THE EPIDEMIOLOGY AND CONTROL OF GASTROINTESTINAL NEMATODES OF SMALL RUMINANTS IN A SEMI-ARID AREA OF KENYA WITH EMPHASIS ON HYPOBIOSIS OF *HAEMONCHUS CONTORTUS*.

BY

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ABSTRACT

A study on the epidemiological dynamics of gastrointestinal nematodes of small ruminants was conducted in a semi-arid area of Kenya over a period of two years. Three major trichostrongylid species were identified; Haemonchus, Trichostrongylus and Oesophagostomum. Trichuris and Strongyloides species were occasionally encountered. Of the major trichostrongylids, Haemonchus spp. was the most prevalent (90%) and accounted for about 80% of the total worm burden. This species was found to undergo hypobiosis at levels that varied with seasons: nil levels of hypobiosis were recorded during the wet months and as much as 80% was recorded during the dry months. Hypobiosis was not investigated in the other nematode species. Evaluation of the relationship between the faecal egg count and the worm burden showed that the two parameters were more highly correlated during the wet months than during the dry months. This was a desirable situation because it is during the wet season that livestock owners in this area need to closely monitor the worm burdens in their animals. Treatment with ivermectin before the onset of the rains not only delayed the onset of egg

shedding but also controlled clinical helminthiasis. In addition, a temporal change in the pattern of the appearance of infective larvae on pasture was observed; the appearance was delayed for about a month after the onset of the rains. The effect of treatment administered during the rains was a temporary and a short-lived relief of infection as evidenced by a brief decline in egg output: it had no detectable impact on pasture infectivity. These results suggested that removal of hypobiotic larvae before they resumed development had the combined benefit of reducing both the severity of clinical helminthiasis and the level of pasture contamination. This impact was expressed in improved flock performance and particularly in the improvement of birth weights that subsequently enhanced kid and lamb survival rates. Probably due to the high selective pressure exerted on the parasite population in this treatment regime, evidence of loss of susceptibility to ivermectin was detected at the end of the study.

RÉSUMÉ

Une étude a été entreprise, pour une période de deux ans, sur la dynamique épidémiologique des nématodes gastrointestinaux des ovins et caprins d'une région semi-aride du Kenya. Les espèces Haemonchus, Trichostrongylus et Oesophagostomum ont été les plus abondantes des espèces de trichostrongyles identifiées. Les espèces Trichuris et Strongyloides ont été observées à l'occasion. Parmi les plus abondantes espèces de trichostrongyles, Haemonchus spp. était la plus répandue (90%), constituant environ 80% de la charge totale de vers. Haemonchus a démontré qu'il entrait en hypobiose à différents niveaux variant avec les saisons. Durant la saison des pluies, l'hypobiose a été presque inexistante et a augmenté jusqu'à 80% durant la saison sèche. L'hypobiose n'a pas été étudiée chez les autres espèces de nématodes. L'évaluation du lien entre le compte faecal d'oeux et la charge de vers a démontré que la corrélation entre les deux paramètres est bien plus élevée durant la saison des pluies que durant la saison sèche. Ceci est une situation voulue, considérant le fait que les propriétaires de troupeaux de la région auraient besoin de contrôller de près la charge de vers de leurs bêtes durant la saison des pluies. Le traitement à l'invermectin avant la période des pluies n'a pas seulement retardé la production d'oeufs, mais a aussi réprimé l'apparition des symptômes cliniques d'helminthiase. De plus un changement provisoire du mode d'apparition des larves infectives sur les pâturages a été observé. L'infectivité des pâturages a été retardée d'un mois, à partir du début des pluies. Par contre le traitement administré pendant les pluies n'a provoqué qu'une diminution temporaire et de courte durée de l'infection

reflétée par une brève diminution de la production d'oeufs. Aucun effet détectable n'a été enregistré sur l'infectivité des pâturages. Tous ces résultats suggèrent que l'élimination des larves en état d'hypobiose est bénéfique à la fois au niveau des symptômes cliniques d'helminthiase et au niveau du taux de contamination des pâturages. Le bénéfice s'est exprimé par un meilleur rendement du troupeau et particulièrement au niveau du poids à la naissance des agneaux. Toutefois, à cause d'une forte pression sélective inhérente à cette thérapeutique, un début de résistance à l'ivermectin a été décelé à la fin de l'étude.

SUGGESTED SHORT TITLE:

EPIDEMIOLOGY OF SMALL RUMINANT NEMATODES IN SEMI-ARID KENYA

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This work is dedicated to my daughter

Daina Mugure

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

The place of small ruminants in livestock development.

The natural covering of the earth's surface ranges from the canopy of thick rain forests to the sparse vegetation of the desert. The permanent natural grasslands of the world - the steppe, the prairies, the pampas and the savannahs support only grasses because the soil is too thin and dry to support anything bigger. Though the livestock productivity of these lands is comparatively low (Mackenzie, 1980), their vast extents support the better part of the world's herds.

In the tropics, the savannah vegetation cover is characterised by coarse grasses and small thorny shrubs that are unappetizing. In exploiting this kind of resource in livestock husbandry, the first line of pasture utilisation is the sheep whose split lip enables it to bite herbage at the soil level. The grazing habit of the sheep is however selective; they generally avoid rough vegetation but can utilise coarse shrubs by nipping off the soft growing shoots.

The goat has the toughest mouth of all ruminants (Mackenzie, 1980). It can browse with pleasure such repellent vegetation as thorny acacia and sodom's apple (Solanium spp). In proportion to its size, the goat can eat more than twice as much fodder as the cow, almost one-third of its total body capacity being available to accommodate food in the process of digestion (Mackenzie, 1980).

Cattle crop the pastures evenly leaving behind them plenty of herbage material that sheep and goats would find useful and easily accessible. Taking into consideration that a cow will consume as much as 10-15 kg of dry matter each day, it is understandable that she has neither the time nor the patience for anything that frustrates her steady rhythm of grazing, be it short herbage, thorny weeds or unpalatable shrubs. Under less than optimal pasture conditions therefore, the cow will find herself more disadvantaged than the sheep or the goat in accessing adequate dry matter to meet her daily needs. With limited resources therefore, sheep and goat enterprises will most probably be more viable in semi-arid areas than cattle enterprises.

Background to the study:

Within the last two to three decades, it has become increasingly evident that the rapidly growing human population in Kenya can no longer be adequately sustained by the food produced in the prime agricultural lands in the highlands. Consequently there has been a gradual exodus of people from the highlands to the bordering semi-arid lands in search of exploitable land resources to meet their food requirements and other basic needs. Conditions in these areas have been harsh and inhospitable to both man and his domestic animals in view of the diversity and severity of climatic conditions, presence of wild animals and a multitude of parasitic fauna and other pests.

The domestic small ruminants (sheep and goats) appear to perform better than cattle under these conditions and are therefore the main stay of the livestock enterprise. Consequently, like in many other parts of the tropics, small ruminants form a major asset in these areas. However, they are not exempt from various stresses commonly present in these areas that tend to reduce their production potential. In addition to such devastating diseases as contagious caprine pleuropneumonia and cowdriosis, poor nutrition and other maladies, gastrointestinal helminth infections have been a major drawback to flock performance.

Over the years, reports from semi-arid areas such as Marsabit, Kiboko and Turkana have highlighted high mortalities occurring among lambs and kids and to a lesser extent, their dams, during the late dry season or soon after the onset of the rains. The diagnosis has consistently been severe clinical haemonchosis. This pattern of the occurrence of clinical haemonchosis has led to the suspicion that hypobiosis could be involved in the epidemiology of Haemonchus contortus but this has not been positively confirmed. Kenya Agricultural Research Institute (KARI), an organisation charged with the responsibility for agricultural research in the country has (among other mandates) the mandate to improve productivity of livestock in arid and semi-arid regions. It was therefore in this connection, that, as an employee of KARI in the department of animal health, I took leave of absence to do this study for my PhD degree. The study was proposed with the general objectives of investigating the role of hypobiosis in the epidemiology of haemonchosis in semi-arid areas and of formulating control strategies to maximise flock performance. The general hypothesis was that removal of Haemonchus contortus with ivermectin at the hypobiotic stage would lower subsequent worm burden levels, reduce the resulting pasture re-contamination and consequently enhance flock performance. The study also included the determination of the level of Haemonchus contortus susceptibility to ivermectin after exposure for the two years of the study.

STATEMENT OF AUTHORSHIP

The field experimental set-up, data collection, laboratory analyses and the manuscript preparation were all done by myself. As a CIDA (Canadian International Development Agent) summer student in Kenya, Joyce Njoroge performed the in vitro ivermectin susceptibility assay on infective larvae that I provided from the experimental flocks.

This statement of authorship is presented in conformity with the regulations of the Faculty of Graduate Studies and Research, McGill University.

LITERATURE REVIEW

A GENERAL OVERVIEW

Epidemiology of nematodes:

Trichostrongylid nematodes have simple direct life cycles. Infective larvae (L_3) on pastures are ingested by grazing animals and passed to the abomasum where they develop to L_4, L_5 and adult worms. At sexual maturity (in Haemonchus this is attained about 3 weeks post-infection) female worms start laying eggs which are passed out with the host's faeces. Within the faecal material and under suitable conditions on pasture, eggs hatch into L_1 which then moult into the next stage, L_2 and finally the L_3. The L_3 are non-feeding but are very active as they migrate on moist herbage where they are ingested by a new host.

In the abomasum, the L_3 moult to L_4 in 3-4 days after ingestion. The early L_4 stage is normally a period of very rapid growth. However, during hypobiosis, development ceases soon after moulting and the larvae acquire a hypometabolic state. In this state, the parasite has a low death rate and can accumulate in large numbers in the abomasum of infected host during the grazing season without causing apparent damage (Armour and Duncan, 1987).

Hypobiosis:

Extensive literature is available on the gastrointestinal nematodes of ruminants in temperate countries with regard to epidemiology, pathology and control methods (Soulsby, 1966; Michel, 1974; Gibbs, 1986). Specifically, a very interesting aspect of the biology of <u>Haemonchus</u> <u>contortus</u> is the tendency of the immature worm to go into a state of arrestment (hypobiosis), a phenomenon whereby the development of the nematode is temporarily halted at the immature stage (L_4) inside the host during periods of harsh climatic conditions when survival of eggs and infective larvae (L_3) on pasture would be threatened. Hypobiosis is therefore an adaptation of self-preservation that enables the parasite to survive periods of high risk. The phenomenon has been studied in many parts of the world but its extent in Kenya is unknown though its presence was reported by Allonby and Urquhart (1975). Elucidation of its epidemiological pattern and extent may have important implications in the control of nematode infections especially in semi-arid areas of Kenya.

In most living organisms, growth and development are discontinuous processes. This is particularly well illustrated in nematodes where they go through a series of four molts, each usually associated with a period of lethargus in their growth. Such interruptions in growth are an integral part of the life-cycle of these organisms and occur at the appropriate times.

Successful transfer from an infected host to a susceptible host is a very important achievement in the parasitic way of life. Any adaptive characteristic geared towards facilitating this achievement is crucial in the epidemiology of such infections (Gibbs, 1986). Hypobiosis is one such adaptation, widely spread among parasitic nematodes of domestic animals. Essentially, this phenomenon facilitates the persistence of larval stages in the host thereby enabling the parasite to capitalise on optimal conditions for transfer at the opportune time. It is known to occur in more than 30 species of parasitic nematodes (Michel, 1968).

The term "hypobiosis" simply means slowing down of life processes (Gibbs, 1986). It was first coined by Gordon (1970) who later (Gordon, 1973) included such terms as arrested, retarded, inhibited and suppressed development to be applicable to worms that had not completed their development in the host within the commonly accepted interval for the species. Other related terminology commonly used in studies of nematode growth and development include such terms as suspended, stunted development, cryptobiosis, diapause, dormancy and quiescence. Differences between these phenomena (if any) especially at the physiological level are to date, very obscure.

Michel (1974) described hypobiosis as "the temporary cessation of the development of nematodes at a precise point in early development and usually under certain circumstances, or .. certain times of the year, often affecting only a proportion of the worms". From this definition, it should be appreciated that the phenomenon represents complex associations with the possibility of a multiplicity of interactions between the host, the parasite and the environment.

In a review of the requirements for the growth of nematodes, Rogers and Sommerville (1968, 1969) concluded that when certain critical requirements are not met, development will be inhibited to a certain degree. In addition, host immunity was thought to be a primary cause of retarded development though the exact mechanism remained obscure. In a further discussion, Rogers and Sommerville (1969) observed with particular interest the fact that inhibition occurred at a highly specific point in the development of nematodes and invariably seemed closely associated with moulting. They therefore concluded that, because most nematodes stop growing when they moult (lethargus), this might be a critical stage in their life cycle when they are specially susceptible to arresting factors.

However, Schad (1977) emphasised that although hypobiosis involved a temporary cessation of development, there was more than the brief halt in growth associated with moulting. The period of arrestment could be extended over prolonged durations of time without loss of viability at the opportune time of resumed development.

Recognition of the population of arrested worms is often a problem when examining natural infections in a host. The population of hypobiotic worms must be differentiated from comparable stages undergoing normal rates of development (Gibbs, 1986). Consequently, uniformity in the developmental stage of a population of immature worms is thought to be the most important criterion for hypobiosis (Michel, 1978).

Earlier reports (Armour, 1970) indicated that hypobiotic worms were largely refractory to anthelmintic treatments. However, it was later successfully demonstrated that some anthelmintics which included oxfendazole, fenbendazole and ivermectin were effective in removing arrested worms (Mackenna, 1974; Leaning, 1984; Cole, 1986; Prichard and Ranjan, 1993). This finding had important implications in the control of nematode parasites and especially in the control of peri-parturient rise which is largely associated with hypobiosis (Blitz and Gibbs, 1972; Michel, 1974; Gibbs, 1986) in the temperate regions of the world. The hypothesis of the current study is based on the efficacy of ivermectin in removing the hypobiotic Haemonchus contortus.

In addition to the extensive studies on hypobiosis in the temperate regions, a substantial number of studies have also been conducted in the subtropical regions as evidenced by the reports of Smeal et al. (1980), Waller et al. (1981) and Waller and Thomas (1983). However, relatively little seems to be known about the extent and epidemiological significance of hypobiosis under tropical climatic conditions. Further, the scanty information available is characteristically conflicting. For example, hypobiosis has been reported to be absent in Egypt (El-Azazy, 1990) and Brazil (Charles, 1989) and inconsequential levels have been reported in Kenya (Allonby and Uqurhart, 1975), Zimbabwe (Pandey, 1990; Pandey et al., 1994), South Africa (Boomker et al., 1989; Horak et al., 1991), Malaysia (Ikeme et al., 1987) and Nigeria (Chiejina et al., 1988). Yet other studies in Nigeria (Ogunsusi and Eysker, 1979; Van Geldorp and Schillhorn, 1976) and in the Gambia (Kaufmann and Pfister, 1990) have reported very high levels. It is therefore evident that much more needs to be learned about the role of hypobiosis in the epidemiology of nematodes in the tropics.

THE ROLE OF HYPOBIOSIS IN NEMATODE BIOLOGY

The mechanism and significance of arrested development to the parasitic nematode has been the subject of much speculation (Gibbs,1986). The phenomenon has long been considered to be closely linked with host resistance to helminth infections and with the regulation of the incoming parasites. Conversely, it was postulated that resumption of development was a result of relaxation of host immunity triggered by stress factors such as poor nutrition, gestation, parturition and lactation (Crofton, 1954; Soulsby, 1957).

It was shown in subsequent investigations (Anderson et al., 1965 a & b) that seasonal factors were critical in arrested development and consequently it was suggested that hypobiosis was primarily a means of survival of some nematode species during harsh conditions when development outside the host as well as transmission of free-living stages faced higher risks (Blitz and Gibbs, 1972; Michel, 1974). It was concluded that arrested development was a normal feature of the nematode life cycle that varied in importance depending on the species of nematode. For example, in Haemonchus contortus where eggs and larval stages on pasture are so sensitive that extremes of environmental conditions barely allow continued existence, arrested development is virtually indispensable. However, in Nematodirus spp where the life cycle involves significant survival of resistant eggs and temperature-induced synchronization of egg hatching in spring, hypobiosis is less important (Gibbs, 1986).

In many instances, resumption of development coincides with availability of susceptible young hosts. This observation prompted Gibbs (1982) to postulate that there was a distinct advantage for the parasite to enter a young host as early as possible in its life. Studies by Dineen and Kelly (1973) and Kassai (1986) suggested that larvae entering the young host enjoyed relatively high immune tolerance. In cases where there is a sudden high availability of infective forms (as is the case after parturient rise), a susceptible young host may experience immune paralysis to the advantage of the parasite in its rate of establishment (Gibbs, 1986):

Types of hypobiosis:

In parasitic nematodes, hypobiosis is associated with two underlying causes (Gibbs, 1986):

a). Immune-mediated arrest.

This form has an immunological basis, where incoming larvae entering resistant hosts face constraints on their development as a result of the immune status of the host. This is best referred to as "inhibited" development.

b). Seasonally induced arrest:

This is a manifestation of innate response within larvae to some stimulus independent of the host e.g. environmental temperature. It represents seasonal adaptation and is analogous to diapausal development in insects (Armour and Bruce, 1974; Horak, 1981). It is characterised by a pause in development of the parasite and is directed to prevent maturation and release of eggs when conditions are unfavourable thereby maximizing chances of successful transmission to susceptible hosts at the appropriate time.

Immune-mediated arrest.

The mechanisms controlling inhibition of gastrointestinal parasites of ruminants have been the subject of many investigations (Michel, 1974; Waller and Thomas, 1975). Many authors have implicated host immunity as being instrumental in causing this phenomenon (Ross, 1963; Michel, 1963, 1968). The possibility that inhibited larvae could resume development as the immune status of the host declined has been known for a long time (Gordon, 1948; Michel, 1952; Gibson, 1953).

Experimental investigations especially those of Ross (1963), Donald et al. (1964) and Dineen et al. (1965 a and b) have shown that animals previously exposed to nematode infection harboured more inhibited larvae on challenge than unexposed immunologically naive ones. Among primarily seasonal arrestors like Haemonchus contortus, Adams (1983) demonstrated that host immunity could also play a role. He showed that more arrested larvae were found in a host if challenge infection was superimposed on an existing infection. Michel et al. (1979) and Snider et al. (1981) found similar effects in Ostertagia ostertagi : marked lymphoid cell infiltration of the intestinal mucosa in this case was evidence of an immunological response. In some nematode species, for example, Nematodirus spp (Donald et al., 1964; Dineen et al., 1965a; Thomas, 1978; Eysker, 1980) and Trichostrongylus spp (Michel, 1974; Eysker, 1978; Ogunsusi and Eysker, 1979), the involvement of immunological response as an underlying cause of hypobiosis is highly variable. It is therefore evident that, although host immunity may play an important role in inhibited development, there is remarkable variation in the degree to which individual species of nematodes respond to this stimulus.

Seasonally induced arrest:

Under natural conditions, large numbers of arrested H.contortus larvae have been found in grazing sheep in autumn and winter (Soulsby, 1966; Gibbs, 1968). Similar observations have been reported in non-immune tracer calves infected with O. ostertagi (Anderson et al., 1965a). It appears therefore that some environmental stimulus triggers arrestment during these seasons. In temperate climates, seasonally induced arrest is linked with the advent of cool temperatures. It has been suggested that the chilling of infective larvae of O. ostertagi in autumn and winter might be the primary inducer of arrestment in this species (Armour et al., 1967). This was later confirmed by Armour and Bruce (1974) who suggested that this type of arrest represented a diapause-like phenomenon. Many other investigations (Fernando et al., 1971; Hutchison et al., 1972; Blitz and Gibbs, 1972; Mackenna, 1973; Armour, 1978; Schad, 1982; Waller and Thomas, 1983; Herd et al., 1984a & b) on various nematode species have shown that exposure to gradually falling temperatures for about 5-6 weeks was effective in inducing this type of arrest.

In tropical and sub-tropical areas, seasonally induced arrest is observed with the onset of hot dry conditions. Shimshony (1974) reported this form of arrest of Haemonchus contortus in goats during a dry season in Israel. Peak arrest of O. ostertagi in cattle has been reported in southern USA during the hot dry summer months (Baker et al., 1981, 1984; Williams et al., 1983). In Australia, seasonal occurrence of arrested development in O. ostertagi was maximum in spring (the onset of dry conditions) in permanently grazed cattle (Anderson, 1968; Smeal et al., 1977). In Iraq, Altaif and Kassai (1983) also reported high levels of arrest in O. circumcincta during the hot dry months of summer. In these instances, hypobiosis may have been triggered by the increasing temperatures or by simply the onset of dry conditions associated with summer (Gibbs, 1986).

There has been speculation that factors other than temperature could be involved in seasonally induced arrested development. Moisture and humidity have received considerable thought in this respect. For example, Ogunsusi and Eysker (1979) showed that arrested development of H. contortus in Nigeria occurred towards the end of the rainy season. At this time, humidity is rapidly decreasing and giving way to the hot dry season. It is therefore likely that decreasing environmental moisture could act as a seasonally associated inducer of arrest in certain geographical regions.

Genetic role in seasonal arrestors:

Further investigation on seasonal arrestment has indicated that genetic mechanisms could be involved in its manifestation. This is especially evident with regard to differences in propensity to arrest among different geographic strains of some nematode species (Gibbs, 1986). Two types have been described: an obligatory propensity to arrest that does not require a stimulus to be induced and a stimulus-dependent propensity. The first type was described by Waller and Thomas (1975) when they observed substantial arrest of H. contortus larvae under conditions that precluded the possibility of an environmental temperature effect on the free-living stages. The second type depends upon external stimulus for induction (Armour et al., 1967; Michel et al., 1973; Smeal et al., 1980; Smeal and Donald, 1981, 1982; Watkins and Fernando, 1984).

Metabolism of arrested larvae:

Reduced susceptibility of arrested larvae to anthelmintics has been reported (Armour and Bruce, 1974). Consequently, it has been suggested that arrested larvae may be altered in some way so that they are metabolically less active than normally developing larvae (Anderson, 1977). This has important implications for the use of anthelmintics in the treatment of arrested larvae. However, there have been reports that indicated high levels of activity of some anthelmintics (oxfendazole, fenbendazole and ivermectin) against inhibited larvae (Leaning, 1984; Coles, 1986; Prichard and Ranjan, 1993). This implies that some anthelmintics could be used in treatments targeted on the arrested larvae.

Resumption of development:

There has been considerable speculation on the exact nature of the factors responsible for termination of arrested development. The following opinions have been expressed by various authors:

Immune-mediated arrest:

It would be logical to assume that in immune-mediated arrest, removal of host immunity would lead to resumption of development of arrested larvae. Consequently, the "spring rise" phenomenon has generally been ascribed to loss of immunity (Crofton, 1954; Soulsby, 1957; Thomas, 1978; Eysker, 1980). However, experimental attempts to simulate resumption of development of arrested larvae by immunosuppressing the host have generally not supported this hypothesis (Gibbs, 1968; Prichard et al., 1974; Schad and Page, 1982). In these investigations, the immunosuppressing compounds used were effective in suppressing immunity as evidenced by significant lymphopenia. It was therefore concluded that arrested larvae appeared to resume development spontaneously. However, Behnke and Parish (1979) reported some success in reactivating arrested Nematospiroides dubius larvae in mice by using immunosuppressing compounds. In view of the above facts, it is evident that the role of immunity in hypobiosis may vary depending on the species of the parasite and the host.

Seasonally induced arrest:

Seasonally arrested development in parasitic nematodes has been viewed as being similar to diapause in insects (Armour and Bruce, 1974; Horak, 1981). On the basis of such an assumption, the duration of arrest can be considered to be of a fixed and predetermined length equal to the period of adverse conditions over which the larvae need to be arrested (Gibbs, 1986). Under the circumstances, the resumption of development would therefore be most likely initiated by an internal mechanism within the arrested larvae themselves or by an external stimulus, either host or environmental in origin. Consequently, this would lead to a well synchronised resumption of development at an appropriate time. Indeed this seems to be the case in some of the trichostrongyles like H. contortus (Gibbs, 1968;

Connan, 1978) and O. ostertagi (Armour and Bruce, 1974). However, this trend was contradicted by two major studies conducted by Michel et al. (1976a & b) on the emergence of O. ostertagi from arrest. Results of these studies indicated a pattern of continuous rather than synchronous resumption of development.

It has been shown in some studies (Armour and Bruce, 1974; Michel et al., 1975) that ability to arrest is not a permanent feature and that, with time, larvae conditioned to arrest lose their ability if not ingested by a suitable host in good time. Beyond a certain time, such larvae will not arrest even if acquired by a susceptible and suitable host. This means that, once the larvae are conditioned, the physiological processes that have to be completed before they can resume development, proceed irrespective of whether they are ingested or not. By surgically transferring arrested H. contortus larvae from naturally infected sheep to worm-free sheep, Blitz and Gibbs (1971a & b) clearly demonstrated that there is a fixed end point of arrest. The surgically infected sheep showed marked increase in egg output at the same time as the naturally infected controls. This was further confirmed by Connan (1978) in a study where he naturally exposed two groups of sheep to H. contortus larvae in pasture, two months apart. Resumption of development occurred simultaneously in both groups irrespective of time of exposure. In the same study, Connan (1978) concluded that parturition did not seem to influence resumption of development of arrested larvae.

PRINCIPLES OF STRATEGIC NEMATODE CONTROL

Epidemiological factors:

Occurrence of parasitic diseases may arise as a result of interaction of a number of factors which include an increase in the number of infective larvae on pasture, an alteration in the host susceptibility and an introduction of new infection (Urquhart et al., 1987).

