placed in a glass column containing water at 38°C . The roll was kept in position by means of a small wooden rod (swab stick) and a clothes-peg. The tray was rinsed and the rinsing fluid poured into the column.

: Following incubation at 38°C for 18-20hrs, approximately, 10 ml of fluid were drawn

off into a conical centrifuge tube and centrifuged at 2000 r.p.m. for 2 minutes.

The supernatant was siphoned off leaving approximately 0.2 ml of water containing the final sediment.

9.1.4 *Staining and microscopic examination*

One drop of iodine solution was added to the sediment in the centrifuge tube.

After a minimum of one hour the sediment was transferred to a glass slide and a drop of thiosulphate solution was mixed with it. The parasitic larvae will retain the brown colour much longer than the soil nematodes due to the double cuticle they have which is not in soil nematodes. This facilitates the identification and counting of L₃ under the microscope.

This preparation was examined after a few minutes under a cover glass at a low power magnification.

The larvae were identified using keys found in a standard laboratory manual (MAFF, 1986).

The counting was recorded and expressed as number of L₃ kg⁻¹ dried herbage [= count X 1,000/weight of dry herbage (in grammes)].

Reference: Jorgensen (1975); Mwegoha and Jorgensen (1977); MAFF (1986).

Appendix 9.2 **Modified McMaster technique**

9.2.1 *Procedure*

- : 4 g of faeces were weighed out in plastic cup with accuracy of 0.1 g.
- : Tap water was added in a proportion of 1.0 g faecal: 14.0 ml of water.
- : The contents were stirred up with a wooden spatula, and left for about 30 min. The stirring was repeated after 30 min. to breakup the faeces.
- : The suspension was poured through a piece of gauze (cheese cloth) placed over another plastic cup, and immediately, 10 ml was transferred into a labelled centrifuge tube.
- : The tubes were centrifuged for 7 min, at 1200 rpm (X g).
- : The supernatant was sucked off by a water suction pump and flotation medium (sugar and salt solution) was added to the sediment up 4.0 ml.
- : The sample was stirred by pasture pipette or by a test tube shaker and while stirring a subsample was taken and one chamber was filled.
- : The remaining sample was mixed well again and a second subsample taken and put into the second counting chamber.
- : The counting chamber was allowed to stand for 5 minutes so that the eggs get enough time to float.
- : The two subsamples in the two chambers were examined under a light microscope at 4 X 10 or 10 X 10 magnification.
- : All nematode eggs were counted within the engraved area of both chambers.
- : Egg counts of the two chambers were added together and multiplied by 20 to get eggs per g of faeces (epg).

9.2.2 *Flotation fluid for the Method*

Salt/sugar solution

Sodium chloride (Kitchen salt)	400 g
Water	1000 ml
Sugar	500 g
Specific gravity	1.280

Dissolve the salt in water (saturated solution). Add the sugar to the saturated salt solution.

Stir until the sugar is dissolved.

Reference : Henriksen and Aagard (1976).

Appendix 9.3 Techniques for faecal culture

9.3.1 *Procedure*

- : Faeces, 10 g were mixed with demineralized water (8 mls) until a suitable soft consistency was attained.
- : Vermiculite, 4 g, was added to the faeces suspension and thorough stirred until an ideal moist/porous consistency was attained.
- : The culture was transferred to vessel B (Fig. 11), which was thereafter covered by the double layer of gauze (c). To fix the gauze, and complete the culture chamber (L) the ring A was pressed down in reversed position until the cut lines of A and B are level.
- : The culture chamber (L) was incubated in the moist box fastened in apposition 1-2 cm above the filter paper as indicated in Fig. 11.
- : After incubation for 14 days at room temperature, the culture chamber was transferred for baermanisation to the conical glass vessel (H), which was filled with

tap water (20-25 °C). The holder (J) ensures the correct position of the chamber and further move makes room for a pipette (K) to be passed besides the chamber to the bottom of the vessel.

: The aggregate was left for 24 hrs. at room temperature, during which period the larvae will have migrated through the meshes of the gauze and settled at the bottom of the glass vessel. For collecting the larvae, 0.2-0.4 ml of the deposit was pipetted off and put into a test tube.

: 2-3 drops of iodine solution was added to the deposit in the test tube.

: After a minimum of one hour the deposit was transferred to a glass slide and a drop of thiosulphate solution was mixed with it. Parasitic larvae retained the brown colour due to double cuticle they have. This facilitates the identification and counting of L₃ under the microscope.

: The deposit was examined under 10 X 10 magnification.