1. Increase in the number of infective larvae:

Temperatures in the tropics are normally high enough to allow hatching and development of eggs to L_3 all year round. However, translation of L_3 from faeces to the pasture herbage is an intermittent process that depends on the wetting of the faecal material, usually by rain. Consequently, the wave of L3 migration onto pasture corresponds to the rainfall pattern (Wink et al., 1983). The level of this contamination is strongly influenced by stocking density and this is particularly true for nematode infections where no multiplication of the parasite takes place outside the host (Urquhart et al., 1987).

2. Alteration of host susceptibility:

Sheep and goats become more susceptible to most helminth infections during late pregnancy and early lactation due to the periparturient relaxation in immunity (Urquhart et al., 1987). In addition, in many countries, parturition may be synchronised to occur when the weather is most favourable for pasture growth. Incidentally, this is also the time that is most conducive for the development of the L_3 on pasture. The epidemiological significance of this is that the system ensures increased contamination of the environment with infective larvae at a time when the number of susceptible hosts is increasing.

Nutritional status has been found to influence host tolerance to parasitism (Blackburn et al., 1991, 1992). Well fed animals are better able to tolerate parasitism than animals on a poor plane of nutrition. For example, sheep and goats infected with blood-sucking Haemonchus contortus may be able to maintain their haemoglobin level as long as their iron intake is adequate. However, if iron intake becomes sub-normal, their haemopoetic systems may become exhausted and this could result in death.

3. Introduction of new infection:

This can occur in two ways: by introducing stock into an environment infected with parasite species that the animals have not been exposed to before or by recruiting infected stock into a clean herd. Such helminth-naive animals are highly susceptible and outbreaks of parasitic diseases are consequently, very common.

Approach to control:

Several decades of experience in helminth control have shown that eradication of helminth infections is not practical (Brunsdon, 1980). The thrust for control strategies has therefore shifted towards principles of control that focus on keeping parasite populations below levels that adversely influence profitable animal production. There are two ways in which such strategies could be implemented - (1) the use of chemotherapy and (2) the use of integrated techniques that utilise the knowledge of the ecology and epidemiology of parasites.

Livestock owners have been known to exclusively rely on broad-spectrum anthelmintics to control nematode parasites in their herds. While usually affording protection against serious disease and mortality, such treatments are not frequently effective in preventing the exposure of animals to high levels of infective larvae on pasture (Brunsdon, 1980). This is because the host-parasite-environment relationship is so complex that sole reliance on anthelmintics does not successfully interrupt the transmission cycle of nematode parasites. Consequently, production losses still occur as a result of re-infection in the interval between treatments.

Another approach to chemotherapeutic control of nematodes has focused on the removal of hypobiotic larvae. Some anthelmintics, for example oxibendazole, fenbendazole and ivermectin have been found to be highly effective in removing hypobiotic larvae (Herd et al., 1984b; Leaning, 1984; Prichard and Ranjan, 1993). It has been shown that treatment of ewes at winter housing suppressed the peripaturient rise and the subsequent worm loads (Procter and Gibbs, 1968; Herd et al., 1983). However, a post-lambing treatment was necessary during spring to remove worms arising from overwintered larvae on pasture and any uncleared hypobiotic worms during pre-lambing treatment.

Besides being only partly effective in controlling parasites, the use of anthelmintics poses the threat of drug resistance, chemical residues in animal products and ecological pollution (Waller, 1993). It has therefore become imperative that sustainable parasite control programmes that integrate the knowledge of the ecology and epidemiology of the parasite be developed and implemented in an attempt to minimise the use of anthelmintics. The central principle of such strategic control schemes is to prevent or limit contact between parasite and host with the aim of preventing the build-up of dangerous levels of infective larvae on pasture and to anticipate the periods during which large numbers of infective larvae are likely to occur. This principle was earlier conceived by Gordon (1948, 1957) who stated that "the primary objective of helminth control is to prevent or reduce pasture contamination rather than to remove worms which in many cases have a very short life span anyway". This objective can be achieved by the integration of grazing management, chemotherapeutic intervention and acquisition of immunity.

Grazing management decisions can strongly influence the extent and severity of helminthoses. Such decisions include the choice of stocking density, time of reproductive events (especially parturition and weaning), alternate grazing and the variation in the relative proportions of young and adult livestock (Morley and Donald, 1980). These factors will enable manipulations of stock and pasture management in integrated control programmes to produce safe pastures for susceptible animals at appropriate times. In strategic treatment trials, Anderson

(1972, 1973), Southcott et al. (1976) and Donald et al. (1978) have shown that the effectiveness of a single anthelmintic treatment in reducing parasite populations in grazing sheep can be prolonged if treatment is given when the rate of reinfection from pasture is greatly reduced or treatment is combined with movement to safe pastures. In the tropics, availability of infective larvae on pasture is lowest during the long dry spells.

In future, it is expected that other options, for example, helminth vaccines, resistant breeds of livestock and biological control methods will become available. Such methods will revolutionise the current thinking on nematode parasite control of livestock (Waller, 1993; Donald, 1994).

***Anthelmintic resistance*:**

For a drug-naive population of a given parasite, the gene or genes expressing resistance are usually present at very low frequencies (Prichard et al., 1980). Selection for resistance occurs when the frequency of these genes increases as a result of survivors of treatment making progressively greater contributions in succeeding generations. Procedures that accelerate this selection, for example, excessive use of anthelmintics especially with underdosing, naturally promote rapid emergence of resistance. However, withdrawal of the selection pressure may allow reversion to a susceptible population. Several suggestions on management and use of drugs have been made as a counter measure against development of resistance (Wescott, 1986) and these include:

1). Using a drug with high efficacy and regularly confirming this efficacy by post-treatment analysis of egg output.

2). Administering the recommended doses and avoiding underdosing. It should be remembered that the recommended dose is the lowest amount of the drug that is capable of killing the parasite. This means that the margin between the effective dose and an underdose is quite narrow.

3). Avoiding excessive use of the drug - select the minimum frequency of treatment that will be effective in keeping the worm burden at a level that does not adversely affect animal performance.

4). Practising a slow rotation of unrelated anthelmintics.

5). Combining particular products in order to prolong their useful life.

6). Not relying exclusively on anthelmintics by incorporating management systems that facilitate reducing the frequency of treatments per year.

PARASITISM AND METABOLISM OF RUMINANTS

Gastrointestinal nematode infections are a major cause of impaired productivity in ruminants (Sykes and Coop, 1976, 1977; Bliss and Todd, 1976; Leyva et. al., 1982; Steel et al., 1982; Michel et al., 1982; Thomas and Ali, 1983; Sykes and Juma, 1984; Abbott et al., 1986). Further, it has been shown that even subclinical levels of infections can cause considerable losses (Allonby and Urquhart, 1975; Bremner, 1982; Sykes, 1983; Holmes, 1985).

Adverse impacts of parasitism on productivity are expressed in a number of ways which include loss in body weight (Sykes and Coop, 1976, 1977; Abbott et al., 1986), reduced milk production (Michel et al., 1982; Thomas and Ali, 1983; Kloosterman et al., 1985) and poor quality and quantity of wool (Steel et al., 1982). The underlying causes of such poor performance have been associated with the influence of parasitism on host food intake; gut motility, digestion and absorption, protein, energy and mineral metabolism as well as water and electrolyte balance.

Decreased food intake:

Parasitised animals usually express certain degrees of inappetance. Up to 20% reduction in food intake has been reported in lambs infected with T. colubriformis (Sykes and Coop, 1976; Coop et al., 1982) and the degree of inappetance has been shown to vary with the level and duration of infection (Abbott et al., 1986). However, reasons for the occurrence of inappetance are unknown and despite efforts by many authors to offer explanations, none has been found to be entirely satisfactory. Pain has been suggested to be an important factor (Andrew, 1939; Gibson, 1955), but this is difficult to assess. Changes in abomasal pH associated with Ostertagia spp infections (Ritchie et al., 1966) and with intestinal trichostrongylosis (Baker and Titchen, 1982) have also been suspected to play a role in inappetance but this is yet to be confirmed. Altered plasma concentration of gastrointestinal hormones and especially cholecystokinin (CCK) has similarly been implicated as a cause of reduced food intake in parasitised ruminants (Symons and Hennessy, 1981; Titchen, 1982). However, radioimmunoassay tests have failed to confirm changes in CCK in inappetant sheep infected with trichostrongylosis and it is therefore uncertain whether this hormone plays a role in causing reduced food intake.

Gut motility, digestion and absorption:

In addition to reduced feed intake, parasitised animals have frequently been found to have reduced utilisation of nutrients relative to parasite-free contemporaries. Although a number of studies have demonstrated changes in gastrointestinal motility (Roseby, 1977; Bueno et al., 1982), pH of gastrointestinal tract (Anderson et al., 1981), gastrointestinal secretions (Symons and Hennessy, 1981) and decreased activity of a variety of digestive enzymes (Jones, 1983), it has been difficult to positively associate these changes with impaired digestion and absorption. Generally, these studies have indicated that impaired digestion and absorption are not important causes of poor utilisation of nutrients by parasitised ruminants.

Protein and carbohydrate metabolism:

In gastrointestinal parasitism, substantial protein losses occur in terms of exfoliated epithelial cells, plasma, mucus and red blood cells (Bremner, 1969; Holmes and Macleans, 1971; Dargie, 1975; Abbott 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; Sykes and Coop, 1976; Roseby, 1977). It has further been demonstrated that protein synthesis is reduced in skeletal muscles of parasitised sheep (Symons and Jones, 1975). Subsequent to these findings, it has been concluded that due to inappetance, gastrointestinal losses of proteins and increased rates of gastrointestinal tissue metabolism as a result of accelerated repair of damaged epithelial lining, there is a net movement of amino acid nitrogen from muscle and skin to the liver and gastrointestinal tract which decreases available nitrogen for growth, milk and wool production (Symons, 1985).

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).

Mineral metabolism:

A number of studies have shown that muscle growth and mineralisation are impaired in parasitised sheep (Reveron et al., 1974; Sykes and Coop, 1976; 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.

Water and electrolyte balance:

Diarrhoea is a common feature in ruminants parasitised with most major gastrointestinal helminths. In abomasal 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 Macleans, 1971; Parkins et al., 1973). Associated with this stage of infection is the 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 diarrhoea 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, so low that their net water loss is actually lower than the non-infected animals (Bremner, 1982). Such increased water retention by parasitised animals indicates that tissue loss attributable to parasitic infections can not be strictly determined from losses in body weight alone (Halliday et al., 1965; Entrocasso et al., 1986b; Abbott et al., 1986).

ANTHELMINTIC CHARACTERISTICS OF IVERMECTIN

Ivermectin is one of the most potent anthelmintics with a broad-spectrum of activity towards nematode and arthropod parasites (Burg et al., 1979; Chabala et al., 1980; Campbell et al., 1983). It belongs to a family of highly potent anthelmintics, the avermectins , that were first reported in the late 1970's. Its unusual combination of activity against both arthropods and nematodes gives it a special place among anthelmintic groups. In addition, its spectrum of activity covers both adult and immature worms including hypobiotic larvae (Armour et al., 1980) but has no activity against tapeworms or flukes (Prichard, 1983). It can be administered both orally and subcutaneously and is safe for pregnant animals. Administered subcutaneously, ivermectin is active against ectoparasites like ticks, mites and lice.

It was initially thought that ivermectin acts by blocking the inhibitory gamma-aminobutyric acid response and interfering with the chloride ion kinetics of the muscle cells of parasitic nematodes thereby killing them by paralysis (Kass et al., 1980; Duce and Scott., 1985; Bloonquist et al., 1987; Gration and Harrow, 1987; Bookish and Walker, 1986; Robertson, 1989; Martin and Pennington, 1989; Holden-Dye and Walker, 1990). Recent evidence indicates that it acts by opening a glutamate-dependent chloride channel of neuromuscular membranes of both nematodes and arthropods (Rohrer et al., 1992; Cully et al., 1993; Prichard, 1994). This results in the paralysis of the worm.

At the time ivermectin was introduced, resistance against the major broadspectrum anthelmintics was widely reported. It was therefore received with great expectations for a revamped fight against nematode infections. However, a few years later, cases of resistance against this drug were reported in South Africa (van Wyk and Malan, 1988). Subsequently, more cases were reported in many other countries including the USA (Craig and Miller, 1990), New Zealand (Watson and Hastings, 1990) and Kenya (Mwamachi et al., 1993). H.contortus has been the most common helminth involved in all these cases. A consistent feature of these reports has been the rapid rate at which resistance has developed. For example, in the case reported in South Africa, resistance was observed 33 months after the drug had been introduced in the country. As mentioned earlier, ivermectin is highly efficacious and this is likely to result in high selection pressures which could be the underlying cause for this rapid loss in susceptibility.

CHAPTER 2

GENERAL OBJECTIVES AND EXPERIMENTAL DESIGN

INTRODUCTION

Each chapter in this thesis covers a specific aspect that is embodied in the broad objectives of the entire study. In spite of this approach, the general experimental protocol that led to the development of the data relevant to each chapter was basically similar. The purpose of this chapter is therefore to explain this protocol and to state, in general terms, the major objectives of the whole study. Any special methodologies specific to particular chapters will be described in the respective chapters.

OBJECTIVES

General objectives:

One of the most important goals of Kenya as a developing country is the need to be self-sufficient in food production. This is clearly expressed in the National Food Policy Paper No. 4 of 1981 and the Sessional Paper No. 1 of 1986 both of which recognise the role of new technologies in sustainable food production. In view of the fact that only 25% of Kenya is suitable for arable farming, there is a real challenge for developing management technologies that can be utilised to exploit resources in the non-arable areas. One of the ways to do this is to increase productivity of livestock that can survive in such areas by improving management aimed at reducing losses due to diseases. This is the long-term objective of this study.

Specific objectives:

Occurrence and severity of clinical haemonchosis among sheep and goat flocks raised in the semi-arid regions of Kenya is highly seasonal, being most severe during the long rains and least important during the prolonged dry periods. The problem is also insignificant during the short rains. An important characteristic of the clinical manifestation is that it occurs so soon after the onset of the rains that it can not be accounted for by fresh infection acquired from the pastures. Hypobiotic larvae have therefore been suspected as the source of this infection. This 2-year study was therefore designed to address the following objectives which were thought as being pertinent in the epidemiology of Haemonchus contortus in semi-arid areas of Kenya:

1. To investigate the significance of hypobiosis in the epidemiology of haemonchosis by estimating its extent and seasonal pattern in both sheep and goats.

2. To evaluate the level and seasonal pattern of pasture contamination and to assess the consequential host worm burdens.

3. To compare the effects of treatment administered before the rains and treatment administered during the rains on the level and pattern of pasture contamination and on the performance of the flocks. Ivermectin was used in both treatment regimes.

4. To evaluate the susceptibility of Haemonchus contortus population to ivermectin after exposure over the course of the study.

It was hypothesised that hypobiotic larvae were the source of infection that caused clinical haemonchosis at the beginning of the rains and that if these larvae were removed before they resumed development, both clinical disease and pasture contamination could be reduced significantly.

EXPERIMENTAL DESIGN

Geographical location:

This study was conducted at the national Animal Husbandry Research Centre, Naivasha, Kenya, an area known to be endemic for haemonchosis. The area is at an altitude of approximately 1700 m. and has a semi-arid climate with strong desiccating winds during the dry seasons. The rainfall pattern is bimodal (Fig. 3.1) with the long rains in March-June and the short rains in October-November. The short rains are very unpredictable with wide variation from year to year. In some years they fail to come altogether. The average annual rainfall stands at about 600 mm. The maximum temperatures are between 20^0C and 30^0C and the minimum between 12^0C and 18^0C with little variations between seasons. Relative humidity ranges between 50% and 80%. Natural vegetation is comprised of dwarf acacia bushes and three main types of grasses - Cynodon pletostachyum, Cynodon dactylon and Pannisetum clandestinum.

Experimental animals:

150 ewes and lambs (Red Maasai) and 150 does and kids (Small East African goats) were ear-tagged and randomly allocated to three flocks (designated A, B and C) of 100 animals each. Randomization was done along weight classes (10-15 kg., 15-20 kg. over 20 kg) and nursing dams. Three rams and three bucks were added to each flock for breeding purposes. In order to minimise flock fertility variation associated with males, these rams and bucks were rotated through the three flocks every fortnight. Before being introduced into a new flock, they were treated with albendazole (5 mg/kg: 'Valbazen, Smith Kline Animal Health Ltd. - UK). They were also regularly checked for nematode faecal egg output which was confirmed negative throughout the period of the study. The flocks were maintained on separate paddocks of 25 hectares each. Fencing was done in such a way that the flocks did not mix. At night, the animals were housed in sheds erected in their respective paddocks and during the day they were let to graze freely under the supervision of three herdsmen as an extra precaution against possible mixing. Water and mineral salts were provided ad libitum and during the dry season, lucerne hay was provided as supplementary feeding. However, supplementary feeding is not a normal management strategy in this area.

In addition to the three flocks grazing in the permanent pastures, another flock comprising 25 ewes, 2 rams, 25 does and 2 bucks was maintained indoor on concrete floor that was cleaned daily. At the beginning of the study, this flock was treated twice with albendazole (5mg/kg: 'Valbazen' Smith Kline Animal Health Ltd.- UK) at 2-week intervals and thereafter regularly examined for nematode eggs. It was designated the "Tracer Breeding Flock" and was exclusively fed on commercial lucerne and Rhodes grass hays which were regularly tested for infective larvae to confirm them negative. Water and mineral salts were provided ad libitum.

Experimental Protocol:

Weather data:

Weather data was recorded routinely by the research station staff. This included rainfall, minimum and maximum temperatures and air humidity.

Strategic control regimes:

Flock A was treated with ivermectin (200μg/kg 'Ivomec' Merck, Sharp and Dohme Ltd. - Switzerland) 4 weeks before the onset of the rains (both short and long rains) and flock B was treated with the same drug 4 weeks after the onset of rains. Flock C served as the control and was therefore not treated (Table 1) except for occasional salvage treatments with albendazole (5mg/kg: 'Valbazen' Smith Kline Animal Health Ltd. - UK).

Table 1. Scheme of treatment administered to the three flocks over the experimental period.

Flock	Treatment strategy	Time of Study																						
		1991					1992												1993					
		A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J
A	Pre-rains		Rx					Rx							Rx						Rx			
B	During rains			Rx						Rx						Rx						Rx		
C	No treatment																							

Rx = Time of treatment

Estimation of adult and hypobiotic worms:

One tracer lamb and one tracer kid were removed from the Tracer Breeding Flock every month and introduced into each of the flocks A, B and C where they were allowed to graze for 3 weeks. They were then withdrawn together with 2 permanent lambs, 2 permanent kids, 1 ewe and 1 doe randomly selected from each flock, and housed together (on the basis of flock of origin) on separate concrete floor pens. They were fed on commercial lucerne hay for 4 weeks after which they were necropsied and the following parameters estimated by standard methods (Anonymous, 1986):

1) Differential total adult worm counts.

2) Total hypobiotic larval count.

Basically, the following steps were used in processing the material for examination:

a). Immediately after the animal was killed, the abdomen was slit open and the abomasum, the small intestine and the large intestines were retrieved and ligatured at their extreme ends and placed into different containers.

b). The abomasum was then opened through the greater curvature and the contents released into a bucket. The mucosa was then cleaned thoroughly under running tap water with the washings being let into the same bucket. This was made up to 2 l. After vigorous stirring, 200ml. of the material was withdrawn into a plastic jar containing 5 ml of 10% formalin. The mucosa of the abomasum was then scraped and placed into a separate jar without formalin and taken to the laboratory where it was digested in a hydrochloric acid-pepsin mixture for 6 hrs at 37^0C. The resulting digest was made up to 2 l and after stirring, 200 ml was withdrawn into a jar containing 5 ml of formalin and preserved for examination. Estimation of adult worm counts and hypobiotic larvae counts in the abomasal contents/washings and the abomasal digest were done under a dissecting microscope at X16. 40 ml of each was examined in aliquots of 10 ml after adding 2-3 drops of Lugol's iodine to stain the worms. Each 10 ml aliquot was placed in a petri dish with parallel lines 5 mm apart on its bottom to enable systematic examination. The total number of worms counted from the 40 ml was multiplied by a factor of

50 to give the total number of worms in the original material. Adult worms were identified to the genera level under the compound microscope.

c).The small intestines were slit longitudinally and the contents put in a bucket and processed in the same way as the abomasum except that there was no HCl-Pepsin digest.

d). The contents of the large intestines were placed in a test sieve with pore diameter of 213μm and tap water passed through gently to remove most of the colouring fluids and coarse debris. The rest was put in a jar containing 5 ml of 10% formalin. In the laboratory, all the worms contained in this material were counted.

e). Neither the small nor the large intestines were examined for hypobiotic larvae.

Estimation of faecal egg output and packed cell volume:

Faecal samples were collected (per rectum) monthly from all animals in each flock and the egg output estimated by modified McMaster method (Whitlock, 1960). The remaining faecal material was separately cultured in an incubator by animal species and by flock at 27^{0}C. The larvae were harvested by baermannization (Anonymous, 1986) and identified by the method of Keith (1953).

All the animals in each flock were bled from the jugular vein once a month and about 5 ml of blood collected in test tubes containing the anticoagulant EDTA. In the laboratory, this blood was used for the estimation of the packed cell volume (PCV).

***Estimation of pasture larval counts*:**

Once a month, herbage samples were collected from each of the paddocks by the method of Tylor (1939) and processed for infective larval counts by a standard method (Anonymous, 1986). Infective larvae were identified by the method of Keith (1953). In addition, pastures were also sampled by trailing the animals as they grazed and sampling the areas and the type of herbage they commonly consumed, clipping the herbage at approximately the same level the animals nibbled it. For the purpose of this sampling, animals were trailed for 8 hours (8.00 am-4.00 pm) every time it was done.

Estimation of flock performance:

Daily and monthly records of each flock were kept on the following parameters:

- Live weights were taken once a month.
- Birth weights were recorded as they occurred.
- Age and weight at first lambing and kidding.
- Lambing and kidding intervals.
- Mortalities as they occurred.

Statistical analyses:

Repeated measures of analysis, one-way analysis of variance, paired t-test, Bonferroni t-test and Mann Whitney U-test were used to compare the various parameters among the three flocks. These tests are contained in SAS packages (Schlotzhauer and Littell, 1991; Freund and Littell, 1991; Littell et al., 1991). The statistical tests for the specific parameters are contained in the relevant chapters. In all cases, level of significance was set at $\alpha = 0.05$.

CHAPTER 3

ARRESTED DEVELOPMENT OF *HAEMONCHUS CONTORTUS* IN NATURAL INFECTION OF SHEEP AND GOATS.

INTRODUCTION

The role of hypobiosis in the epidemiology of gastrointestinal trichostrongylid nematodes is well documented (Anderson et al., 1965b; Gibbs, 1967; Connan, 1968; Armour, 1970; Eysker, 1980; Smeal and Donald, 1984; Eysker and Kooyman, 1993). In the temperate regions of the world, it has consistently been shown that hypobiosis is the primary means of overwintering for Haemonchus contortus (Gibbs, 1967; Blitz and Gibbs, 1971a & b, 1972; Eysker, 1993). Because the infective stage of this nematode is highly susceptible to winter conditions (Kates, 1950; Crofton, 1963), the nematode survives the winter in the inhibited form and resumes development when climatic conditions become favourable for transmission (Anderson, 1972; Reid and Armour, 1972; Gibbs, 1973; Michel, 1974). It was initially thought that inhibition in H. contortus was solely induced by host resistance to worm infection (Michel, 1974; Waller and Thomas, 1975; Adams, 1983), but more recent studies have consistently shown that environmental changes may play a more significant role (Eysker, 1981; Gibbs, 1986).

Studies of inhibited development in H. contortus in the tropical and sub-tropical regions of the world are very limited and show variable results. For example, no hypobiosis was detected in Egypt (El-Azazy 1990) or Brazil (Charles, 1989) while low levels of hypobiosis were found in Zimbabwe (Pandey, 1990; Pandey et al., 1994), South Africa (Boomker et al., 1989; Horak et al., 1991), Malaysia (Ikeme et al., 1987) and Nigeria (Chiejina et al., 1988). In contrast, high levels of hypobiosis were reported in Nigeria (van Geldorp and Schillhorn, 1976; Ogunsusi and Eysker, 1979). It is widely believed that the dry desiccating conditions in these areas act as the stimulus that triggers hypobiosis (Giangaspero et al., 1992).

No detailed studies on hypobiosis have been conducted in the East African region, although Allonby and Urquhart (1975) briefly reported a low level of inhibition in a Merino flock in their study of pathogenic significance of haemonchosis. Experience by farmers and veterinarians in marginal areas of Kenya has shown that acute haemonchosis occurs in sheep and goats (especially the younger stock) immediately after the onset of the "long rains". This occurs so quickly that the source of the acute infection is unlikely to be fresh infection from the pastures. There have been instances when the syndrome has been reported during the dry season even before the onset of the rains. Consequently, the source of this disease has been attributed to the resumption of development of arrested larvae, although this has not been confirmed. The objective of this study was therefore to determine whether arrested development occurs in H. contortus in marginal areas of Kenya and, if so, to evaluate its epidemiological significance.

MATERIALS AND METHODS

Data collected:

As described in Chapter 2, data on hypobiotic and mature worms were collected every month from the permanent animals and tracers grazing in each of the three flocks. In this chapter, these data are examined in relation to seasonal fluctuations in rainfall in order to determine if the level of hypobiosis was significantly influenced by these changes. The data are further examined to find out if strategic treatment with ivermectin had an impact on the seasonal patterns of hypobiosis.

Statistical analyses:

In order to determine the season when hypobiosis was most prevalent, monthly levels of hypobiosis in the 4 classes of animals (ewes, lambs, does and kids) were compared between the wet and the dry months using Wilcoxon-Mann

Whitney U-test (SAS: Schlotzhauer and Littell, 1991) at $p < 0.05$ level of significance. Photographs of pasture vegetation cover were taken every month and based on the state of pasture (lush, dry or bare) and the corresponding amount of rainfall, wet months were defined as those with cumulative rainfall of 50 mm or more distributed over at least 25% of the month; all other months were considered dry. Dew only occurred during the rainy season and lasted for about four hours from daybreak. In addition, in order to determine the relationship between hypobiosis and the amount of rainfall and its duration, levels of hypobiosis were regressed on total amount of rainfall per month and on the number of days of rain. To determine the effect of treatment on hypobiosis, monthly mean numbers of hypobiotic larvae per class of animals were compared among the three flocks using one-way analysis of variance. Finally, levels of hypobiosis in permanent lambs and kids were compared with the levels in the respective tracers within each flock in order to find out if immunity influenced the level of hypobiosis.

RESULTS

Weather data:

The average annual rainfall recorded over the period of this study (676.0 mm) was higher than the long-term average for this area (589.6 mm).

Compared with the average distribution pattern (Fig. 3.1), the rainfall distribution over the period of the study (Fig. 3.2) also showed some marked changes. For example, there was an unexpected heavy rainfall in Dec 1992-Jan 1993, with a total of 243.6 mm (about 40% of annual total). Normally, this is a dry season in this area. Table 3.1 shows the classification of the period of study into wet and dry months and Figs. 3.3- 3.6 show the condition of the pastures during the long, short, dry and the unexpected rain periods.

The minimum and maximum temperatures were fairly constant (Fig. 3. 2) with means of 13^0C and 23^0C respectively. Relative humidity was measured from the air and ranged between 46% and 81%.

Nematode species found:

Results of the adult worm counts in permanent animals indicated that H. contortus was both the most prevalent (90-95% - Fig. 3.7) and the most abundant (80-85% - Fig. 3.8) nematode. Other major nematode species identified were Trichostrongylus spp. and Oesophagostomum spp. Occasionally, Strongyloides spp. and Trichuris spp. were encountered.

Recovery of hypobiotic worms:

About 90% of hypobiotic worms in this study were recovered from the abomasal contents and washings in all animal classes. This observation was consistent in the three flocks.

Adult and hypobiotic worms in flock C:

The monthly profiles of adult and hypobiotic Haemonchus contortus worm burdens in both the tracers and the permanent animals are presented in Figs. 3.9-3.14. For flock C, there was tendency for hypobiotic worms to predominate over the adult worms during the dry months (Fig. 3.14). The reverse was true during the wet months. One exception to this trend occurred in Dec 92-Jan 93 when, despite the high rainfall, the level of hypobiosis remained high. Statistical comparison of the levels of hypobiosis between the two seasons (Table 3.2) in flock C revealed significant differences in the ewes and kids but not in the lambs and does. Percent hypobiosis in the tracers and the corresponding permanent animals (Fig. 3.17) were comparable. Regression of hypobiosis on the cumulative monthly rainfall (Fig. 3.15) and the number of days of rain (Fig. 3.16) yielded a negative relationship with low correlation coefficients in both cases.

Effects of treatment on hypobiotic and adult worms in Flocks A and B:

In Flock A, treatment significantly reduced the number of hypobiotic worms as compared to treatment in Flocks B and C. Examination of the trend of adult and hypobiotic worms in the two flocks (Figs. 3.10 & 3.12) revealed that treatment did not change the seasonal pattern but significantly ($p<0.05$) influenced the actual numbers of both parasite stages (Tables 3.3 & 3.4). Percent hypobiosis in the tracers and the corresponding permanent animals (Fig. 3.17), in the three flocks, were comparable.

DISCUSSION

This discussion is focused on the findings of an investigation of the epidemiology of <u>Haemonchus</u> <u>contortus</u> in naturally infected sheep and goats grazing on an unimproved pasture in a semi-arid climatic region of Kenya. <u>H. contortus</u> was the most common nematode in helminth infections in small ruminants raised under traditional husbandry practices in this area. Several authors (Allonby and Urquhart, 1975; Devendra, 1981) have emphasised the significance of this parasite in sheep and goat production in tropical and sub-tropical regions of the world and this study supports this view (see also Chapter 5).

The standard procedure for the recovery of hypobiotic worms is by artificial digestion of abomasal mucosa in a pepsin-HCl medium. Results of this study revealed that about 90% of the hypobiotic worms were found in the abomasal contents and washings and only about 10% were found in the mucosal digest. This suggested that hypobiotic worms of H. contortus might be loosely attached to the abomasal mucosa or simply suspended in the luminal digesta as suggested by Blitz and Gibbs (1971a), and not deeply imbedded in the underlying abomasal tissue as reported by de Chaneet and Mayberry (1978). Recently, Eysker and Kooyman (1993) also demonstrated large numbers of hypobiotic H. contortus in the abomasal contents and washings. From these observations, it was clear that majority of hypobiotic worms of H. contortus could be missed if examination was confined to the mucosal digest alone. It is probable that this could be the underlying reason behind reports of low levels or absence of hypobiosis in some tropical regions (Charles, 1989; Pandey, 1990; El-Azazy, 1990).

Results from this study indicated that adult and hypobiotic worm populations co-exist in proportions that varied with seasons: adult worms increased during the wet seasons and decreased during the dry seasons whereas the hypobiotic ones decreased during the wet seasons and increased during the dry seasons. This is in agreement with the results of Pandey (1994) in a study of goats raised in the highveld regions of Zimbabwe. Analysis of the dynamics of the seasonal levels of hypobiosis in the present study revealed a positive association with dry conditions, suggesting that hypobiosis is necessary for the survival of the parasite under the harsh conditions inherent in dry seasons. The fact that the level of hypobiosis remained unchanged during an aberrant heavy rain spell implies that the stimulus to resume development is not simply a matter of the presence or the absence of rain. It was suggestive of a built-in process in the parasite, that appropriately responds to weather changes in a pre-programmed way. Similar conclusions were made by Blitz and Gibbs (1971b), Connan (1978) and Williams et al. (1983) in different experimental designs. For example, Connan (1978) exposed two groups of sheep to H. contortus larvae on pasture two months apart. Resumption of development occurred simultaneously in both groups irrespective of time of exposure.

Although treatment did not significantly influence the seasonal pattern of adult and hypobiotic worms in either flock A or B compared with C, the actual numbers of both parasite stages were significantly reduced in flock A but not in flock B. Except for the temporary reductions in adult and hypobiotic worms following treatments, the numbers of adult and hypobiotic stages in flock B were similar to those in flock C. Treatment in flock B therefore, did not significantly affect the number of hypobiotic larvae presumably because, by the time it was administered, the larvae had already resumed development and matured into adult worms. Overall, judging from the level of faecal egg output, treatment administered before the rains in flock A was more advantageous than that administered in flock B after the onset of the rains.

Hypobiosis in Haemonchus contortus appears to occurs in two modes. The first mode, commonly found in the temperate regions of the world, is where the parasite survives the winter exclusively in the hypobiotic form inside the host (Connan, 1971; Blitz and Gibbs, 1972; Waller, 1975). This mode has also been reported in the tropical region of West African Savannah in the Gambia where H. contortus in N'Dama cattle was found to survive the dry seasons exclusively as hypobiotic worms (Kaufmann and Pfister, 1990). The other mode, mainly encountered in tropical regions, is where the adult parasite in the host co-exists with the hypobiotic worms (Schillhorn van Veen, 1978; Ogunsusi and Eysker, 1979; Vercruysse, 1985; Pandey et al., 1994). These reports, including the results of this study, suggest that hypobiosis may not be absolutely necessary for the survival of H. contortus in sheep and goats in these areas. Although its epidemiological role may not be as indispensable as it is in the temperate regions, the fact that hypobiosis was significantly more predominant during the dry season than the wet season, indicates that it plays an important role in the survival of the parasite during dry periods.

The role of the concurrent adult worm population together with hypobiotic worms remains to be defined. Vercruysse (1985) suggested that the epidemiological significance of hypobiosis was to reduce the numbers of adult worms below the threshold required to stimulate immune expulsion and therefore, by implication, not all worms need become hypobiotic. If, according to this explanation, hypobiosis is driven by the number of adult worms present, then maximum hypobiosis should be expected at time of maximum transmission. This is contrary to the observations of the current study and other studies in the tropics (Kaufmann and Pfister, 1990; Pandey et al., 1994) where hypobiosis has been found to be minimal during the time of optimal transmission.

Some reports (Allonby and Urquhart, 1975) have suggested that hypobiosis may not be important in the epidemiology of H. contortus in the tropics where bimodal rainfall patterns are common. The argument has been that the period between the "short" and the "long" rains seasons is too short to warrant the parasite to go into the hypobiotic state. Results in this study showed that, at the advent of long rains, the level of hypobiosis was significantly reduced compared to the level that existed in the preceding dry spell. However, similar changes were not observed at the advent of the short rains and the reason for this observation may be attributed to differences between the two rain seasons. One of the most important differences between the long and the short rains is the distribution of rainfall: during the long rains, the amount of rain is spread out over about 25% of the month whereas during the short rains, it is distributed over only about 10% of the month. Moreover, in this area, there are 4 months of long rains and only 2 months of short rains. Characteristically, during the short rains season, rain falls in brief but heavy bouts followed by dry spells. Consequently, conditions during the short rains seasons effectively remain stressful for the survival of the parasite in spite of the presence of some rain. It is therefore suggested that, in influencing the epidemiology of H. contortus in this area, distribution of rainfall is a more important factor than total cumulative rainfall.

One of the most important effects of poor rainfall distribution is its influence in determining the vegetation growth. Although total cumulative rainfall may be high during the short rains, its impact on herbage growth is negligible because of it poor distribution. And because vegetation cover is critical for nematode transmission, the poor herbage growth during the short rains is not conducive for this transmission. Consequently, the parasite remains in a hypobiotic state. Such a situation was particularly evident in Dec 1992-Jan 1993 when unexpected high rainfall did not lead to an increase in the worm burden and the high level of inhibition remained unchanged. The negligible worm burdens acquired by the tracers during the short rains further confirmed that conditions in the pastures were not suitable for larval survival.

Fig.3.18 shows that the level of hypobiosis for the tracers and the permanent lambs and kids was similar, suggesting that immunity did not significantly influence the level of hypobiosis in this area.

Table 3.1 Rainfall distribution data recorded during the period of study showing wet and dry months according to the criterion of 8 days of rain with cumulative rainfall of 50 mm and compared to classification by seasons.

Month	Season	Cumulative rainfall per month (mm)	Number of days of rain per month	% Days of rain per month	Month Wet/Dry
Aug 91	D	22.4	6	20	D
Sept	D	47.4	6	20	D
Oct	SR	39.4	7	23.3	D
Nov	SR	14.1	3	10	D
Dec	D	10.5	4	13.3	D
Jan 92	D	0	0	0	D
Feb	D	0	0	0	D
Mar	LR	142.3	14	46.7	W
Apr	LR	117.9	12	40	W
May	LR	119.5	11	36.7	W
Jun	LR	51.3	9	30	W
Jul	D	102.4	10	33.3	W
Aug	D	8.5	2	6.7	D
Sept	D	40.4	3	10	D
Oct	SR	38.1	4	13.3	D
Nov	SR	23.2	2	6.7	D
Dec	D	84.2	9	30	W
Jan 93	D	159.4	8	26.7	W
Feb	D	30.7	3	10	D
Mar	LR	8.7	3	10	D
Apr	LR	62.8	8	26.7	W
May	LR	33.7	9	30	D
Jun	LR	138.7	10	33.3	W

Legend:
D=Dry LR=Long rains SR=Short rains W=Wet

Table 3.2. Comparison of the degree of inhibition (%) in flock C between wet (W: n = 9) and dry (D: n = 13) months among the 4 classes of animals (Wilcoxon-Mann Whitney U-test).

Animal class	Season	Mean%±S.E	p
Lambs	W	26.2±8.4	0.1107
	D	45.3±6.5	
Kids	W	22.0±7.8	**0.0126***
	D	47.4±6.5	
Ewes	W	19.4±7.1	**0.0048***
	D	54.2±5.3	
Does	W	26.3±9.1	0.0849
	D	48.2±7.7	

* Significantly different at $p<0.05$

Table 3.3. Comparison of monthly mean populations of hypobiotic larvae recovered per class of animals in the three flocks.

	Ewes	Lambs	Does	Kids
Flock	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
A	263.2±44.4^{a}	101.1±19.1^{a}	252.6±40.8^{a}	126.0±25.3^{a}
B	1240.9±252.4^{b}	765.91±79.8^{b}	638.6±150.4^{b}	604.5±170.6^{b}
C	1081.1±275.2^{b}	1127.3±479.4^{b}	575.0±133.9^{b}	1022.7±275.4^{b}

Different superscripts indicate significant differences at $p<0.05$.

Table 3.4. Comparison of monthly mean populations of adult worms recovered per class of animals in the three flocks.

	Ewes	Lambs	Does	Kids
Flock	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
A	245.6 ± 21.0^{a}	112.6 ± 21.7^{a}	186.5 ± 28.4^{a}	143.6 ± 20.1^{a}
B	972.7 ± 184.9^{b}	631.8 ± 168.6^{b}	1286.4 ± 275.6^{b}	1038.6 ± 169.4^{b}
C	1035.2 ± 132.3^{b}	1479.5 ± 213.8^{b}	1025.0 ± 185.6^{b}	1272.7 ± 155.1^{b}

Different superscripts indicate significant differences at $p<0.05$.

Fig. 3.1. Long-term rainfall distribution in Naivasha calculated from 15-year rainfall record kept by the National Animal Husbandry Research Centre - Kenya Agricultural Research Institute, Naivasha.

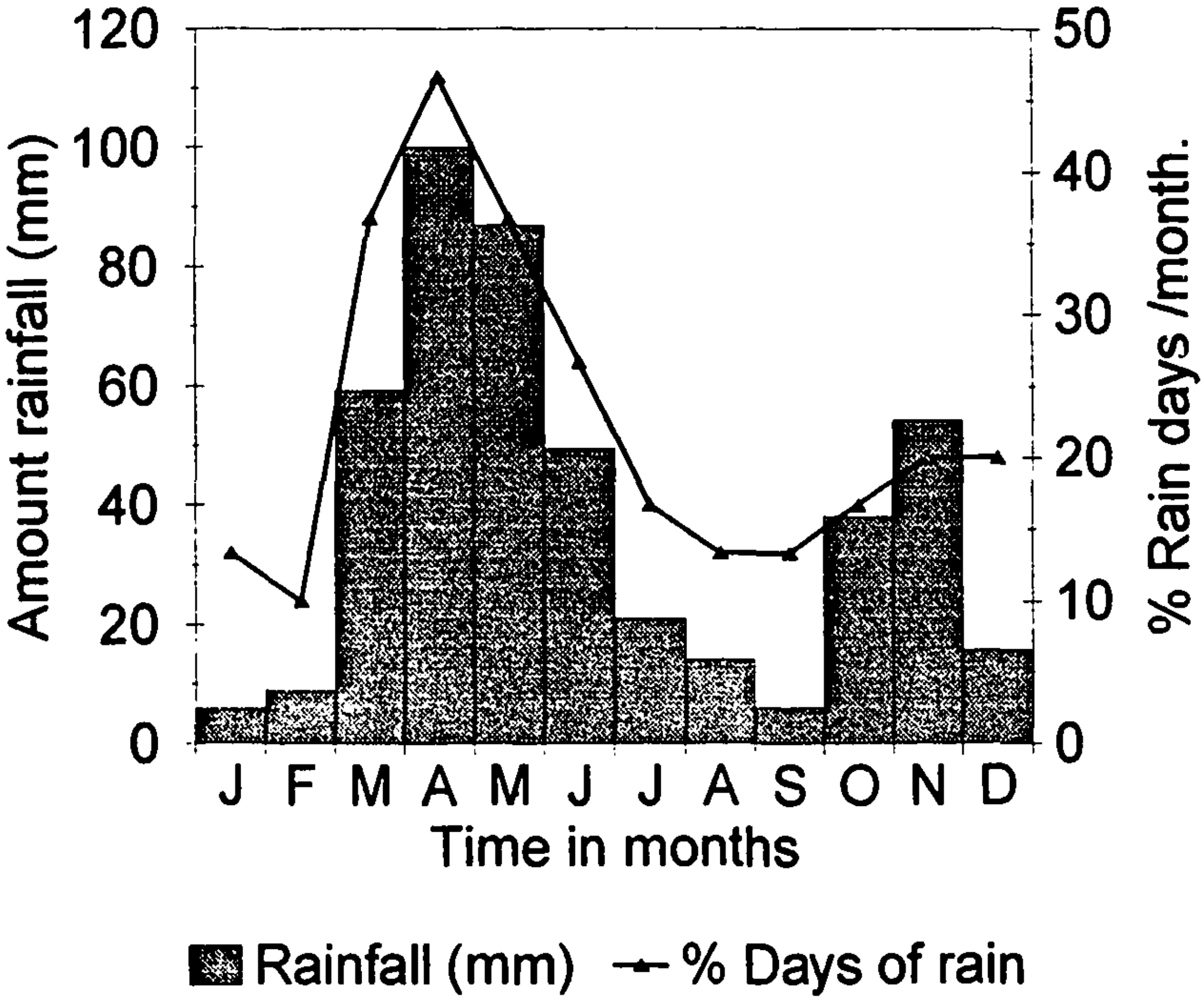
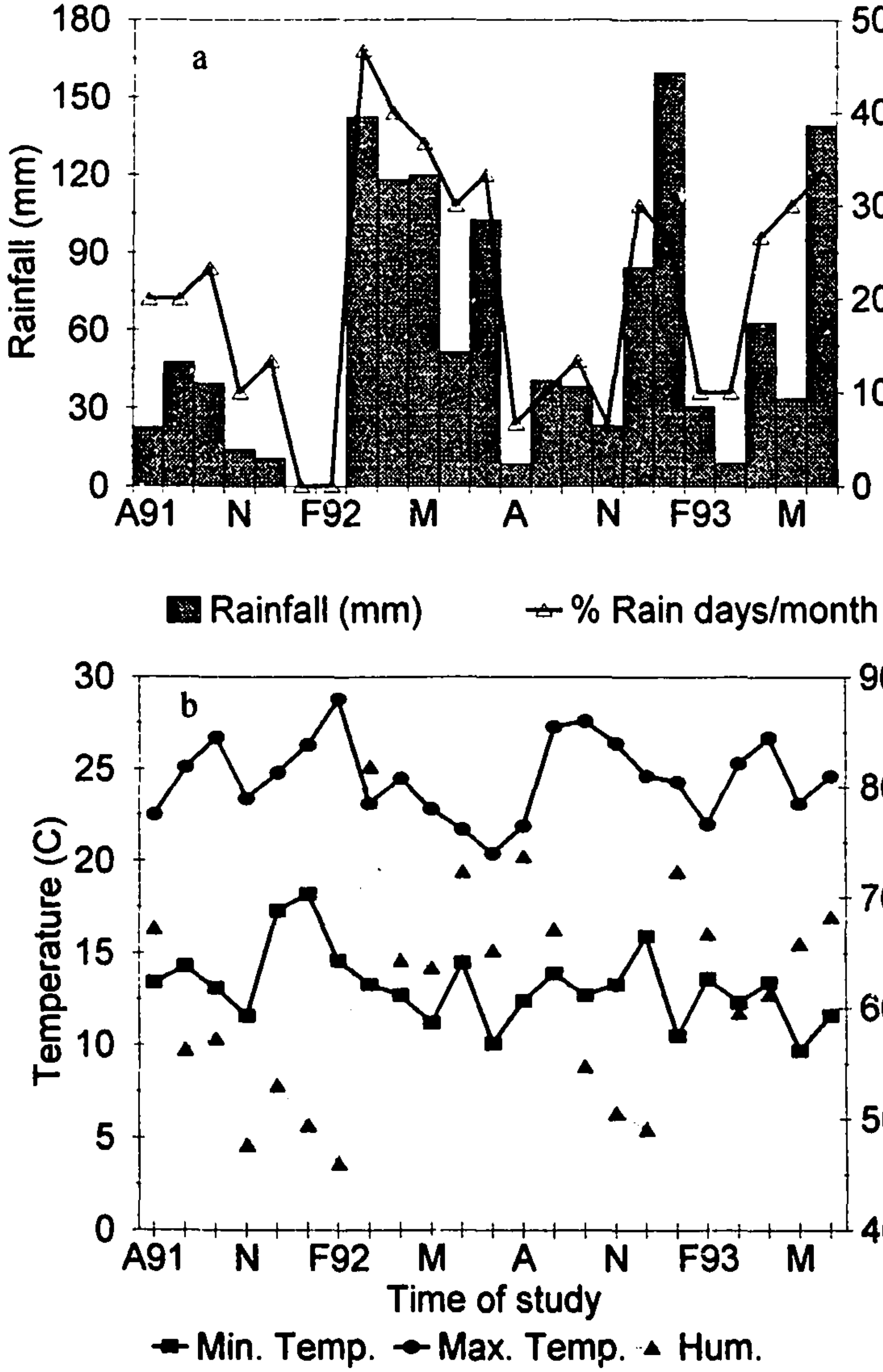

120
100
80
60
40
20
0
Amount rainfall (mm)
50
40
30
20
10
0
% Rain days /month.
J F M A M J J A S O N D
Time in months
Rainfall (mm)
% Days of rain

Fig. 3.2. Rainfall distribution (a), temperature and humidity (b) recorded during the period of this study. The percentage of rain days per month represents an index of rainfall distribution.



a
Rainfall (mm)
% Rain days/month
180
150
120
90
60
30
0
50
40
30
20
10
0
A91 N F92 M A N F93 M
Rainfall (mm)
% Rain days/month
b
Temperature (C)
Humidity (%)
30
25
20
15
10
5
0
90
80
70
60
50
40
A91 N F92 M A N F93 M
Time of study
Min. Temp.
Max. Temp.
Hum.

Fig. 3.3. Herbage cover during the short rains. The emerging green sprouts (early bites) are evident but the ground is virtually bare. This photograph was taken in November 1992, a month that was classified as a dry despite being within the short rains season.

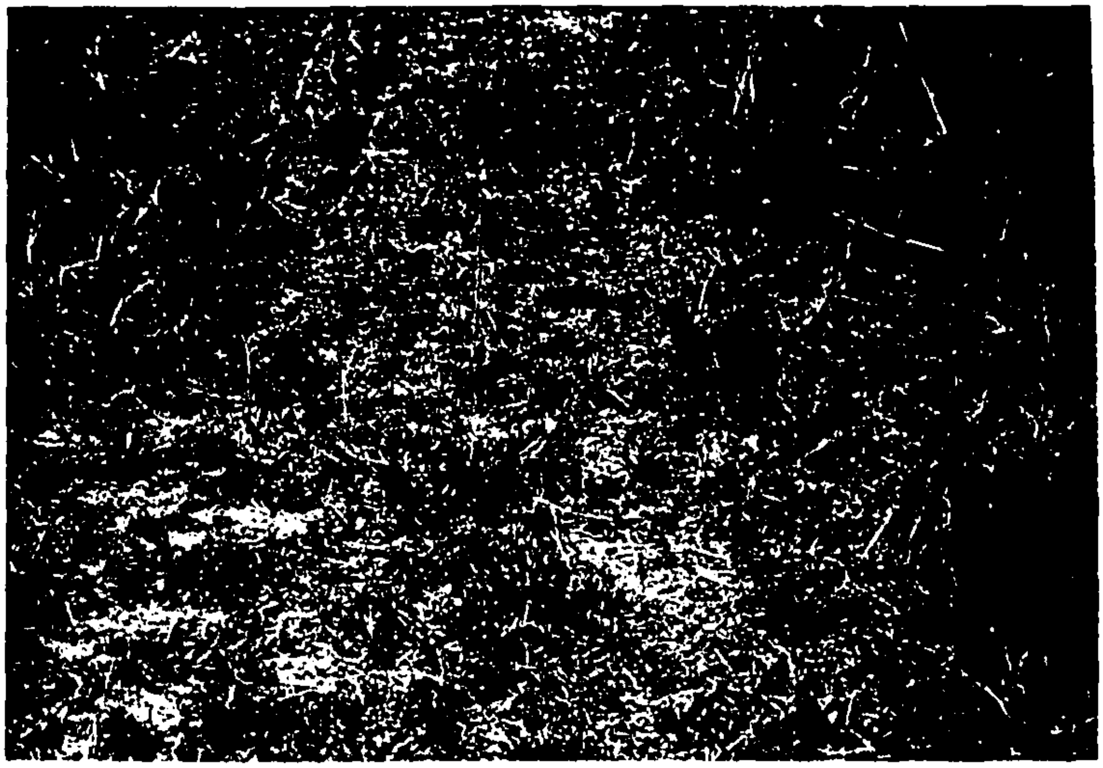


Fig. 3.4. The thick herbage cover that develops during the long rains. The photograph was taken in April 1992, a month classified as wet.