: Steps 8 and 9 were repeated until 100 larvae were identified. The counts for each species provided an estimate of the composition (%) of the parasite population in the host.

9.3.2 *Equipment*

: Culture chamber was made from a disposable polystyrene cup. The cup was divided into two parts of equal height (A and B) by horizontal cut (See Fig. 28). Four to five perforations having been made in the bottom of the lower part (B) by means of an 18 G needle. Circular gauze (C), 10 cm in diameter.

: Moisture box with cover, made from an ordinary plastic box of suitable volume (D). A sheet of filter paper soaked in water (E) was spread on the bottom, and a plate (F)

with circular holes fitting the culture chamber or, alternatively, rings (G) made from the middle third of disposable polystyrene cups, identical to those which the culture chambers were made, were placed upon the filter paper.

: vermiculite

: conical glass vessel (H)

Reference : Henriksen and Korsholm (1983).

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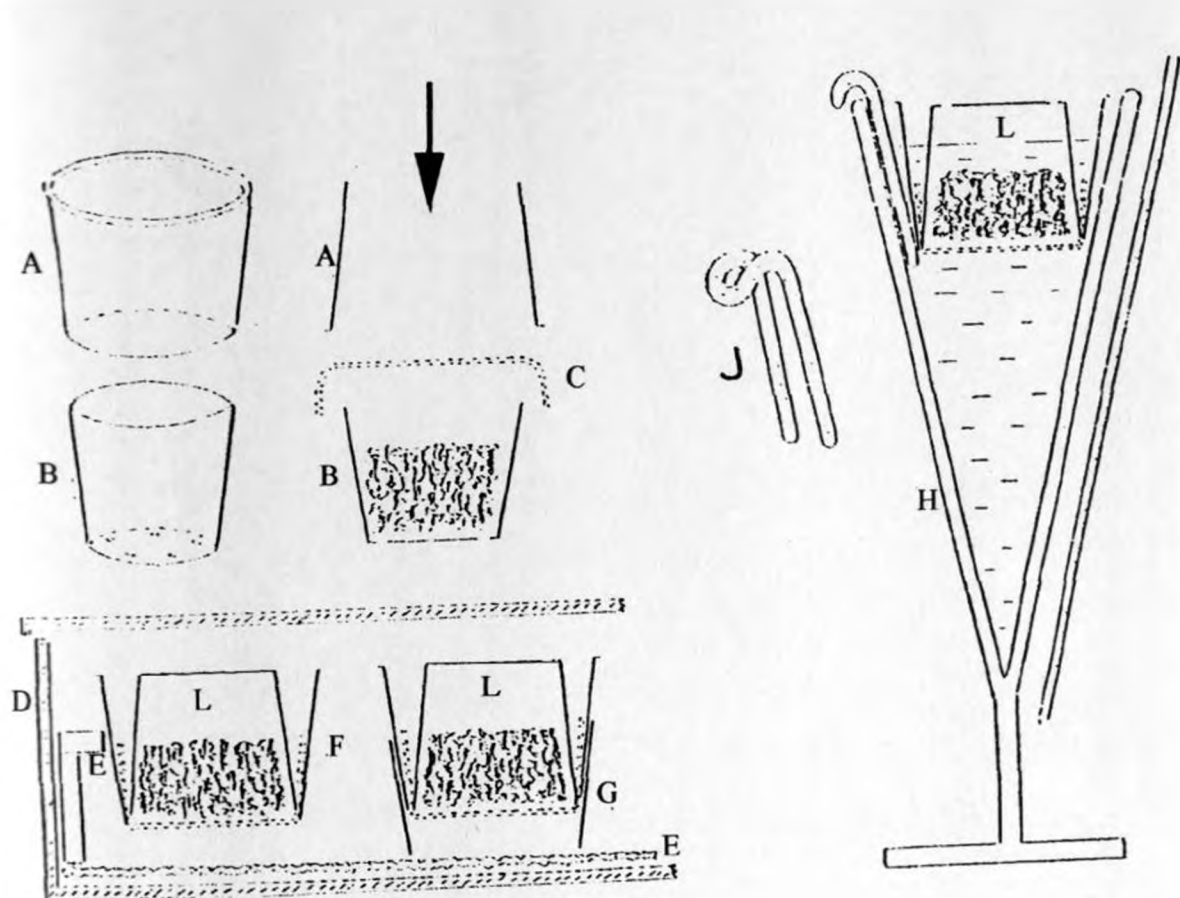


FIG. 11 Schematic representation of the equipments and the technique