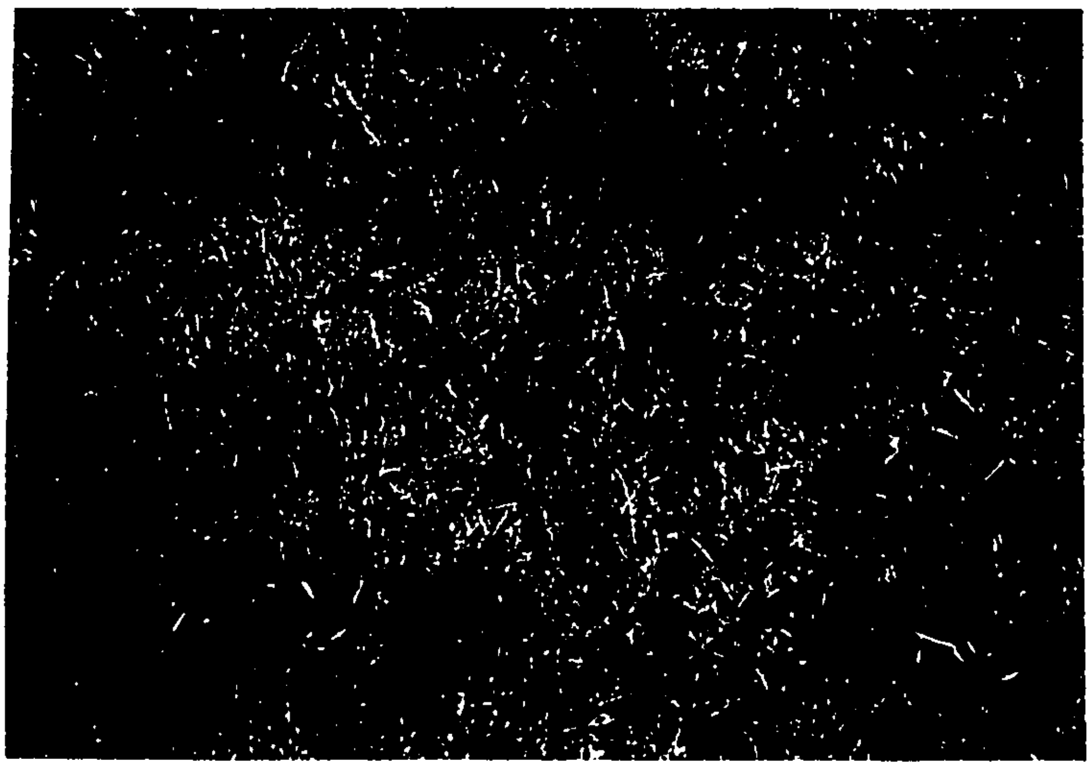


Fig. 3.5. The state of pastures during the dry season. The ground is typically bare; animals rely on "standing hay" for grazing at this time of the year. The photograph was taken in January 1992, a dry month.

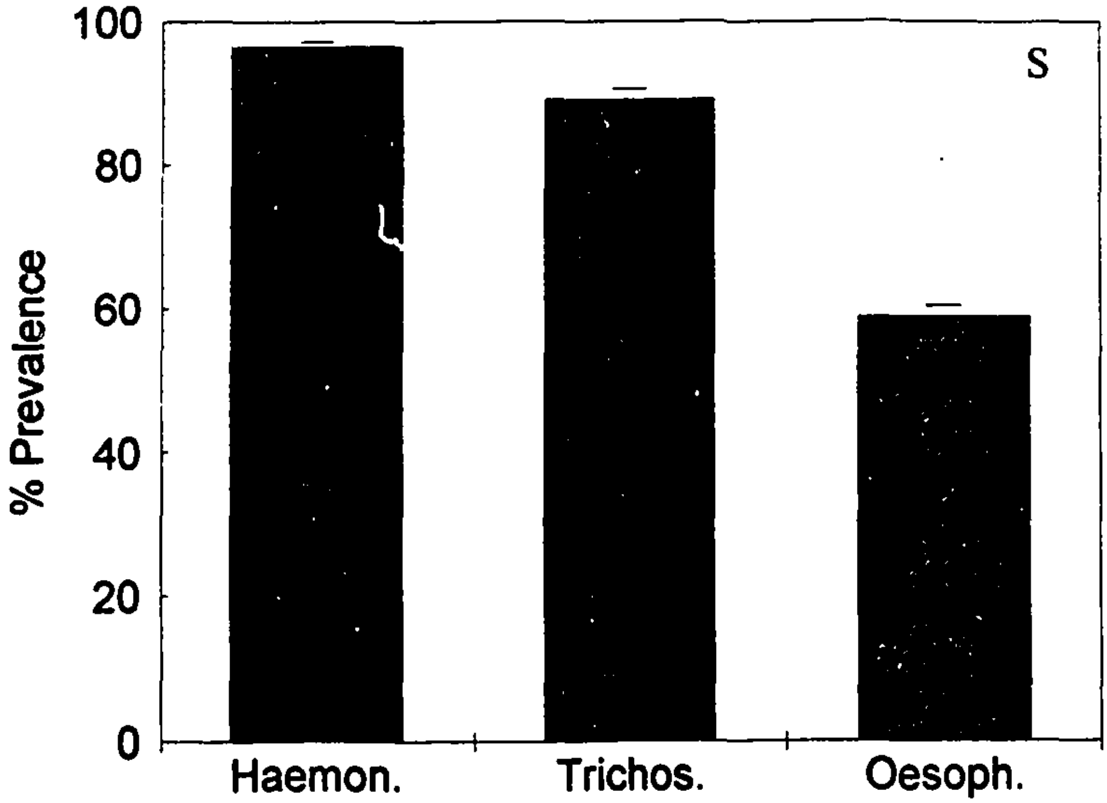
Fig. 3.6. The sprouting herbage during the unexpected heavy rainfall in Dec. 92/ Jan. 93, a period that is normally within a dry season; this year, the two months were classified as wet.

Fig. 3.7. Average prevalence of the major trichostrongylid nematode species in sheep (S) and goats (G). As part of baseline epidemiological information, these data were generated from the permanent control flock (Flock C). The error bars represent monthly variation over the 22 months of the study.

Haemon. = *Haemonchus* spp.

Trichos. = *Trichostrongylus* spp.

Oesoph. = *Oesophagostomum* spp.


S
% Prevalence
100
80
60
40
20
0
Haemon.
Trichos.
Oesoph.

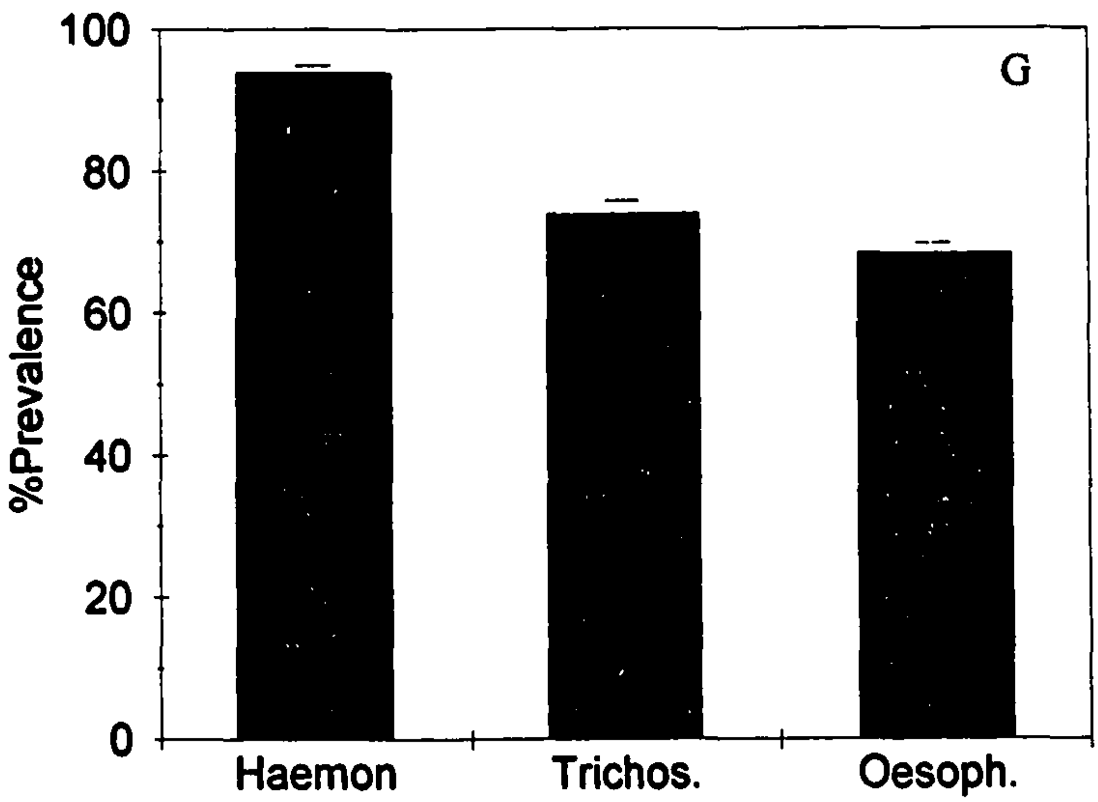
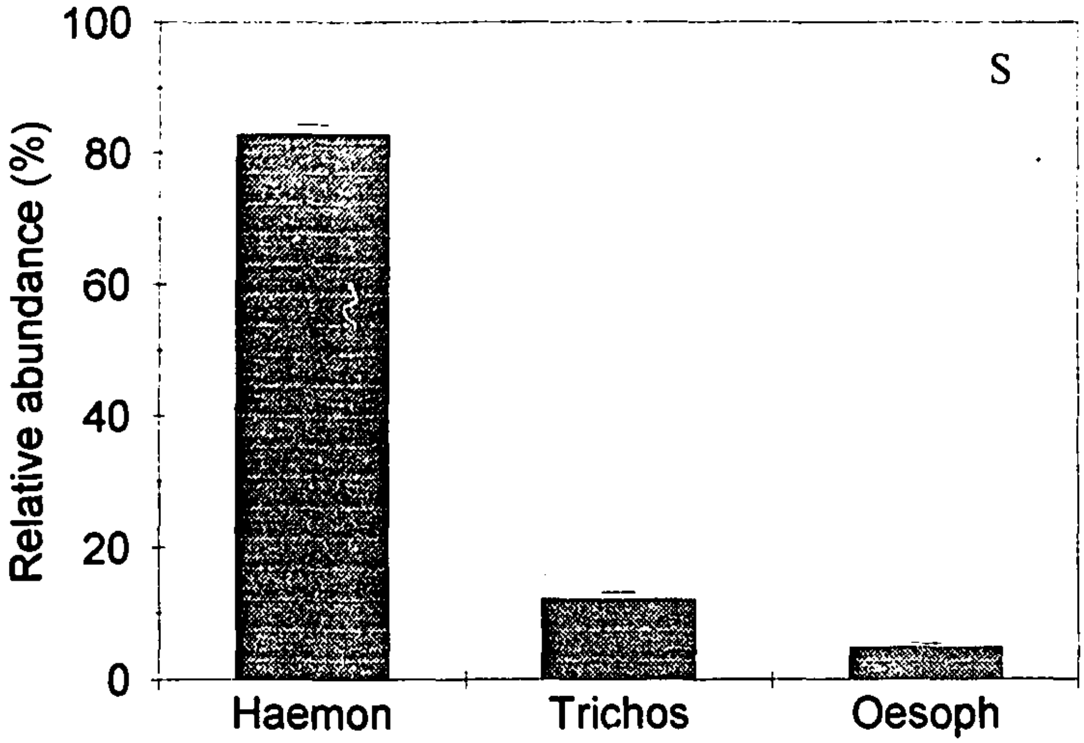

G
%Prevalence
100
80
60
40
20
0
Haemon
Trichos.
Oesoph.

Fig. 3.8. Relative abundance of the major trichostrongylid nematode species in sheep (S) and goats (G). These data were generated from the control flock as part of baseline epidemiological information. Error bars represent monthly variation over the 22 months of the study.

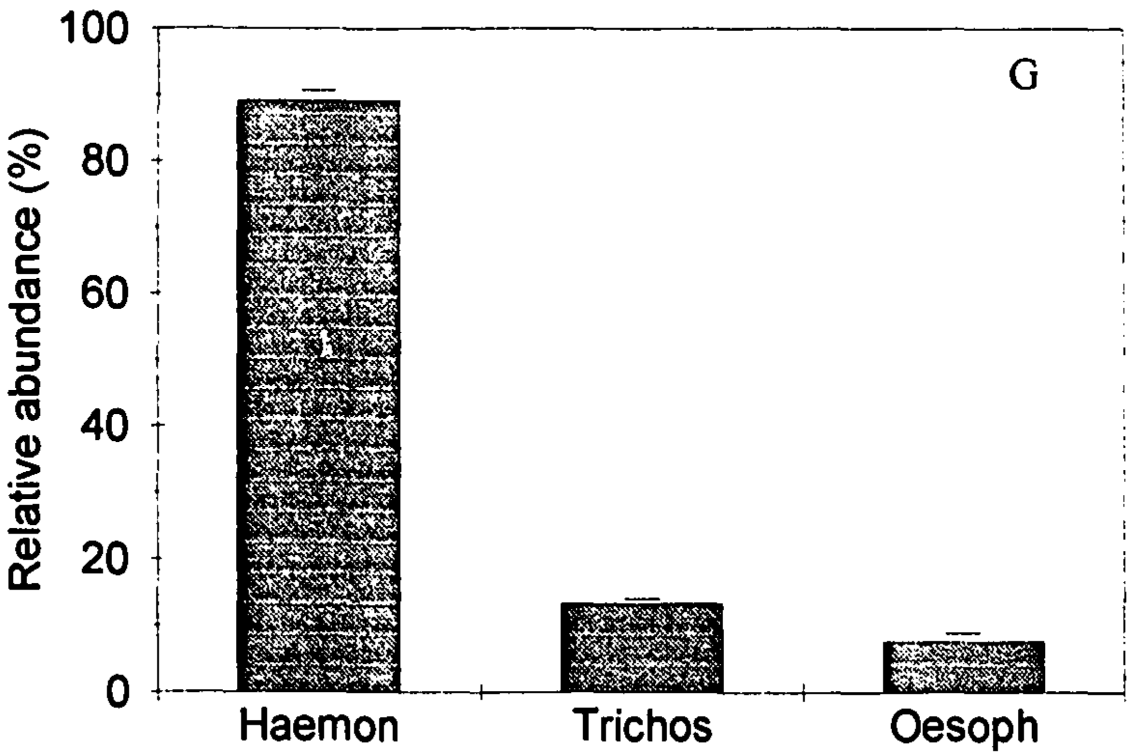
Haemon. = *Haemonchus* spp.

Trichos. = *Trichostrongylus* spp.

Oesoph. = *Oesophagostomum* spp.

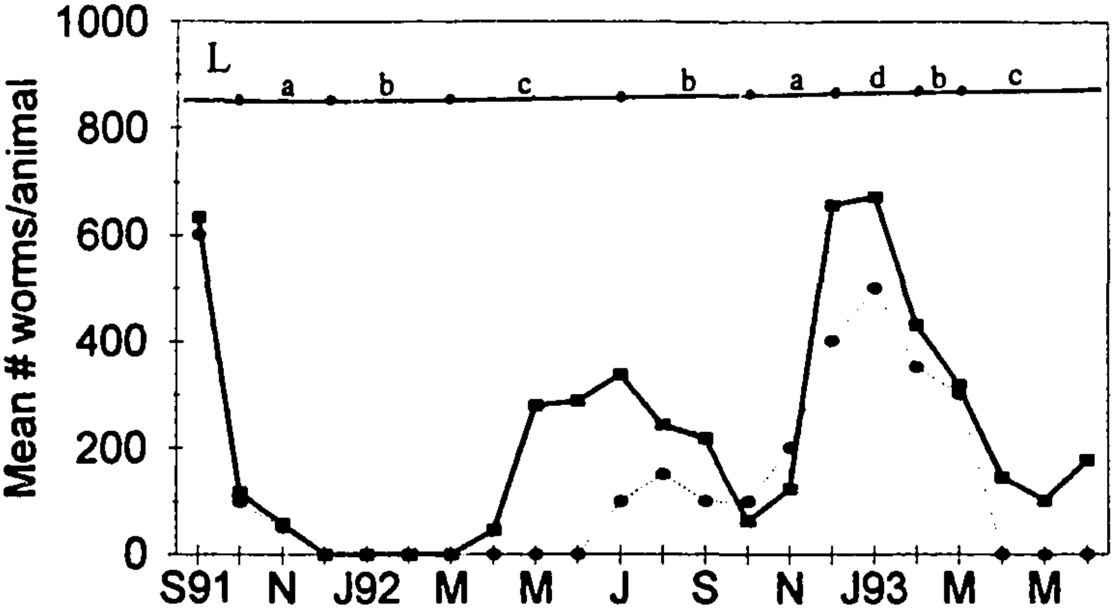


S
Relative abundance (%)
100
80
60
40
20
0
Haemon
Trichos
Oesoph

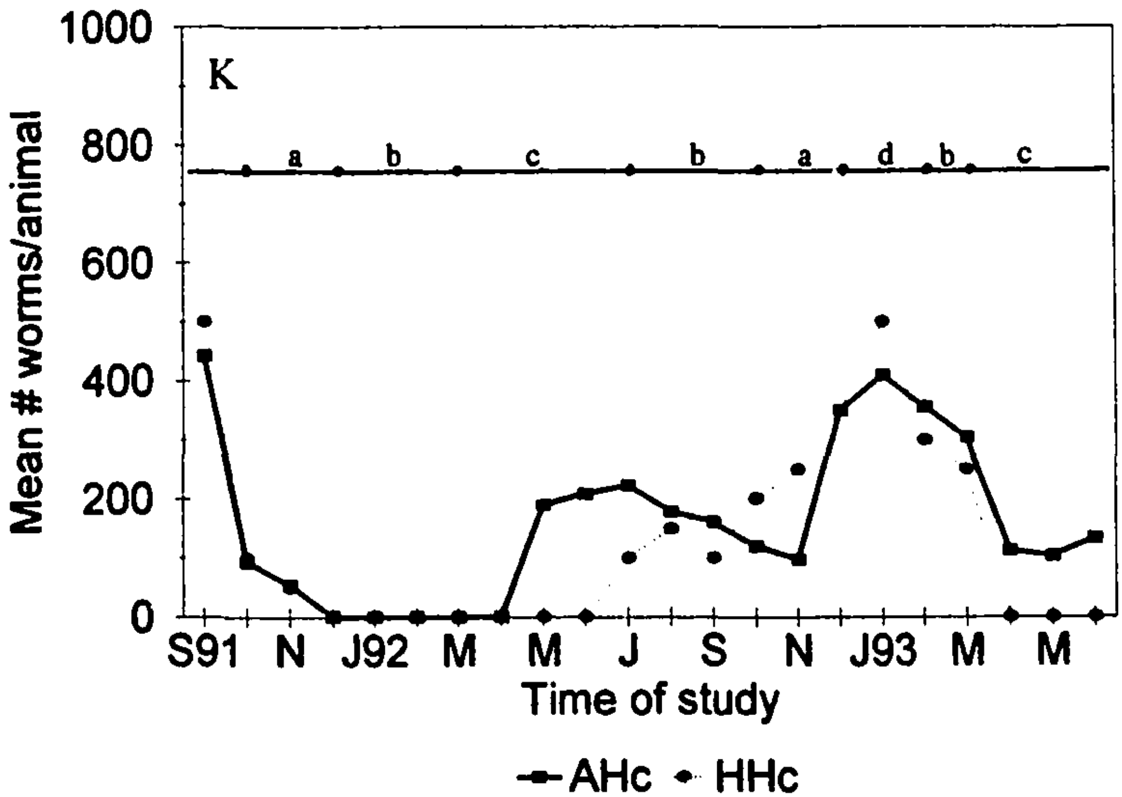


G
Relative abundance (%)
100
80
60
40
20
0
Haemon
Trichos
Oesoph

Fig. 3.9. Seasonal pattern of adult (AHc) and hypobiotic (HHc) Haemonchus contortus worm burdens in tracer lambs (L) and tracer kids (K) in Flock A. Each data point is an average of two animals. Labels a, b and c represent the three seasons: the short rains, the dry period and the long rains respectively; d represents the unexpected rain in a dry period.

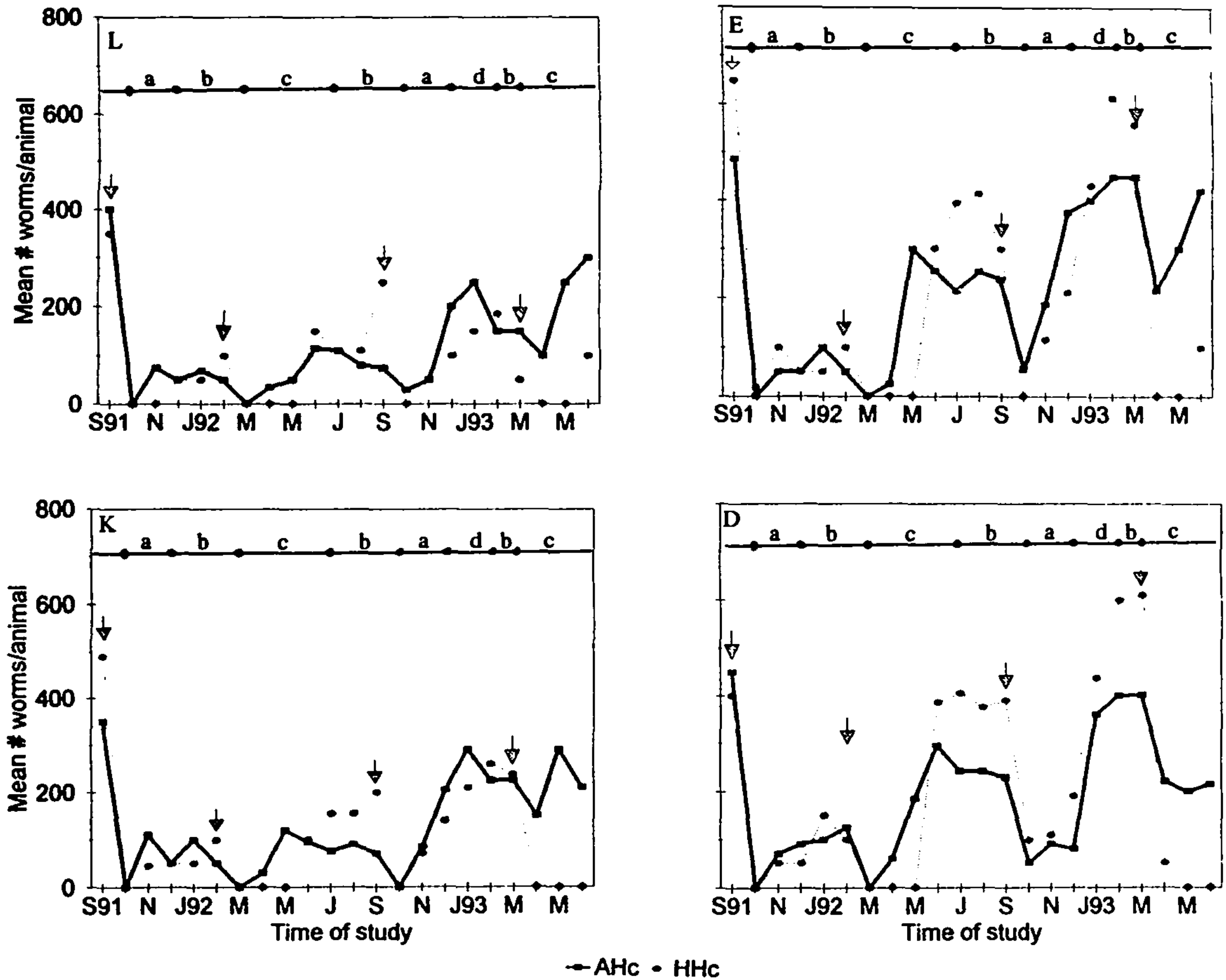


1000
800
600
400
200
0
Mean # worms/animal
L
a b c b a d b c
S91 N J92 M M J S N J93 M M



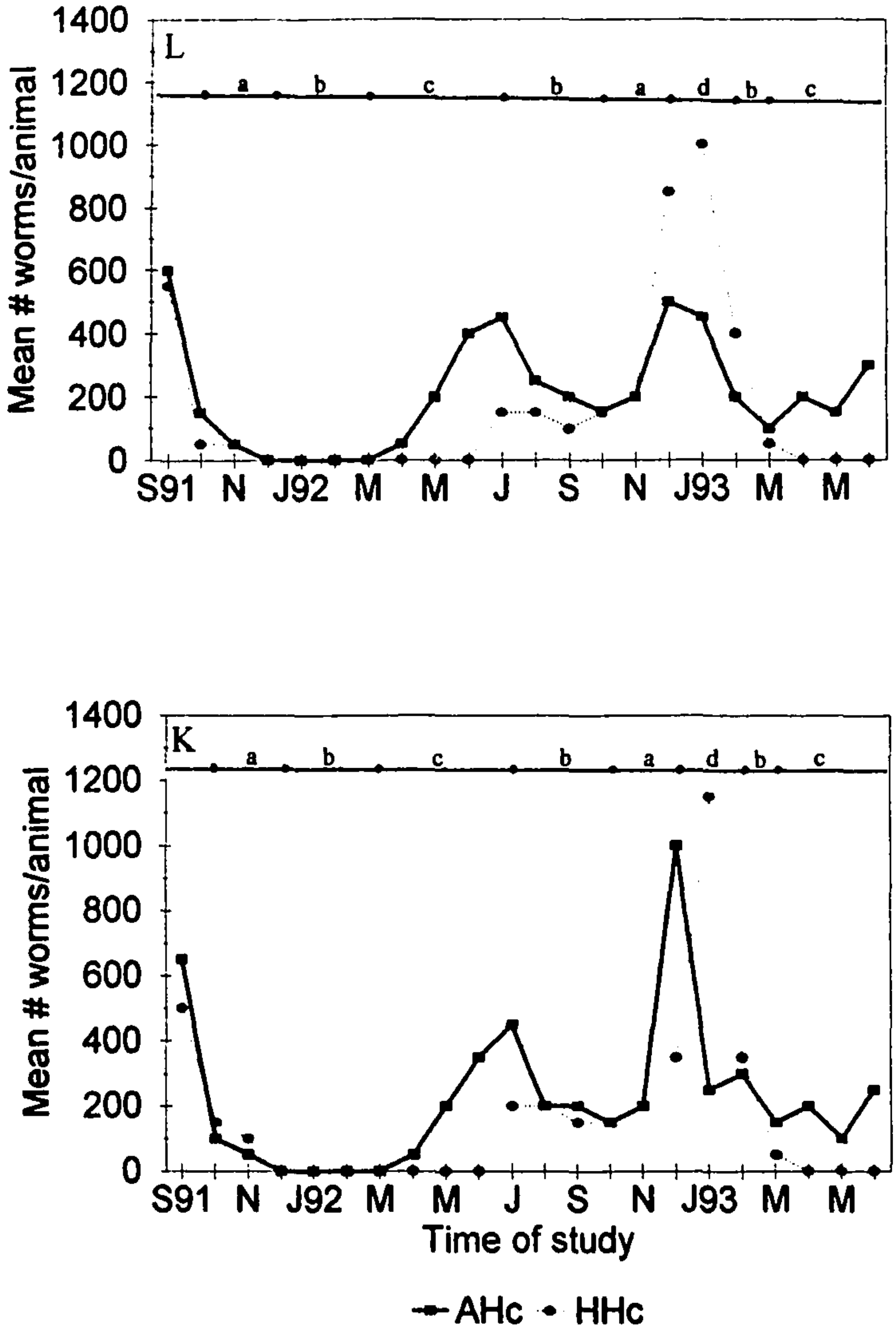
1000
800
600
400
200
0
Mean # worms/animal
K
a b c b a d b c
S91 N J92 M M J S N J93 M M
Time of study
AHc HHc

Fig. 3.10. Seasonal pattern of adult (AHc) and hypobiotic (HHc) Haemonchus contortus worm burdens in permanent stock - lambs (L), kids (K), ewes (E) and does (D) in Flock A. Each data point represents the mean of 2 animals in lambs and kids and one animal in ewes and does. Arrows indicate the time of treatment. Labels a, b and c represent the three seasons: the short rains, the dry period and the long rains respectively; d represents the unexpected rain in a dry period.



L
E
K
D
a b c b a d b c
800
600
400
200
0
Mean # woms/animal
S91 N J92 M M J S N J93 M M
Time of study
AHc
HHc

Fig. 3.11. Seasonal pattern of adult (AHc) and hypobiotic (HHc) Haemonchus contortus worm burdens in tracer lambs (L) and tracer kids (K) in Flock B. Each data point is an average of 2 animals. Labels a, b and c represent the three seasons: the short rains, the dry period and the long rains respectively; d represents the unexpected rain in a dryperiod.



1400
1200
1000
800
600
400
200
0
Mean # worms/animal
L
a b c b a d b c
S91 N J92 M M J S N J93 M M
1400
1200
1000
800
600
400
200
0
Mean # worms/animal
K
a b c b a d b c
S91 N J92 M M J S N J93 M M
Time of study
AHc HHc

Fig. 3.12. Seasonal pattern of adult (AHc) and hypobiotic (HHc) Haemonchus contortus worm burdens in permanent stock - lambs (L), kids (K), ewes (E) and does (D) in Flock B. Each data point represents the mean of 2 animals in lambs and kids and one animal in ewes and does. Arrows indicate the time of treatment. Labels a, b and c represent the three seasons: the short rains, the dry period and the long rains respectively; d represents the unexpected rain in a dry period.

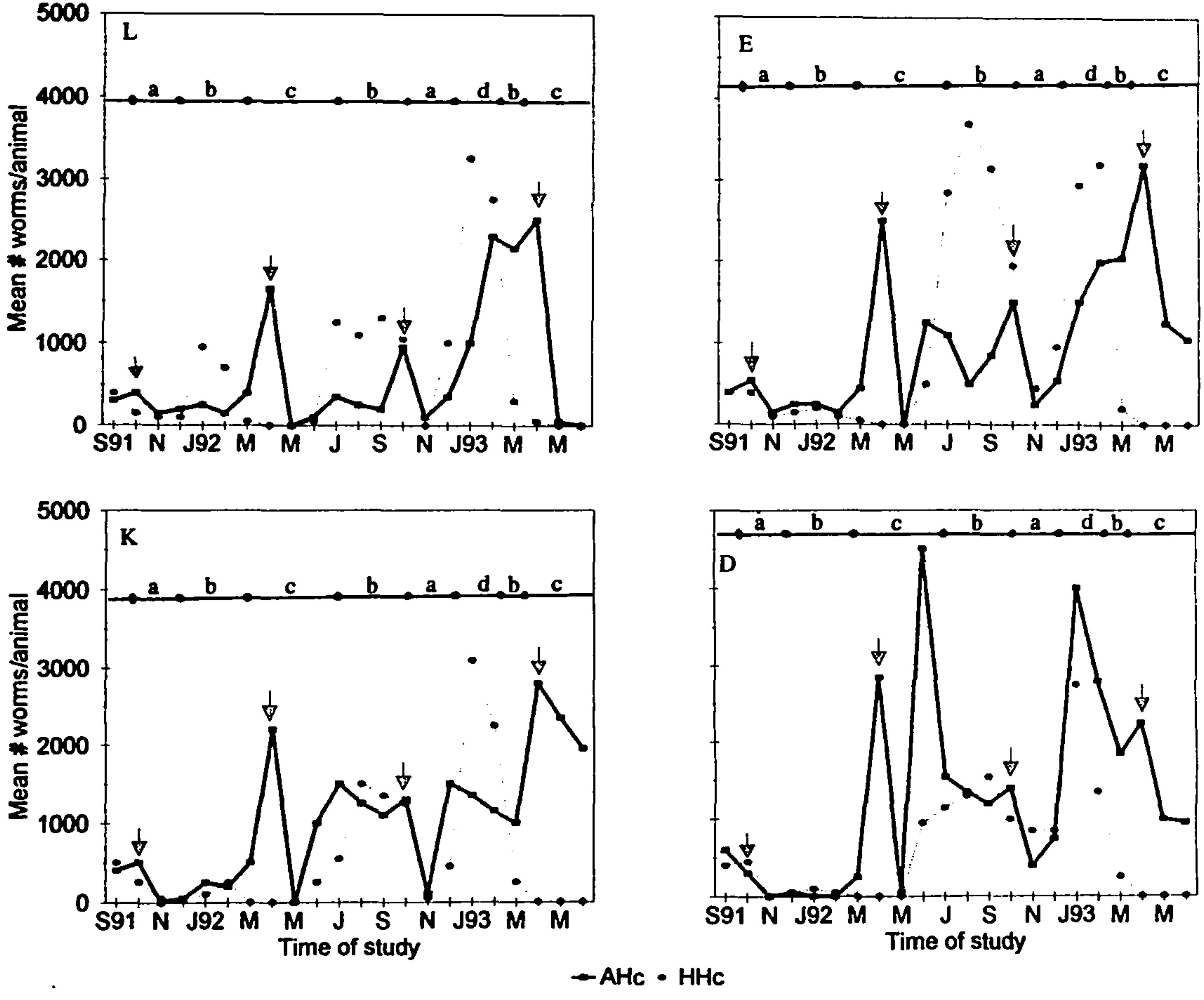

L
E
K
D
a
b
c
d
Mean # worms/animal
0
1000
2000
3000
4000
5000
S91
N
J92
M
M
J
S
N
J93
M
M
Time of study
AHc
HHc

Fig. 3.13. Seasonal pattern of adult (AHc) and hypobiotic (HHc) Haemonchus contortus worm burdens in tracer lambs (L) and tracer kids (K) in Flock C. Each data point is an average of two animals. Labels a, b and c represent the three seasons: the short rains, the dry period and the long rains respectively; d represents the unexpected rains in a dry period.

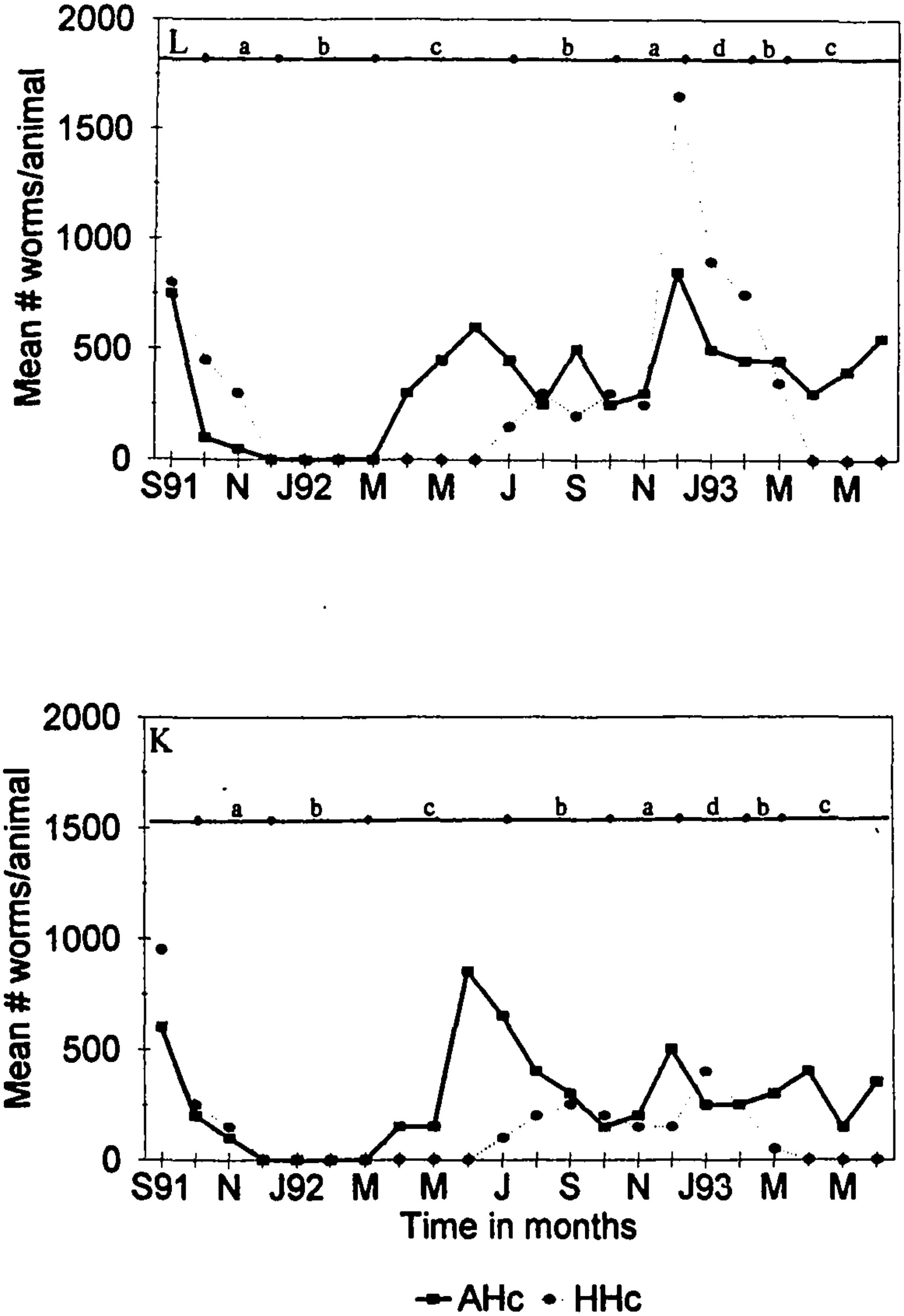
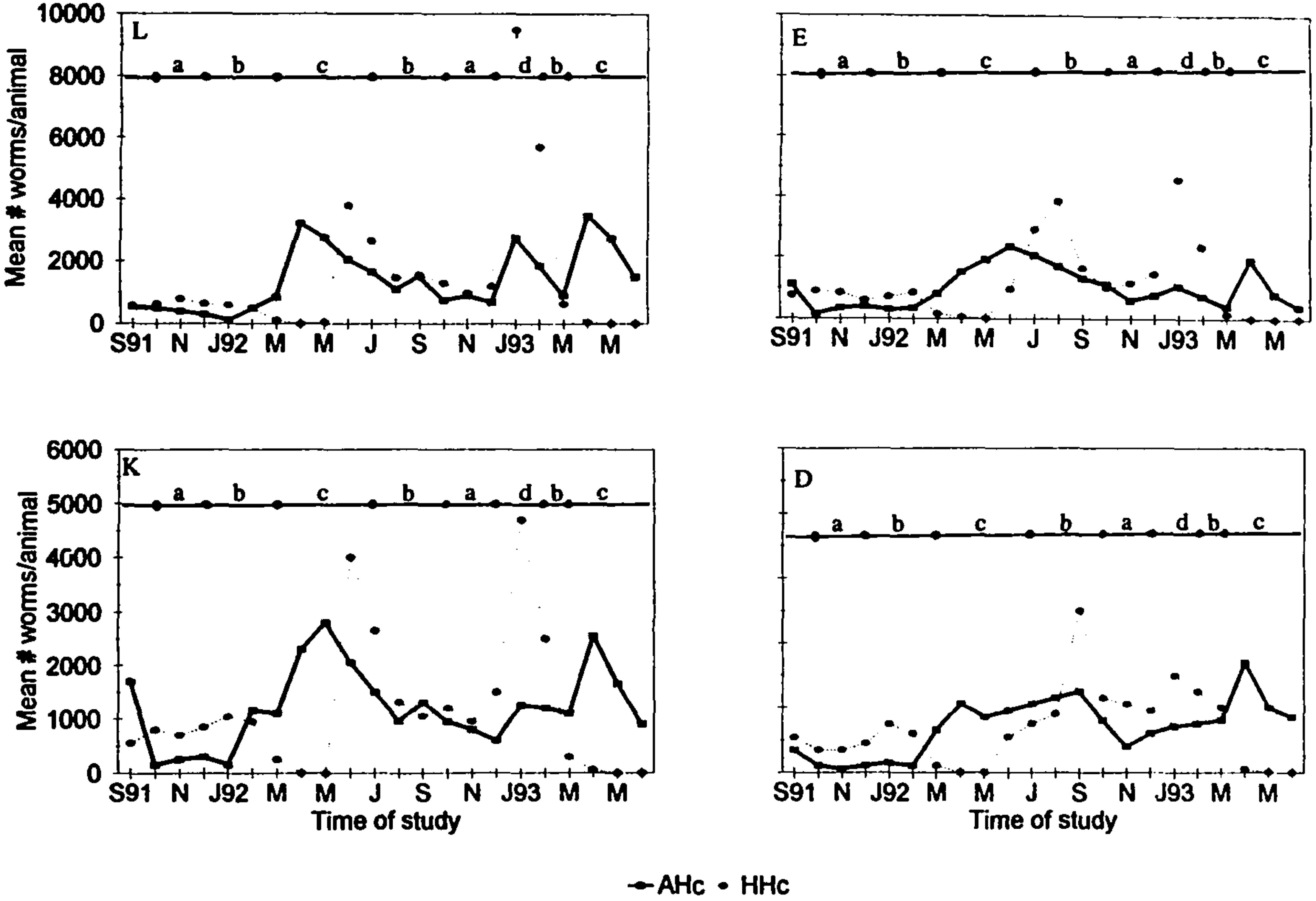

L
a b c b a d b c
2000
1500
1000
500
0
Mean # worms/animal
S91 N J92 M M J S N J93 M M
K
a b c b a d b c
2000
1500
1000
500
0
Mean # worms/animal
S91 N J92 M M J S N J93 M M
Time in months
AHc HHc

Fig. 3.14. Seasonal pattern of adult (AHc) and hypobiotic (HHc) Haemonchus contortus worm burdens in permanent stock - lambs (L), kids (K), ewes (E) and does (D) in Flock C. Each data point represents the mean of two animals in lambs and kids and one animal in ewes and does. Arrows indicate the time of treatment. Labels a, b and c represent the three seasons: the short rains, the dry period and the long rains respectively; d represents the unexpected rains in a dry period.



L
E
K
D
a b c b a d b c
10000
8000
6000
4000
2000
0
6000
5000
4000
3000
2000
1000
0
Mean # worms/animal
S91 N J92 M M J S N J93 M M
Time of study
AHc HHc

Fig. 3.15. Regression line showing the relationship between the level of hypobiosis and the total amount of rainfall per month. These data were generated from the permanent control flock (Flock C). Each data point is an average from 8 animals.

$R^2 = 0.0257$ $p = 0.4763$

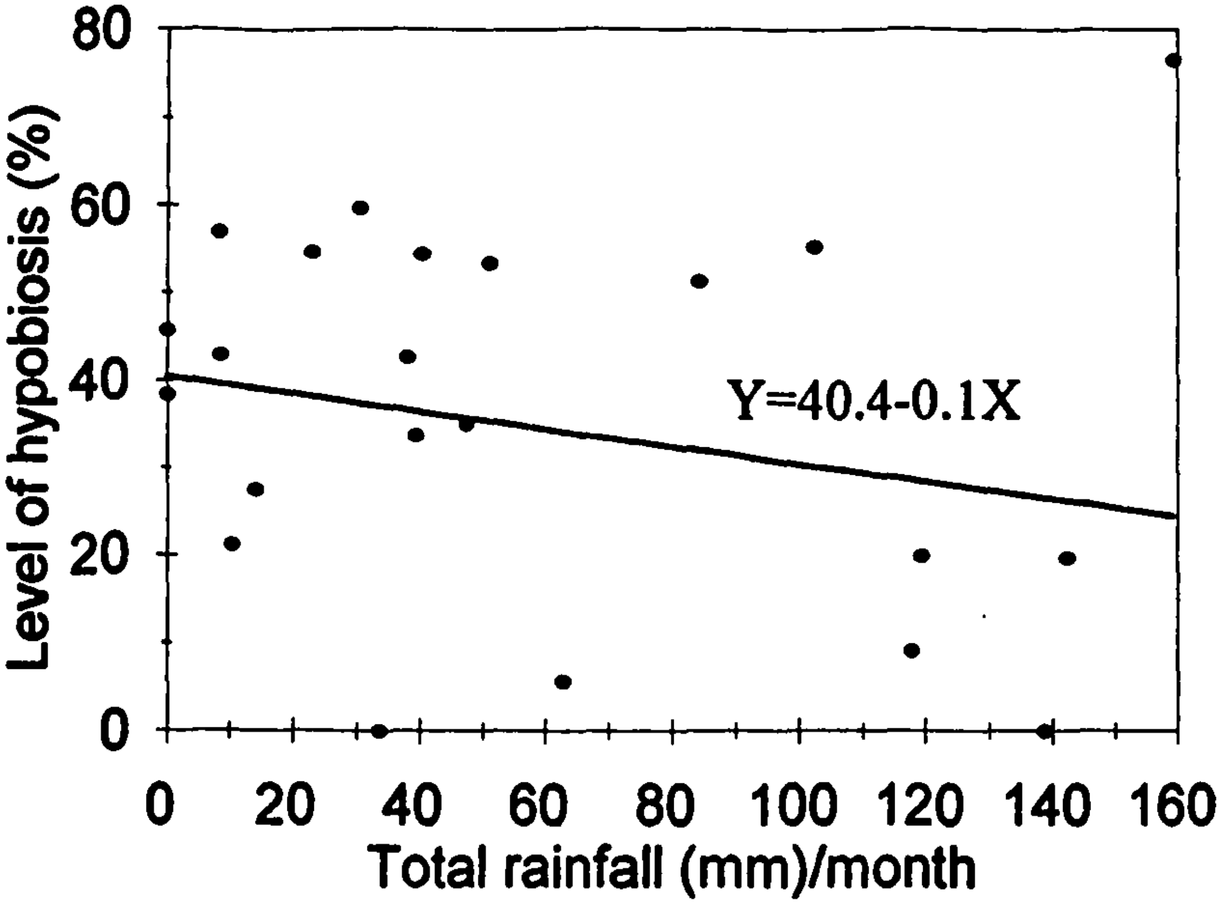

Level of hypobiosis (%)
80
60
40
20
0
Y=40.4-0.1X
0
20
40
60
80
100
120
140
160
Total rainfall (mm)/month

Fig. 3.16. Regression line showing the relationship between the level of hypobiosis and the number of rainy days per month. This data was generated from the permanent control flock (Flock C). Each data point is an average from 8 animals.

$R^2 = 0.0544$ $p = 0.2962$

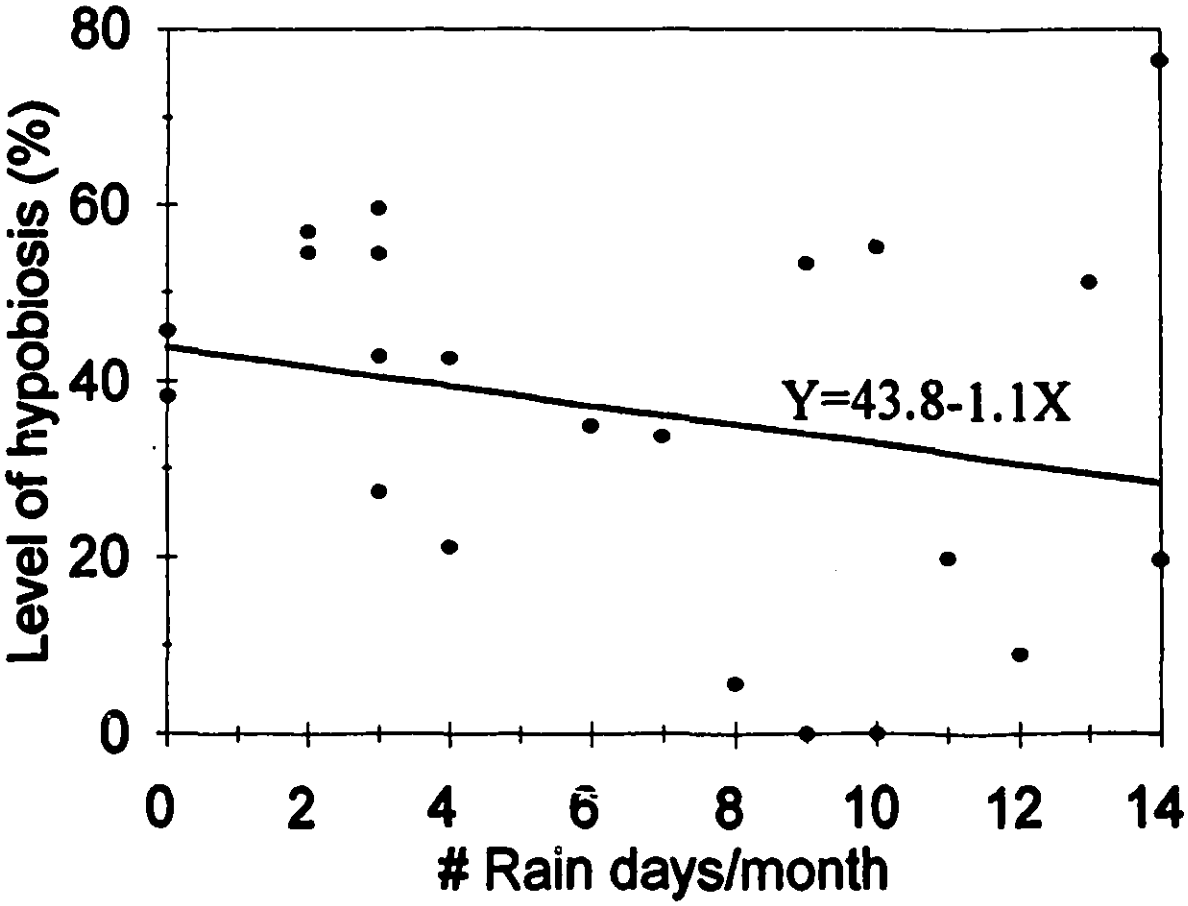
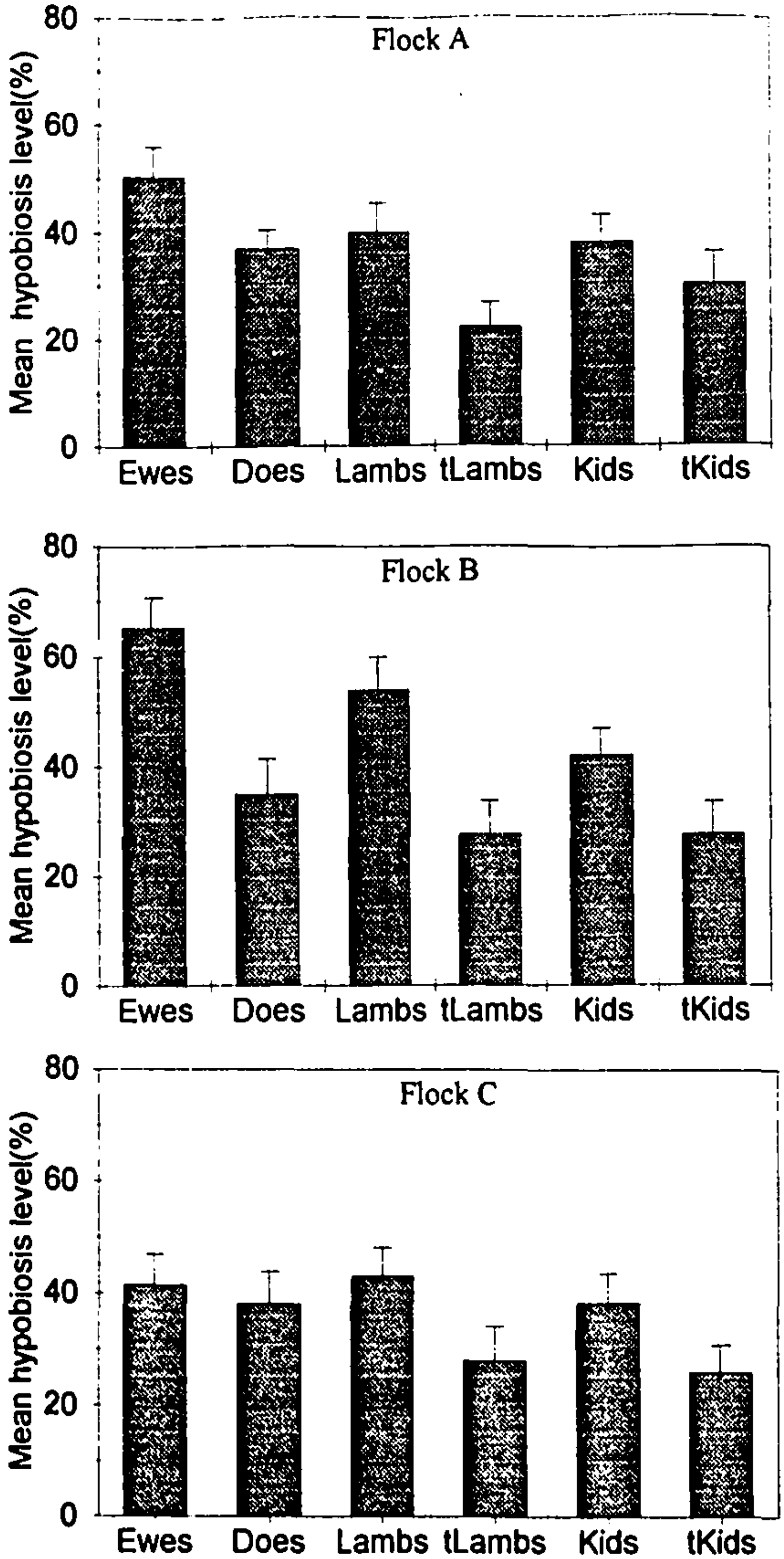

80
60
40
20
0
Level of hypobiosis (%)
Y=43.8-1.1X
0
2
4
6
8
10
12
14
Rain days/month

Fig. 3.17. The overall level of hypobiosis per class of animal over the period of the study . The error bars represent monthly variations.


Flock A
Mean hypobiosis level(%)
0
20
40
60
80
Ewes
Does
Lambs
tLambs
Kids
tKids
Flock B
Mean hypobiosis level(%)
0
20
40
60
80
Ewes
Does
Lambs
tLambs
Kids
tKids
Flock C
Mean hypobiosis level(%)
0
20
40
60
80
Ewes
Does
Lambs
tLambs
Kids
tKids

CHAPTER 4

THE POPULATION DYNAMICS OF INFECTIVE LARVAE ON PASTURE AND THE IMPACT OF ANTHELMINTIC TREATMENT ON PASTURE INFECTIVITY

INTRODUCTION

The abundance and seasonal availability of infective stages of trichostrongylid nematodes on pasture is a key factor in the occurrence and severity of nematode infections in grazing animals (Southcott et al., 1976). Their populations on pasture fluctuate widely from one season to the next (Couvillion et al., 1993) and therefore knowledge of their seasonal dynamics provides information on the precise timing of infections (Grenfell et al., 1987) which is crucial in considerations of control strategies.

The ability to accurately detect and quantify the infective larvae on pasture is critical to the understanding of ecology and epidemiology of trichostrongylid nematodes (Boag and Thomas, 1971, 1975, 1977; Waller and Thomas, 1978) and eventually to the design of strategic control programmes (Callinan et al., 1982; Paton et al., 1984; Thomas et al., 1986; Paton and Boag, 1989). Several methods have been used to determine the level of infective larvae on pasture. The two most commonly used techniques are the random pasture sampling (Taylor, 1939; Lancaster, 1970; Boag and Thomas, 1971; Donald and Waller, 1973) and the tracer method (Kates, 1950; Tetley, 1959; Muller, 1968; Anderson, 1972 ; Donald et al., 1978).

The objective of this study was to evaluate the effect of two treatment strategies on seasonal infectivity of pastures in a semi-arid area of Kenya by use of tracer and pasture random sampling methods. In addition, a modified pasture sampling method which mimicked grazing behaviour of animals was used. For the purpose of this study, this latter method was designated as the "Trail" method.

MATERIALS AND METHODS

Experimental protocol:

Flock A was treated with ivermectin (200µg/kg: 'Ivomec' Merck, Sharp and Dohme Ltd. - Switzerland) during the dry season 4 weeks before the onset of the rains (both long and short rains): in February-March for the long rains normally expected in April, and in September for the short rains expected in October. Flock B was treated with the same anthelmintic 4 weeks after the onset of the long rains and about 2 weeks after the onset of the short rains. Flock C was not treated except for occasional salvage treatments with albendazole (5mg/kg: 'Valbazen' Smith Kline Animal Health Ltd.- UK).

Faecal and herbage samples were collected and processed each month as explained in chapter 2. The numbers of infective larvae in pasture samples were calculated by the following formula:

$$\# \text{ L3/kg herbage} = \frac{1000}{\text{Wt. of dry herbage(gm)}} \times \# \text{ larvae counted}$$

Larval counts in each paddock were plotted against time and the trends compared among the flocks.

The number of worms in tracers were estimated as explained in chapter 2.

Statistical analysis:

Faecal egg count data were normalised by log-transformation: $LogX=Log_{10}(X+1)$ where X was the monthly egg count of individual animals. The transformed data were compared among the flocks by one-way analysis of variance.

RESULTS

Trends of pasture larval counts and egg output:

Trends of pasture larval counts as estimated by the three methods are shown in Figs. 4.1-4.3. The overall pattern indicated rising larval counts during the wet months and declining counts during the dry months. As measured by the three methods of estimation, this trend was similar and consistent in all the three flocks.

However, closer examination of the period Dec.92-Jan.93 revealed an important deviation from this general pattern. Whereas the direct pasture sampling methods (W-transect and Trail) showed low larval counts, the tracer method showed peak worm counts that were even higher than during the previous long rains. This was true for flocks B and C but not so much for flock A where moderately high larval count was detected by the direct sampling methods.

Similar to the pasture larval count, the egg output trend in the permanent flock showed peak counts during the wet season and low counts during the dry period (plots A in Figs. 4.1-4.3). The period Dec.92-Jan.93 was once again noteworthy in that, despite the high rainfall and total worm counts in the tracers, there was no corresponding peak in egg output in the permanent flocks.

Impact of treatment on pasture larval counts and worm burden:

Flock-wise comparisons of the pasture larval counts, under each sampling method are shown in Fig. 4.4. Whereas flocks A and B were similar, they generally had lower counts than flock C. However, flock A differed from flocks B and C in the timing of the appearance of larval counts on pasture during the long rains. In flock A, infective larvae appeared on pasture a month later compared with the appearance in the other two flocks. Statistical comparison of total worm counts acquired by the tracers (Table 4. 1) showed that the three flocks were similar.

DISCUSSION

The three methods used for the estimation of pasture infectivity were consistent in the evidence that maximum number of infective larvae were available on pasture during wet months. The pasture infectivity as measured by the tracer method in the period Dec.92-Jan.93 was interesting in the sense that it was even higher than the infectivity during the preceding long rains and yet it was virtually undetected by the other methods in flocks B and C; however, it was detected in flock A. The reason for this is unclear. The rain was unexpected at this time and it came in torrents for about a week. This resulted in fresh herbage sprouts that are normally attractive to the grazing animals (commonly known as "first bites"). Attraction to this lush vegetation leads to grazing at ground level with the consequence that animals are exposed to higher larval intakes which include larvae migrating from the soil. Grazing animals are known to change their grazing behaviour with the amount and quality of herbage available on pasture (Taylor, 1954; Bryan and Kerr, 1988). In spite of the increased total worm counts in tracers, suggesting substantial larval intake, the egg output of the permanent flocks remained unchanged. This can be explained by the findings of Chapter 3 that, at this time, the level of hypobiosis still remained high suggesting that the majority of the ingested infective larvae did not develop to egg-laying adult worms. In addition, it also implied that the permanent animals had a certain level of immunity.

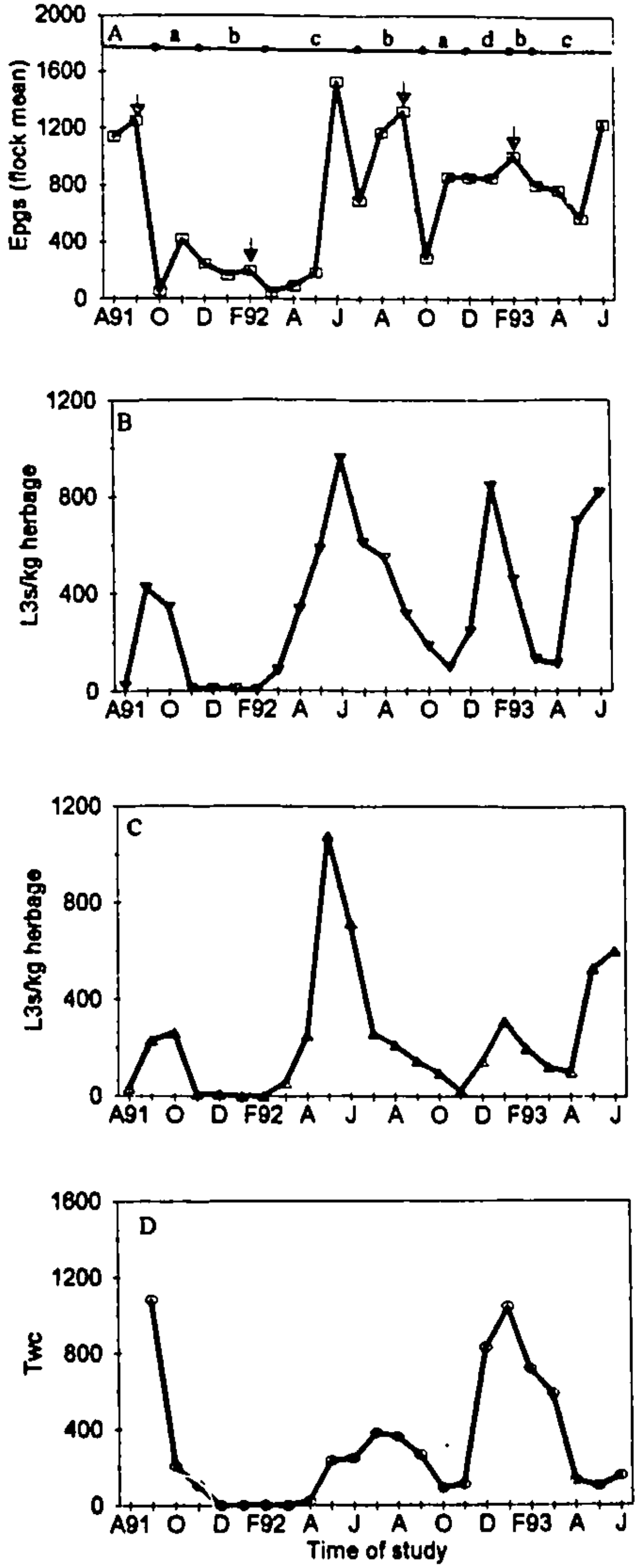
In the context of this study, the tracer method would appear to be the most appropriate method of estimating levels of infectivity because the tracers would be expected to closely simulate the grazing pattern of the permanent animals. However, results of the direct pasture sampling method showed that this method also provided important information particularly in detecting differences in infectivity due to different treatment regimes.

Infective larvae arising from the hatching of nematode eggs deposited on pasture in faeces constitute the primary source of infectivity. In addition, migration of infective larvae from the moistened soil onto herbage could be a secondary source of infectivity. In Chapter 3, it was shown that the level of hypobiosis sharply declined during the long rains, a situation that led to increased number of adult worms as a result of maturation of hypobiotic larvae. In the current experiment, results showed that, with the onset of the long rains, the appearance of pasture larval counts rose immediately in flocks B and C. This meant that the treatment administered to flock B did not affect the source of infectitvity; by the time treatment was administered, the hypobiotic larvae had already matured and were shedding eggs as adults. The situation was different in flock A. There was a definite temporal shift in the appearance of pasture larval counts that was evident using all three methods of estimation. This implied that the hypobiotic worms had effectively been removed by the treatment applied before the onset of the rains. The subsequent slow larval build-up on pasture could be attributed to any remaining hypobiotic worms or infective larvae migrating from the soil.

Table 4.1. Comparison of mean total worm counts recovered from tracers per month . There were no significant differences among the three flocks in either lambs or kids.

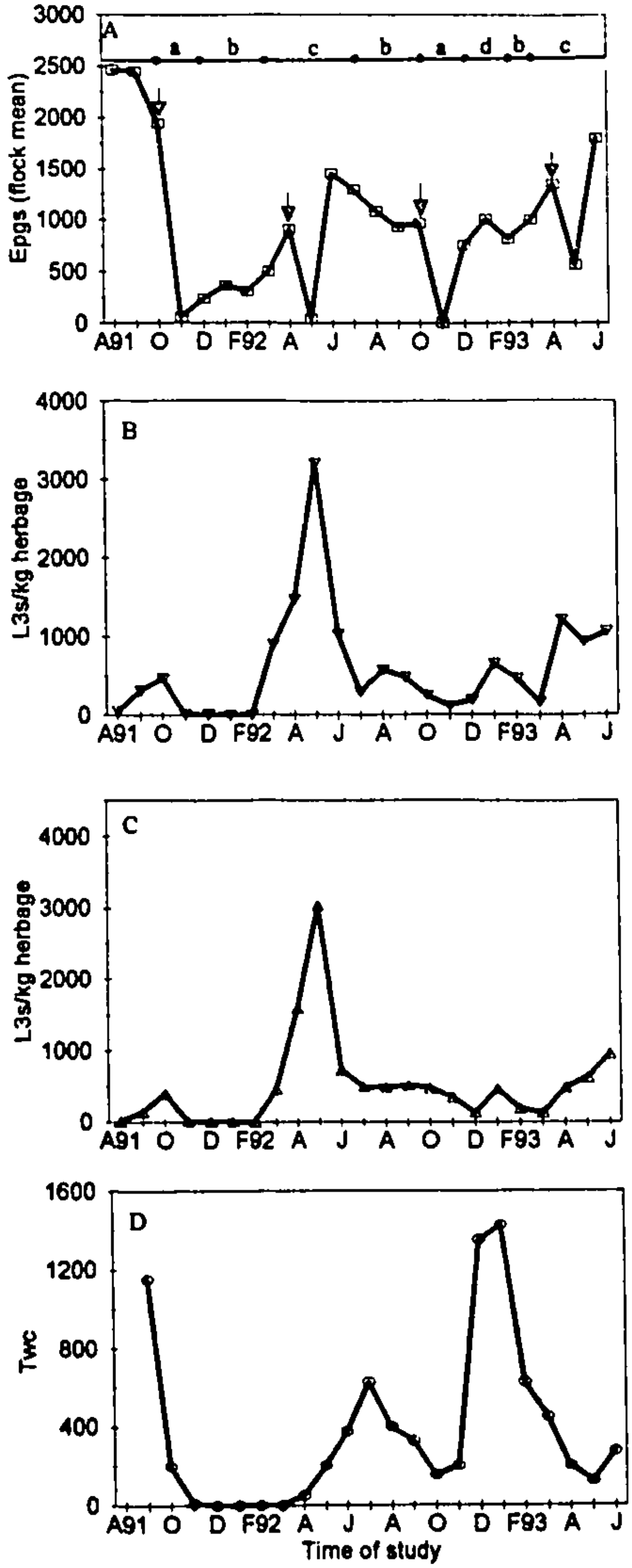
Flock	Mean±S.E Lambs	Mean ±S.E Kids
A	340.6±82.6	265.0±61.3
B	375.3±90.6	174.6±61.8
C	572.7±134.5	397.7±76.9

Fig. 4. 1. Seasonal pattern of pasture larval count in **Flock A** as measured by the three methods: W-Transect (B), Trail (C) and Tracers (D). Treatment time points (Arrows) are shown on the seasonal egg output pattern (A) of the permanent animals. Labels a, b and c represent the three seasons: the short rains, the dry and the long rains respectively; d represents the unexpected rains within a dry season.



A
a b c b a d b c
Epgs (flock mean)
2000 1600 1200 800 400 0
A91 O D F92 A J A O D F93 A J
B
L3s/kg herbage
1200 800 400 0
A91 O D F92 A J A O D F93 A J
C
L3s/kg herbage
1200 800 400 0
A91 O D F92 A J A O D F93 A J
D
Twc
1600 1200 800 400 0
A91 O D F92 A J A O D F93 A J
Time of study

Fig. 4.2. Seasonal pattern of pasture larval count in **Flock B** as measured by the three methods: W-Transect (B), Trail (C) and Tracers (D). Treatment time points (Arrows) are shown on the seasonal egg output pattern (A) of the permanent animals. Labels a, b and c represent the three seasons: the short rains, the dry and the long rains respectively; d represents the unexpected rains within a dry season.



A
a
b
c
d
Epgs (flock mean)
L3s/kg herbage
Twc
B
C
D
A91
O
D
F92
A
J
F93
Time of study

Fig. 4.3. Seasonal pattern of pasture larval count in **Flock C** as measured by the three methods: W-Transect (B), Trail (C) and Tracers (D). Treatment time points (Arrows) are shown on the seasonal egg output pattern (A) of the permanent animals. Labels a, b and c represent the three seasons: the short rains, the dry and the long rains respectively; d represents the unexpected rains within a dry season.

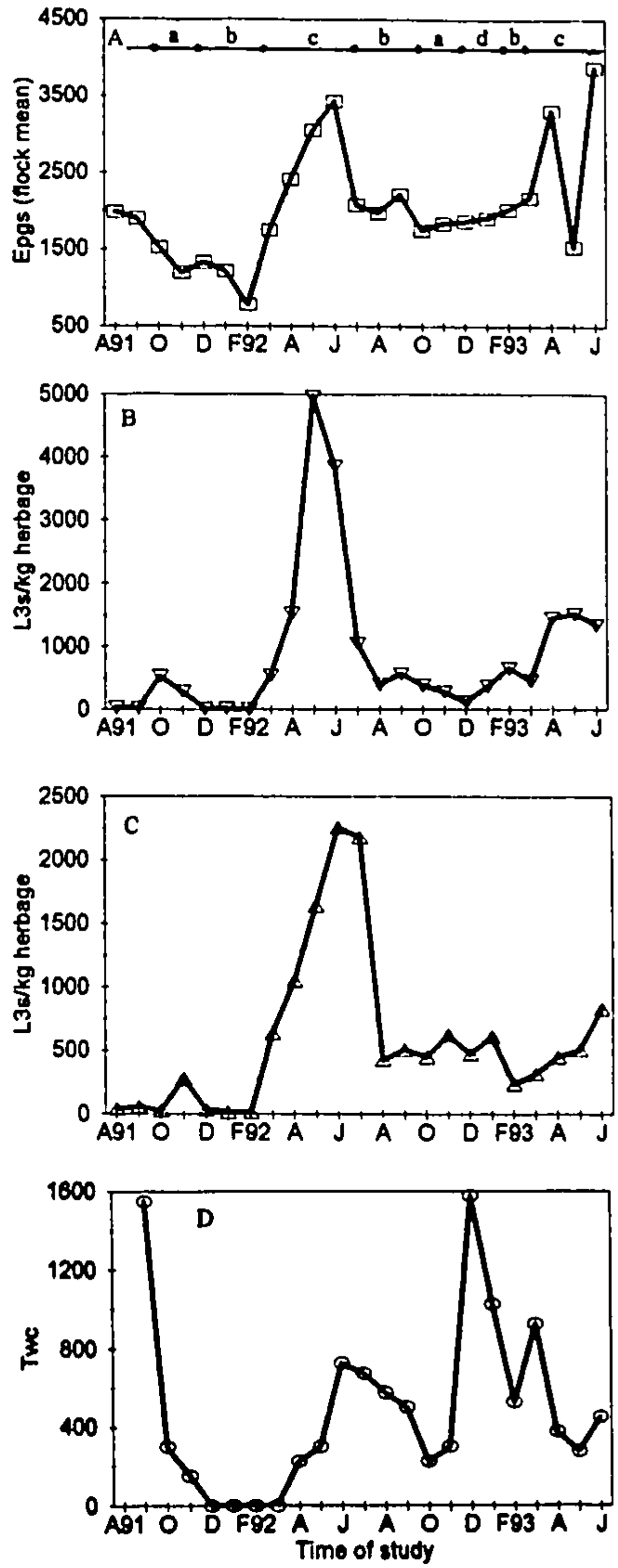
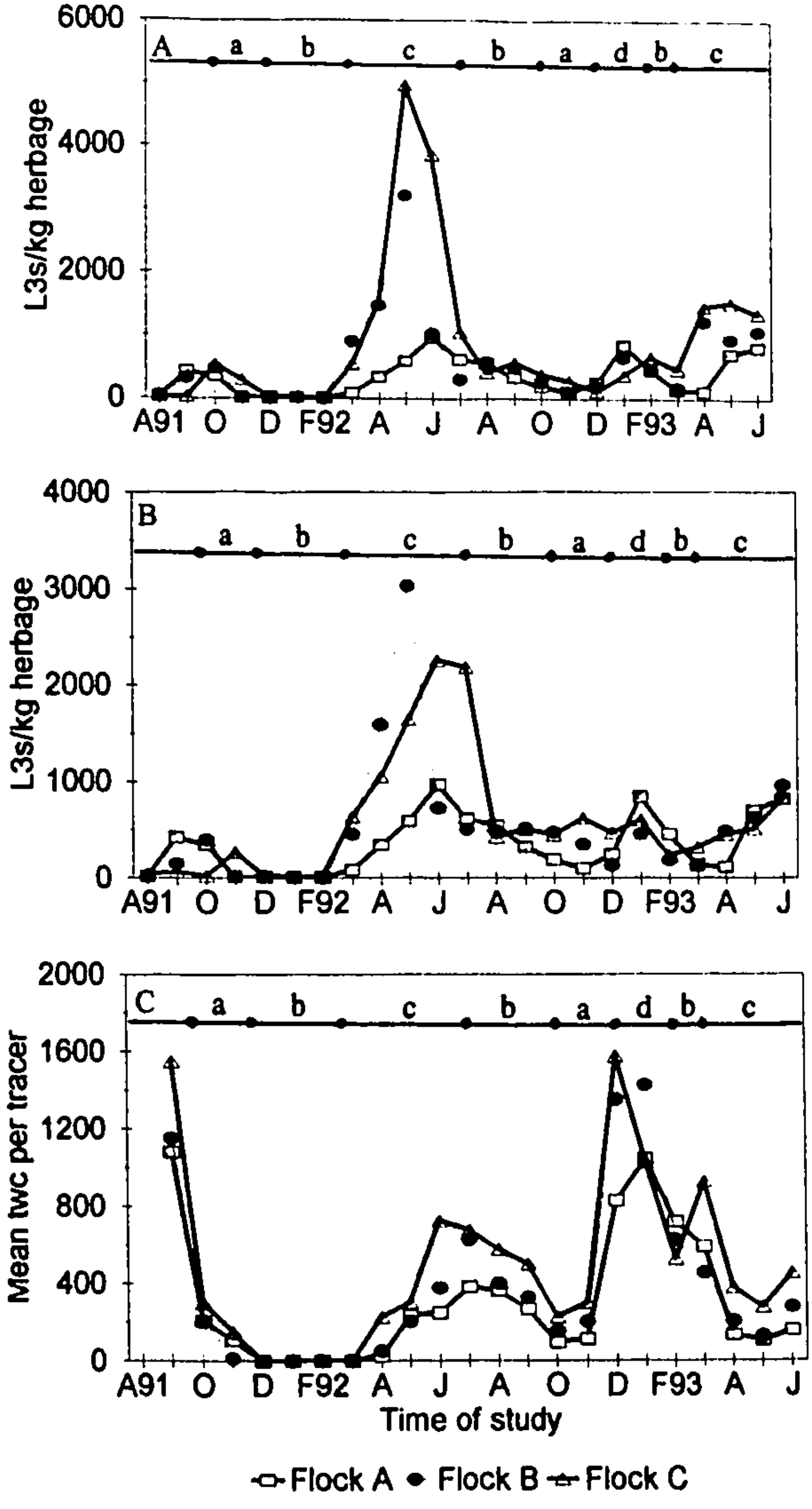

A
a
b
c
b
a
d
b
c
Epgs (flock mean)
4500
3500
2500
1500
500
A91
O
D
F92
A
J
A
O
D
F93
A
J
B
L3s/kg herbage
5000
4000
3000
2000
1000
0
C
L3s/kg herbage
2500
2000
1500
1000
500
0
D
Twc
1600
1200
800
400
0
Time of study

Fig. 4.4. Comparison of the seasonal pattern of pasture infectivity among the three flock as estimated by : W-Transect method (A), Trail method (B) and the Tracer method (C). Labels a, b and c represent the three seasons: the short rains, the dry and the long rains respectively; d represents the unexpected rains within a dry season.



6000
4000
2000
0
L3s/kg herbage
A
a b c b a d b c
A91 O D F92 A J A O D F93 A J
4000
3000
2000
1000
0
L3s/kg herbage
B
a b c b a d b c
A91 O D F92 A J A O D F93 A J
2000
1600
1200
800
400
0
Mean twc per tracer
C
a b c b a d b c
A91 O D F92 A J A O D F93 A J
Time of study
Flock A Flock B Flock C

CHAPTER 5

EFFECTS OF DIFFERENT ANTHELMINTIC TREATMENT REGIMES ON FLOCK PERFORMANCE

INTRODUCTION

Eradication of gastrointestinal parasites of small ruminants is impractical (Brundson, 1980). The main objective of controlling these parasites is therefore to minimise associated economic losses (Michel 1972, 1976; Morley and Donald, 1980; Jorgensen, 1983). The aim is to contain parasite populations at levels that do not significantly affect production of the host animals. In control programmes, helminth populations can be maintained at such levels by strategic use of anthelmintics or by limiting parasite-host contact through pasture management (Brundson, 1980).

Interaction of parasitism and production is evident from reports associating peri-parturient rise to decreased milk production in the ewe (Connan, 1973). Furthermore, Darvil et al. (1978) observed that anthelmintic treatment of ewes was associated with increased weight gains in their lambs. They concluded that treatment had an effect of increased milk production on the part of the ewes which was expressed by increased weight gains in the lambs. Earlier, Munro (1955) and Owen (1957) had demonstrated a high correlation between ewe milk yield and lamb growth rate.

In the tropics, the level of production is low and this is associated with a number of factors which include poor nutrition, disease including parasitism and poor management. While poor nutrition is considered the most critical factor, disease and parasitism constitute a major source of economic loss (Devendra, 1981), especially in areas with poor veterinary services. In Kenya, for example, it has been estimated that haemonchosis alone causes an annual loss of about US$ 26 million in sheep and goat production (Allonby, 1976) while in Nigeria about 40-60% of the lambs die due to gastrointestinal nematode infections (Schillorn van Veen and Brinckman, 1975; Eysker and Ogunsusi, 1980).

Sheep and goats constitute a major source of proteins for human nutrition in these regions (Devendra, 1981). It is therefore critical that sound helminth control programmes be implemented through proper management in order to enhance flock productivity. In marginal areas of Kenya, sheep and goat owners traditionally treat their flocks for helminthoses during the rains when clinical disease is already evident. This clinical manifestation appears so quickly after the onset of the rains that it cannot be explained by infection acquired from the pastures. Hypobiotic larvae have therefore been suspected to be the source of this infection. In chapter 3, it was shown that, during the dry seasons, hypobiotic larvae account for as much as 70% of the total worm burden. To minimise this infection, hypobiotic larvae must be removed before they resume development. The objective of this study was therefore to compare flock performance under three different treatment strategies: traditional treatment administered during the rains, treatment administered before the onset of the rains and no treatment at all. Treatment administered before the onset of the rains was aimed at removing the hypobiotic larvae.

MATERIALS AND METHODS

Experimental protocol:

The study was conducted over a period of 2 years starting from August 1991 to June 1993. Flock A was treated with ivermectin (200μg/kg: 'Ivomec' Merck Sharp and Dohme Ltd.- Switzerland) 4 weeks before the expected onset of the rains and Flock B was treated 4 weeks after the onset of the long rains and 2 weeks after the onset of the short rains. Flock C was the control and therefore no treatment was administered except for occasional salvage treatments with albendazole (5mg/kg: 'Valbazen' Smith Kline Animal Health Ltd.- UK). Effects of treatments were compared by evaluating the following performance parameters in each flock:

Faecal egg counts, packed cell volume and weight:

Faecal and blood samples were collected from all the animals every month and processed by standard methods (Anonymous, 1986) for the estimation of egg counts and packed cell volumes respectively. Body weights of all animals were also recorded every month. These parameters were compared among the three flocks by repeated measures analysis of variance (SAS: Littell et al., 1991).

Growth:

Body weights of lambs and kids from each flock were recorded monthly from birth to 9 months of age. Nine months was the average age at which reproductive maturity was attained in both sheep and goats. For kids, the test period was March 1992-November 1992 and for the sheep July 1992-March 1993. These were the peak lambing and kidding periods when adequate numbers of lambs and kids of uniform age were available. Growth was compared by repeated measures analysis of variance.

Birth weights, age and weight at first parturition, lambing and kidding intervals:

Birth weights of lambs and kids in each flock were recorded within six hours after birth for births taking place during the day. Weights for night births were recorded the following morning. Ages of ewes and does at their first conception were determined by subtracting the gestation period (5 months) from the ages at their first parturition. It was not possible to detect early embryonic deaths. For ewes and does in their second and subsequent parturitions, lambing and kidding intervals were calculated. These measurements were compared by one-way analysis of variance.

Mortalities:

Using data from mortality and birth records of each flock, survival probabilities of all lambs and kids surviving at every age (1 month intervals) from 1 month to 9 months were determined. Mortalities were also determined in association with first and subsequent parturitions by separating mortalities of lambs and kids born by maiden ewes and does from mortalities of lambs and kids born by ewes and does in their second and subsequent parturitions.

RESULTS

Faecal egg output and packed cell volume:

Comparison of the faecal egg output (Table 5.1) and the packed cell volume (Table 5.2) of the three flocks over the period of the study indicated that in all classes of animals, Flocks A and B were similar but significantly higher ($p<0.05$) than Flock C for the PCV and significantly lower for the egg output.

State of body condition:

Body weights of ewes and does were used as a measure of the body condition of the animals. Results (Table 5.3) showed that weights in flock A were significantly higher than weights in flock C ($p<0.05$). Comparison of body weights between flocks A and B and between flocks B and C showed that these weights were similar.

Impairment of the health status:

The growth performance of the lambs and kids between the ages 1-9 months was used as an inverse measure of infection impact of the flocks. Results (Table 5.4a) of the repeated measures analysis of variance indicated that the only statistical difference in weight among both lambs and kids was between flocks A and C ($p<0.05$; A>C); there was no significant difference when flocks A and B or flocks B and C were compared. One-way analysis of variance (Table 5.4b) of the final weight attained at 9 months showed that, in both lambs and kids, flock A attained higher weights than flock C ($p<0.05$) but that there was no difference when weights between flocks A and B or between flocks B and C were compared. The growth curves of the lambs and kids in the three flocks (Fig. 5.1) showed that lambs and kids in flock C had consistently lower weights than those in flocks A and B.

Breeding cycle:

Although breeding occurred throughout the year in both sheep and goats in the three flocks, definite birth peaks occurred at certain times of the year (Fig. 5.6). In ewes, maximum number of births occurred in July-September which constituted about 60-69% (Table 5.9) of all births. In does, the peak period occurred in Februa-y-April when 63-86% of births took place. There were no flock variations in this pattern.

Birth weights:

Birth weights were significantly different ($p<0.05$) among the three flocks in both lambs and kids; the highest were found in flock A and the lowest in flock C (Table 5.5, Fig. 5.2). Frequency distribution of birth weights over lambing and kidding seasons (Figs. 5. 3 & 5.4) showed improved birth weights in flock A in the second lambing season as compared with the first season. In flock B, there was moderate improvement in birth weights during the second lambing season: in flock C, there was a decrease in birth weights in the second lambing season. In flocks A and B, there was no evidence of change in birth weights of kids between the two breeding seasons but in flock C, there were decreased birth weights in the second kidding season.

Rate of attaining reproductive maturity:

Both age and weight at first conception (carried to term) were considered as measures of reproductive maturity. Comparison of age at first conception showed significant differences ($p<0.05$) between pairwise comparisons A-C (A<C) and B-C (B<C) but not A-B in both ewes and does (Table 5.6). Pairwise comparisons of weight at first conception invariably showed no significant differences among the flocks.

Flock fertility:

Flock fertility was measured in terms of lambing and kidding intervals. One-way analysis of variance of these intervals (Table 5.7) showed significant differences ($p<0.05$) in pairwise comparisons A-C (A<C) and B-C (B<C) but not in A-B.

Survival in the flock:

The probability of survival among the lambs and kids in the three flocks is represented in Fig 5.5. The pattern in the lambs was similar among the three flocks which showed improved survival in the second and third months of life followed by decreased survival at the fourth and fifth months of age. Subsequently, survival improved steadily from 6 to 9 months. Among the kids, survival probability followed a similar pattern but Flock C had markedly lower survival as compared with Flocks A and B; survival in Flocks A and B were comparable. Comparison of mortality rates between lambs and kids born of dams in their first parturition and those born of dams in their second and subsequent parturitions indicated that mortality was consistently higher in lambs and kids born of dams in their first parturition (Table 5.8a). Comparison of mortality rate, on the basis of birth weights, indicated that mortality was higher in lambs and kids with low birth weights than in lambs and kids with high birth weights (Table 5.8b).

DISCUSSION

The ultimate consequence of parasitism is to deprive the host of nutrients required to perform its normal physiological functions. Consequently, such a host becomes inefficient in energy utilization (Jorgensen, 1983) and this is translated into economic losses (Ploeger and Kloosterman, 1993) experienced by stock owners. The loss of blood by parasitised animals as shown by the decreased packed cell volume in this study constitutes an important outlet for the loss of resources that the animal needs for productivity. The overall objective of parasite control is therefore to minimise such losses (Michel, 1976).

One way of keeping worm burdens at minimum levels in small ruminants is by anthelmintic treatment. Removal of worm burden enhances the production performance of the host animals by making them more efficient in energy utilisation. The effect of anthelmintic treatment is manifested in diverse ways such as in increased birth weights of new-born lambs and kids or in enhanced growth rate to maturity (Darvil et al., 1978). These effects could occur either directly through increased milk production by the ewe (Munro, 1955; Owen, 1957) or indirectly by alteration of the onset, magnitude and duration of the availability of infective larvae on pasture (Darvil, et al., 1978), facts that determine the level of worm burden. Consequently, depending on the strategic timing of administration, treatment may alter the temporal relationship of larval availability on pasture thereby delaying the early acquisition of helminth infections by the grazing lambs and kids as well as their dams.

In this study, effects of two strategic treatment regimes on sheep and goat flock performance were compared. Treatment during the rains (flock B) is the strategic control method commonly practised in sheep and goat enterprises in marginal areas in Kenya. This treatment is usually administered when clinical helminthosis is already evident about 3-4 weeks after the onset of the rains. Experience has shown that benefits of such treatments are very short-lived as the animals are immediately re-infected from pastures already heavily contaminated with infective larvae. It was shown in chapter 3 that large numbers of hypobiotic larvae were present in the grazing animals during the dry seasons and that these numbers went down to nil during the wet seasons. Clinical helminthosis that occurs in sheep and goats in this area shortly after the onset of the rains is associated with the maturation of these hypobiotic larvae into large numbers of adult worms within a short time. Treatment that was administered before the rains (flock A) was based on the argument that it would reduce the populations of adult worms and hypobiotic larvae and therefore lead to low pasture contamination during the next wet season. As shown in chapter 4, such a treatment altered the temporal pattern of larval availability on pasture. Similar treatment regimes have been shown to have beneficial effects on growth performance of calves in temperate regions when treatment is administered early in the first grazing season (Jones, 1981; Borgsteede et al., 1981; Herd, 1988; Prichard, 1988; Ploeger and Kloosterman, 1993).

Results in this study suggest that some parameters of animal performance were more directly influenced by nematode infection than others. For example, the pair-wise comparisons of mean body weights showed that only A-C comparison was significantly different whereas all the pair-wise comparisons (A-B, A-C and B-C) of birth weights were significantly different. In this particular case, it was evident that treatment before the rains was significantly more beneficial than treatment administered during the rains. This was further confirmed among the lambs by the increased frequency of higher weights in the second compared with the first lambing season in flocks A and B as opposed to an increase in the frequency of lower weights in flock C. This difference was not as evident among the kids probably because the first kidding season fell at the middle of the study period after treatment had already been administered, unlike the lambs where the first lambing season occurred at the beginning of the study before treatment was administered. Under this circumstance, any difference in weights between the two kidding seasons that would have been associated with treatment effects would be less pronounced because the treatment effects had already appeared at the first kidding season. Elsewhere, birth weights have been found to be critical for the survival of kids and lambs (Ahmed and Tantawy, 1960; Sacker and Trail, 1966); lighter kids and lambs are more susceptible to stresses in the early months of life and in contrast, heavier ones experience lower mortalities (Sacker and Trail, 1966). This association was confirmed by the results of the current study where the highest mortalities were recorded among the kids in the control flock: the lowest weights were also recorded amongst these kids. Within a breed, the average birth weight is a reflection of energy levels that sustain the survival and performance of individual animals (Devendra, 1970). Such energy levels could be adversely influenced by parasitism with deleterious consequences in a given flock.

Comparison of weights at first conception (carried to term) showed no significant differences among the three flocks, but comparison of the same animals with respect to age at first conception indicated significant differences between the control and the treated groups. This meant that ewes and does in the treated and the untreated flocks attained sexual maturity at approximately the same weights, but at significantly different ages. This result was an indication that weight is a more critical criterion as a measure of sexual maturity than age. In Israel, Epstein and Herz (1964) reported that goats kept under superior conditions kidded for the first time at 1 year whereas under more primitive management, where disease and parasitism were rife, they did not kid until 2 years of age. In the current study, in both sheep and goats, first births were recorded as early as 11 months (range 11-18) in the treated groups and as late as 24 months (range 17-24) in the untreated group. Taking into consideration that the treated groups also had significantly shorter lambing and kidding intervals, it is evident that within a given time, these groups would yield significantly higher numbers of offspring and hence become more prolific in reproductive performance.

The mortality rates in lambs in the three flocks were generally comparable. About 17% occurred between birth and weaning and about 20% shortly after weaning which was at 4-5 months of age. It was probable that stresses associated with weaning were the overriding factors in this case because treatment did not influence the magnitude of mortality rates. However, among the kids, flock C showed markedly higher mortality rates from birth to maturity as compared to flocks A and B. This was probably due to the low birth weights in this flock (mean = 1.23kg) as compared to the treated groups (mean = 1.57-2.20 kg). Beneficial effects of treatment in reducing mortality were therefore strongly evident in the young stock.

The higher mortality rates in lambs and kids born of first-kidding and first-lambing dams was a further reflection of significantly lower birth weights associated with this group of dams. Comparison of mortality rates among the three flocks revealed substantial differences. For example, among the kids, contribution of the mortality of the kids born by first kidding dams to the overall mortality was 34.2%, 46.9% and 88.9% in flocks A, B, and C respectively. This observation indicated that dams in their maiden lambing and kidding required special management in order to minimise losses through under-weight births. One way of achieving this could be by supplementing them with quality feeds which would increase their milk production.

In Kenya, where this study was conducted, it is generally accepted that sheep and goats breed throughout the year. While this is partly true, results of this work showed that there are definite peak breeding seasons and that these were different for goats and sheep. About 60-69% of births in sheep took place in the period July-September and in goats about 63-86% of births occurred in the period February-April of each year. This is an important factor to be taken into consideration in flock management in this area in order to adequately cater for the young as they get recruited into the flock.

It was strongly evident from the results of this study that, overall, the treated groups had better performance than the untreated control group. In comparing flock performance in treatment administered before the rains and in treatment administered during the rains, results consistently showed that, in all cases, performance in flock A was either equal to or significantly better than performance in flock B. It was therefore evident that treatment administered during the dry seasons offered higher benefits in enhancing flock performance than treatment administered during the wet season when pastures were already heavily contaminated with large numbers of infective larvae.

Table 5.1. Comparison of monthly mean faecal egg output (by animal classes) among the three flocks.

	Ewes	Lambs	Does	Kids
Flock	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
A	956±150[a]	1009±183[a]	809±125[a]	715±102[a]
B	1174±179[a]	1081±161[a]	1474±261[a]	1136±177[a]
C	2174±183[b]	2024±243[b]	2345±214[b]	2104±186[b]

Means with different superscripts are significantly different at $p<0.05$

Table 5.2 Comparison of the monthly mean packed cell volume (%)by animal classes among the three flocks.

	Ewes	Lambs	Does	Kids
Flock	Mean±S.E	Mean±S.E	Mean±S.E	Mean±S.E
A	34.2 ± 0.6^{a}	32.7 ± 0.2^{a}	33.3 ± 0.5^{a}	32.4 ± 0.3^{a}
B	33.1 ± 0.6^{a}	32.1 ± 0.1^{a}	33.6 ± 0.4^{a}	32.3 ± 0.2^{a}
C	29.6 ± 0.5^{b}	29.0 ± 0.4^{b}	28.8 ± 0.4^{b}	29.4 ± 0.4^{b}

Means with different superscripts are significantly different at $p<0.05$

Table 5.3. Comparison of monthly mean body weights (kg) of ewes and does among the thre flocks.

	Ewes	Does
Flock	Mean±S.E	Mean±S.E
A	24.9 ± 0.6^{a}	23.4 ± 0.5^{a}
B	23.7 ± 0.5^{ab}	21.8 ± 0.5^{ab}
C	21.7 ± 0.5^{b}	20.8 ± 0.5^{b}

Means with different superscripts are significantly different at $p<0.05$

Table 5.4a. Comparison of mean liveweight changes (kg) of lambs and kids from birth to 9 months of age.

	Lambs		Kids	
Flock	n	Mean±S.E	n	Mean±S.E
A	53	11.8 ± 0.3^a	45	10.1 ± 0.2^a
B	50	9.7 ± 0.3^b	36	8.5 ± 0.6^b
C	36	8.6 ± 0.4^c	25	7.8 ± 0.8^b

Means with different superscripts are significantly different at $p<0.05$

Table 5.4b. Comparison of weight (kg) attained by lambs and kids at 9 months of age among the three flocks.

	Lambs		Kids	
Flock	n	Mean±S.E	n	Mean±S.E
A	53	18.8±0.5[a]	45	16.9±0.2[a]
B	50	18.4±0.3[ab]	36	16.3±0.4[ab]
C	36	16.6±0.4[b]	25	15.3±0.3[b]

Means with different superscripts are significantly different at $p<0.05$

Table 5.5. Comparison of birth weights (kg) in lambs and kids among the three flocks.

	Lambs		Kids	
Flock	n	Mean±S.E	n	Mean±S.E
A	128	2.20±0.05[a]	106	1.71±0.04[a]
B	116	1.98±0.04[b]	96	1.53±0.05[b]
C	87	1.57±0.05[c]	74	1.23±0.06[c]

Means with different superscripts are significantly different at $p<0.05$

Table 5.6. Comparison of age (months) and weight (kg) at first conception in ewes and does among the three flocks.

Age:

	Ewes		Does	
Flock	n	Mean±S.E	n	Mean±S.E
A	44	15.1±0.3^{a}	41	14.7±0.4^{a}
B	42	15.3±0.4^{a}	32	15.5±0.6^{a}
C	26	20.7±0.5^{b}	27	20.3±0.4^{b}

Weight:

	Ewes		Does	
Flock	n	Mean±S.E	n	Mean±S.E
A	44	21.6±0.2^{a}	41	21.9±0.3^{a}
B	42	21.6±0.3^{a}	32	20.9±0.3^{a}
C	26	21.9±0.3^{a}	27	20.5±0.3^{a}

Means with different superscripts are significantly different at $p<0.05$

Table 5.7. Comparison of lambing and kidding intervals (days) among the three flocks.

	Ewes		Does	
Flock	n	Mean±S.E	n	Mean±S.E
A	53	241.4±0.8^{a}	49	239.5±1.1^{a}
B	44	243.8±0.9^{a}	41	238.1±1.3^{a}
C	37	281.0±1.3^{b}	30	272.3±2.4^{b}

Means with different superscripts are significantly different at $p<0.05$

Table 5.8a. Comparison of mortality rates between lambs and between kids born by primarous dams (first parturition) and those born by multiparous dams (second and subsequent parturitions):

Mortality rates (%)

	Flock A		Flock B		Flock C	
	Parturition Status		Parturition status		Parturition Status	
	1st	2nd.plus	1st	2nd.plus	1st	2nd.plus
Ewes	38.6	7.1	31.8	9.5	50	11.5
Does	34.2	12.3	46.9	9.4	88.9	7.4

Mortality rate was calculated as a percentage of total deaths to total births.

Fig. 5.8b. Comparison of mortality rates of lambs and kids on the basis of birth weights.

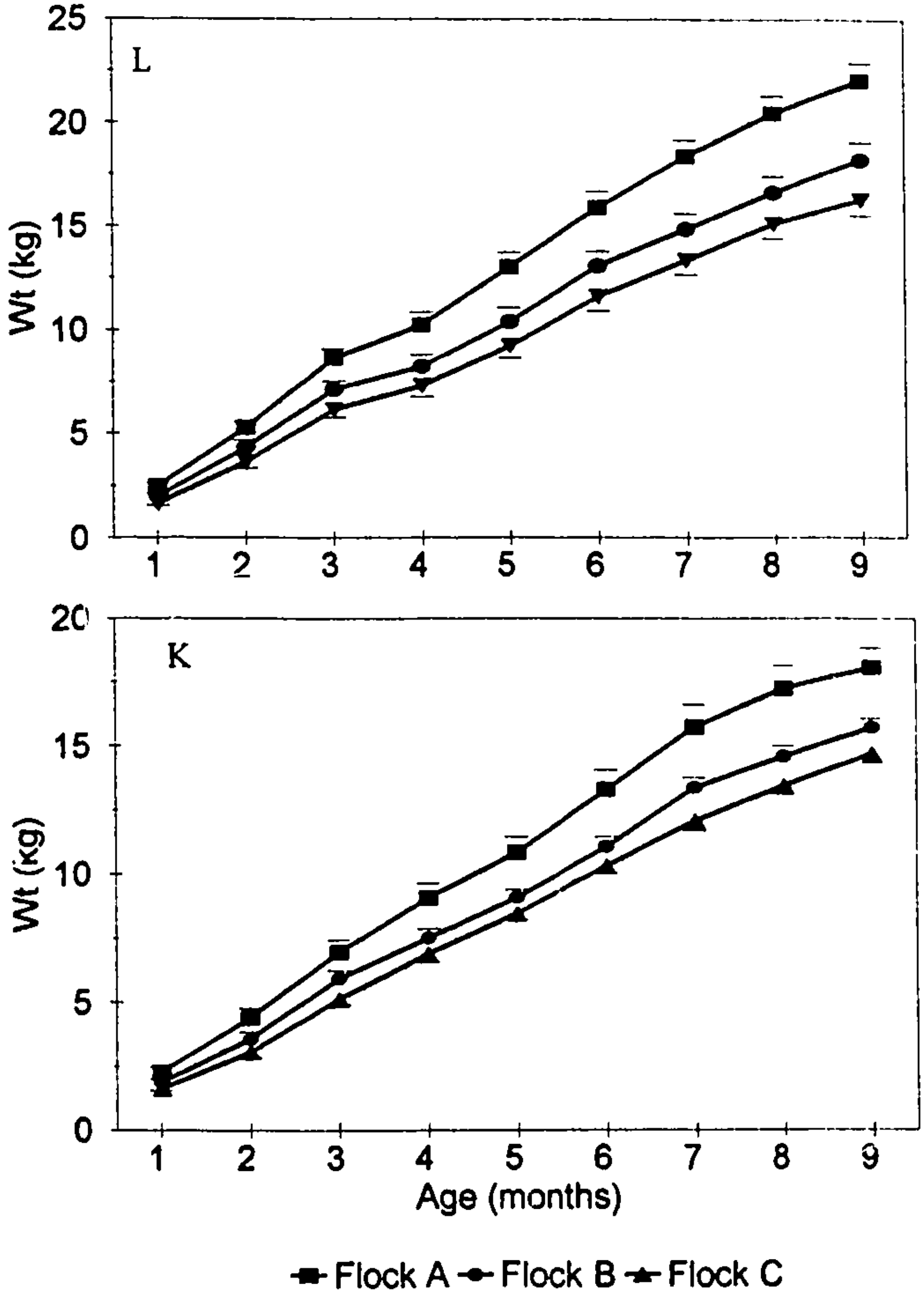
	% Mortality					
	Flock A		Flock B		Flock C	
Birth Wt (kg)	Lambs	Kids	Lambs	Kids	Lambs	Kids
0.5	-	-	-	-	85.7	93.8
1.0	80.0	56.3	55.6	66.7	32.0	60.0
1.5	36.0	17.8	17.5	21.2	6.9	33.3
2.0	9.3	12.9	6.4	6.3	7.7	16.7
2.5	5.6	7.1	4.6	0.0	0.0	0.0
3.0	0.0	-	-	-	0.0	-

Gaps indicate that no births were recorded in the particular weight and animal classes.

Table 5.9. Percentages of total births in each flock that occurred during the peak breeding season in both ewes and does.

	% Births in peak breedingseason	
Flock	Ewes (July-Sept.)	Does (Feb. - April)
A	66.4	85.9
B	60.3	69.8
C	69.0	63.5

Fig. 5.1. Comparison of growth pattern in lambs (L) and kids (K) among the three flocks. Lambs and kids had the highest growth in flock A and lowest growth in flock C; growth in flock B was intermediate. Growth was statistically different among the three flocks at $p<0.05$ level of significance.



L
Wt (kg)
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
K
Wt (kg)
20
15
10
5
0
Age (months)
Flock A
Flock B
Flock C

Fig. 5.2. Comparison of birth weight distribution in lambs(L) and kids (K) among the three flocks over the period of study. In both lambs and kids, flock A had the highest birth weights followed by flock B. Flock C had the lowest weights. The weights were significantly different among the three flocks at $p<0.05$.

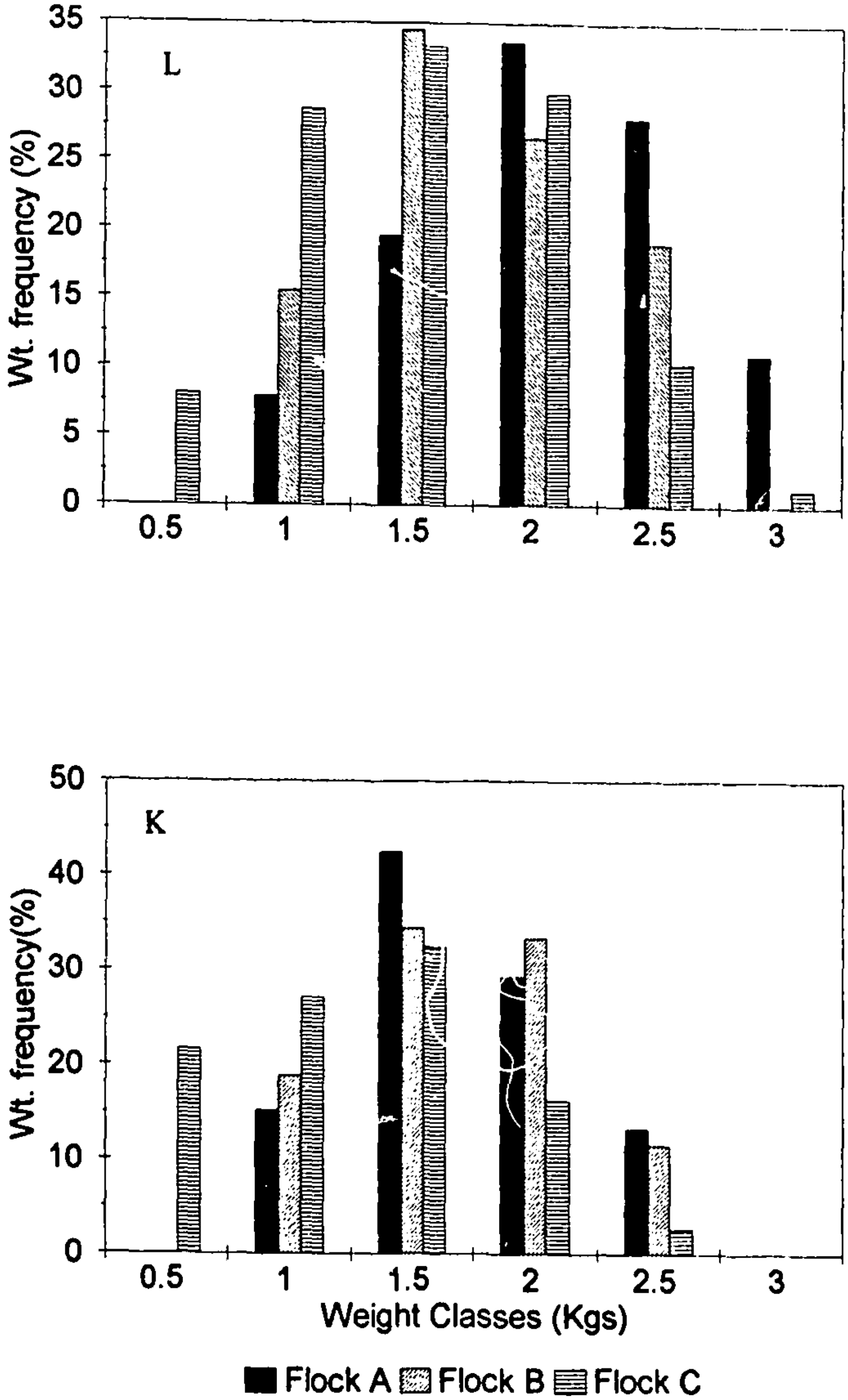
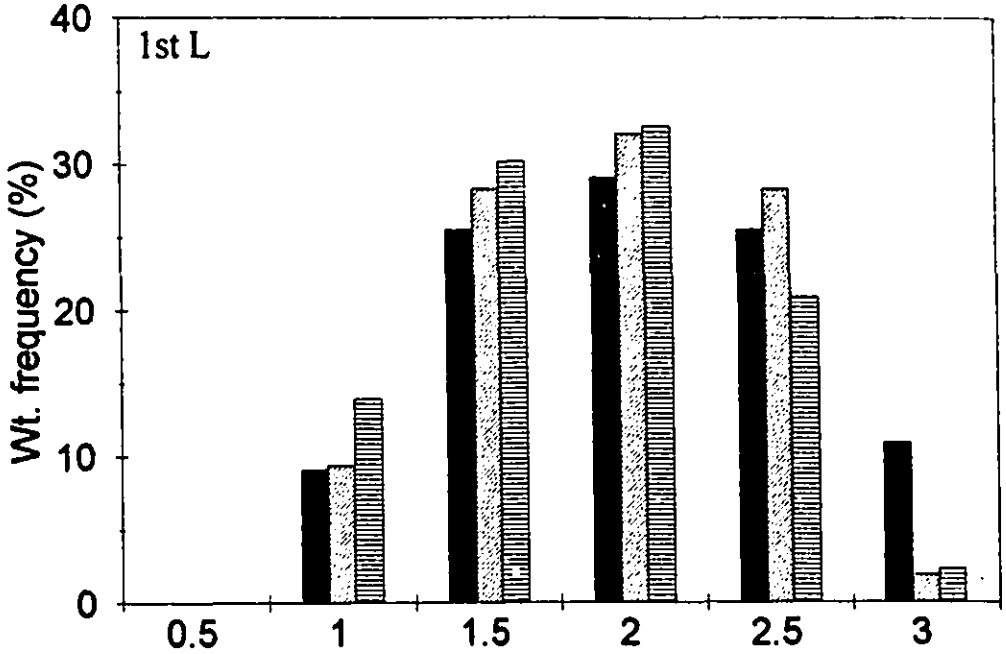
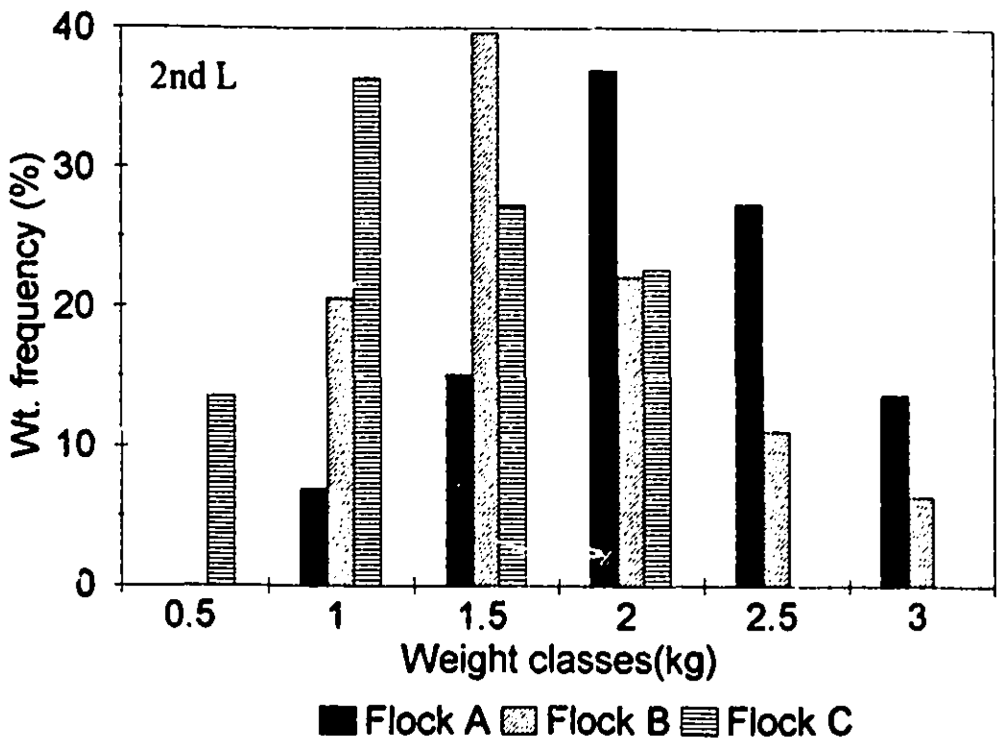

L
Wt. frequency (%)
35
30
25
20
15
10
5
0
0.5
1
1.5
2
2.5
3
K
Wt. frequency(%)
50
40
30
20
10
0
0.5
1
1.5
2
2.5
3
Weight Classes (Kgs)
Flock A
Flock B
Flock C

Fig. 5.3. Comparison of the frequency distribution of birth weights between the first (1st L)and second (2nd L) lambing seasons among the three flocks. There was improvement in birth weights in the second lambing season in flocks A and B but not in flock C which, instead showed lower weights.

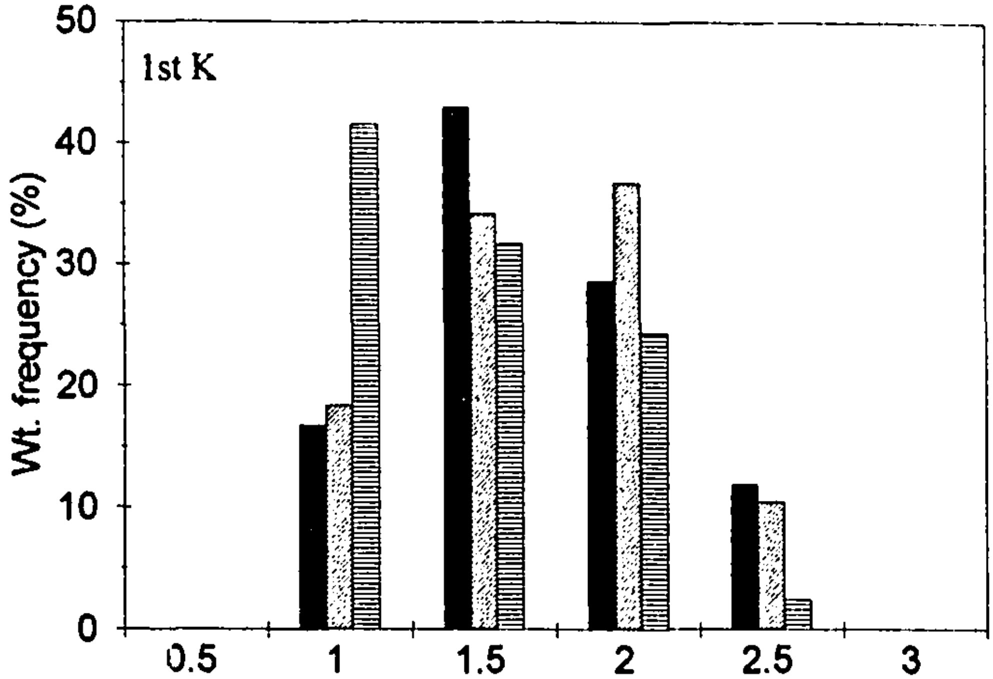


1st L
Wt. frequency (%)
0.5
1
1.5
2
2.5
3



2nd L
Wt. frequency (%)
Weight classes(kg)
Flock A
Flock B
Flock C

Fig. 5.4. Comparison of the frequency distriburion of birth weights between the first (1st K) and second (2nd K) kidding seasons among the three flocks.There was no apparrent change in birth weights in any flock between the two breeding seasons.


1st K
Wt. frequency (%)
0
10
20
30
40
50
0.5
1
1.5
2
2.5
3

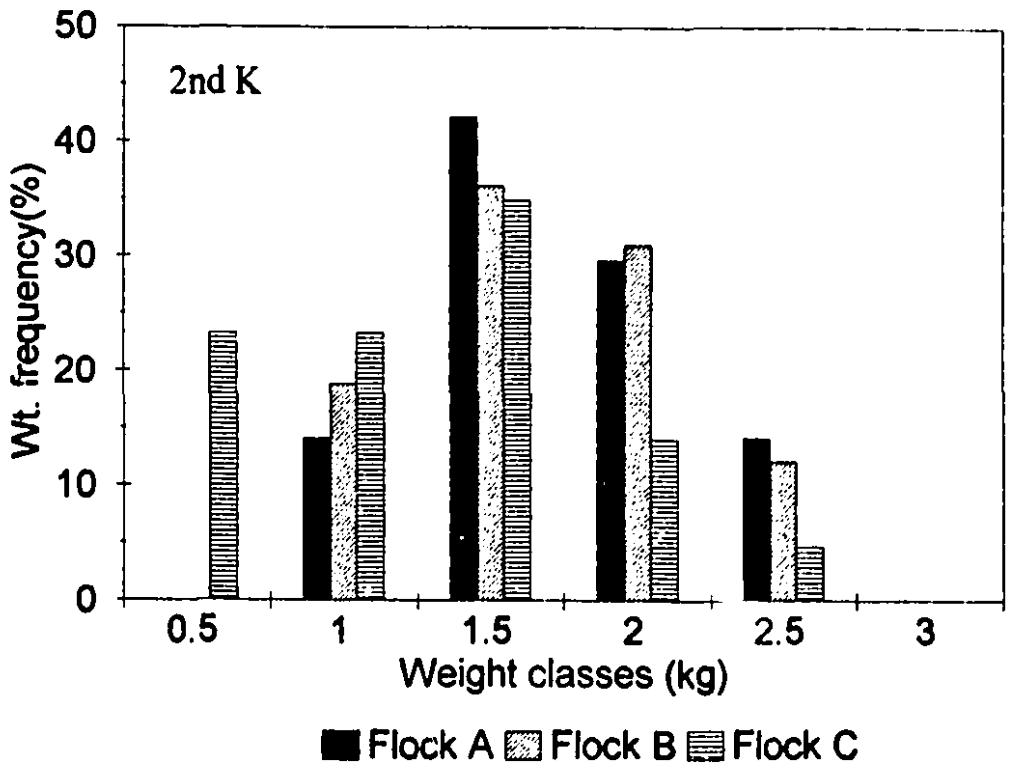

2nd K
Wt. frequency(%)
0
10
20
30
40
50
0.5
1
1.5
2
2.5
3
Weight classes (kg)
Flock A
Flock B
Flock C

Fig. 5.5. Comparison of the monthly probability of survival in lambs (L) and kids (K) from birth to 9 months of age among the three flocks. Lamb survival was comparable among the three flocks but kid survival was substantially lower in flock C than in the other two flocks.

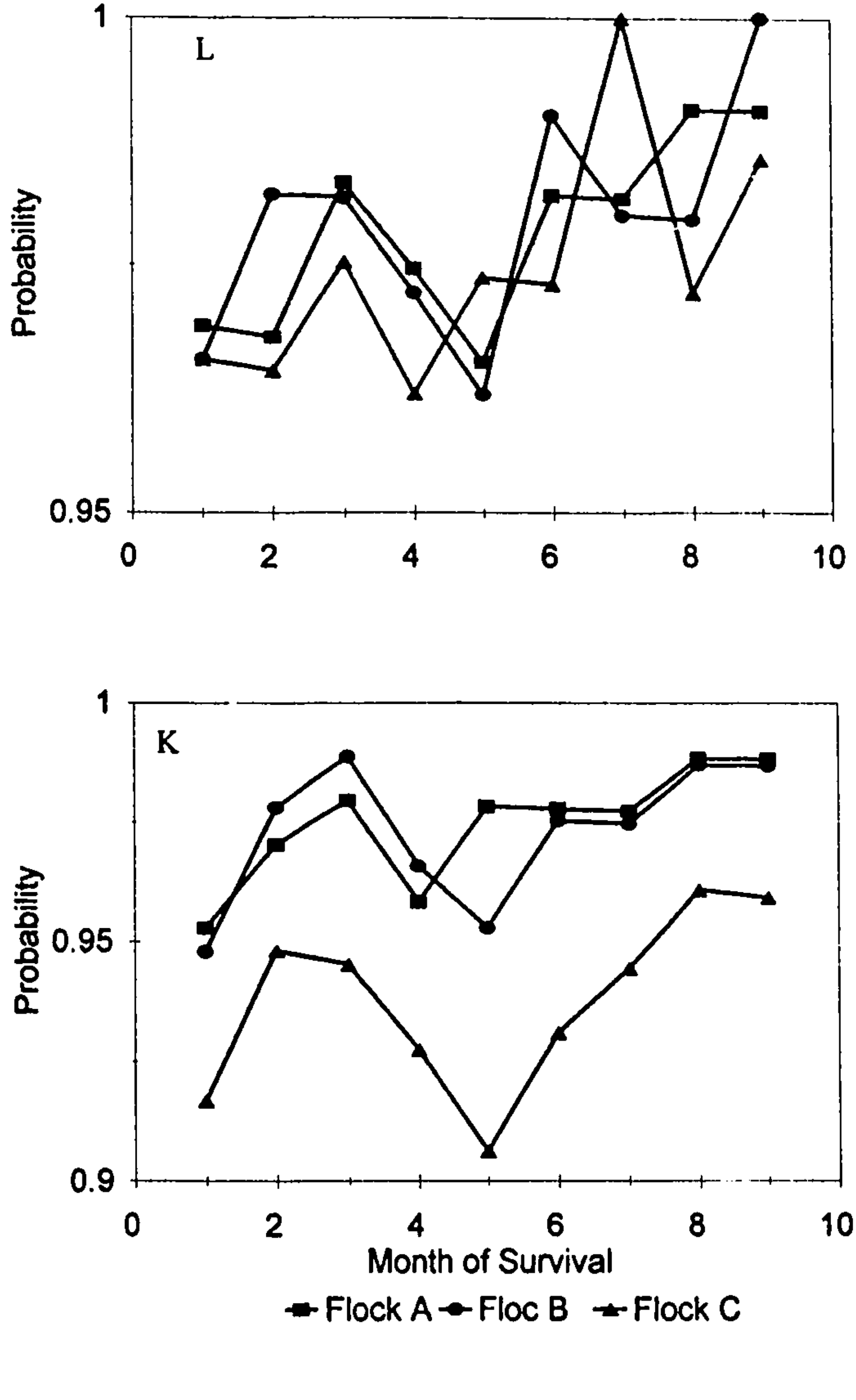
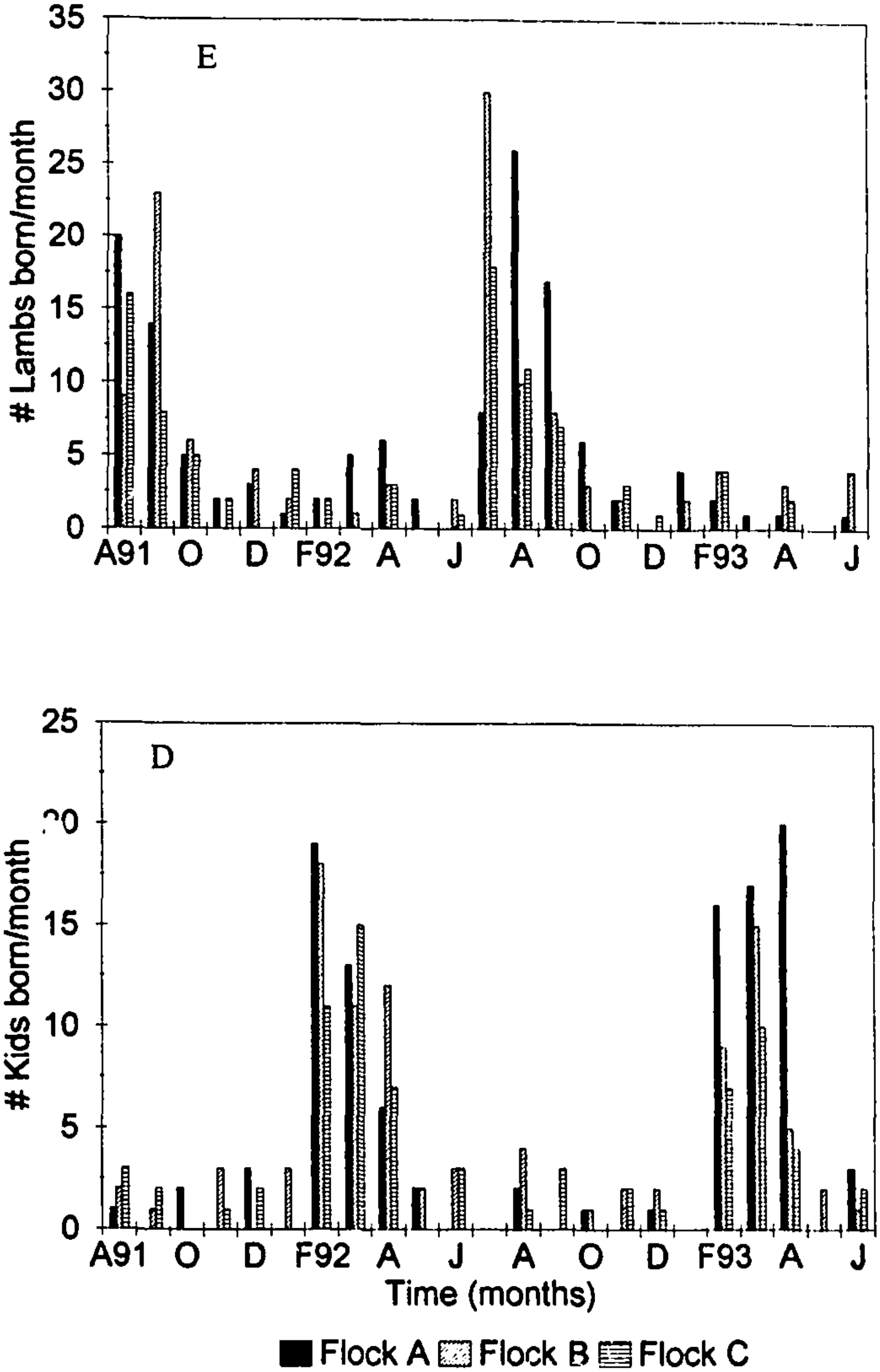

L
1
0.95
Probability
0
2
4
6
8
10
K
1
0.95
0.9
Probability
0
2
4
6
8
10
Month of Survival
Flock A
Floc B
Flock C

Fig. 5.6. Breeding seasons in ewes (E) and does (D) in the three flocks. Despite the occurrence of lambing and kidding throughout the year, definite breeding peaks were observed in both species of animals. The time of the year when this occurred was different for both sheep and goats - July/Aug./Sept. for ewes and Feb./Mar./Apr. for does.


E
Lambs born/month
35
30
25
20
15
10
5
0
A91 O D F92 A J A O D F93 A J
D
Kids born/month
25
20
15
10
5
0
A91 O D F92 A J A O D F93 A J
Time (months)
Flock A Flock B Flock C

CHAPTER 6

FIELD EVALUATION OF FAECAL EGG COUNT AS AN INDEX OF WORM BURDEN IN SHEEP AND GOATS

INTRODUCTION

Relationship between faecal egg output and the worm burden harboured by individual hosts as well as populations of hosts has been a subject of interest to both parasitologists and epidemiologists for a long time (Augustine et al., 1928; Earle and Doering, 1932; Gilles, 1964; Croll et al., 1982; Chai et al., 1981; Sinniah, 1982; Bundy et al., 1985; Sithithaworn et al., 1991 and Sreter et al., 1994). Both technical (Scott and Headlee, 1938; Scott, 1946) and biological (Earle and Doering, 1932; Anderson and May, 1984) problems pertaining to the use of egg output as a reliable indicator of intensity of infection have frequently been highlighted. Specifically, the number of eggs per gram of faeces depends on several factors which include the amount of faeces passed per day, the concentration of eggs within the faeces, the worm load, the age of the worms and the technique used. The underlying biological causes of such factors could be of genetic, environmental, nutritional or behavioural origin (Anderson, 1986). Consequently, the egg output has been described as a qualitative rather than a quantitative measure of worm burden (Anderson and Schad, 1985).

In spite of these constraints, high correlations (r=0.80 - 0.96) between faecal output and worm burden have been recorded in human helminth studies

(Sithithaworn, 1991 and Elkins, 1991). Similar studies in farm animals (Whitlock et al., 1972, ; Roberts and Swan, 1992; Boag et al 1992, Sreter 1994), though limited, have generated useful information for control programmes. The objective of this part of the study was to evaluate the usefulness of egg output as an indicator of intensity of infection during both wet and dry months.

MATERIALS AND METHODS

Just before slaughter, faecal samples were taken for the estimation of egg output. After slaughter, total worm counts were estimated and the combined data grouped into two categories: 1) those collected during the wet months and 2) those collected during the dry months. Only data from flock C was used for the purpose of this experiment.

Statistical analysis:

The data were log-transformed to normalise them. The relationship between adult worm burden and the faecal egg output was estimated by Pearson correlation coefficient. Linear regression analysis was also performed to determine how closely the changes in faecal output were accounted for by changes in the adult worm burden. Non-linear regression of per capita fecundity on worm burden was performed to estimate the degree of density dependence: it was represented by the slopes (exponential term in the equations of the curves) of the plots. T-test was used to compare the monthly per capita fecundity between wet and dry seasons. These analyses were performed using the Statistical Analysis System (SAS: Freund and Littell, 1991).

RESULTS

The descriptive statistics of faecal egg output and worm burden in both sheep and goats are shown in Table 6.1. Both parameters were significantly higher during the wet months than during the dry season in the two species of animals. The sex ratios indicated that, numerically, male worms significantly predominated over females during the dry months; the numbers were statistically equal during the wet season.

Per capita fecundity:

Results of the comparison of the per capita fecundity between the wet and the dry months (Table 6.2) indicated that this parameter was statistically equal in both seasons. In a non-linear regression analysis, the per capita fecundity was inversely related to the worm burden (Fig. 6.1). The slopes of these plots represented the degree of density dependence which was found to be higher during the dry months than the wet months.

Correlation and linear regression analyses:

Relationship between the worm burden and the faecal egg output is summarised in Table 6.3. Pearson correlation coefficients were higher during the wet season than during the dry months. Linear regression plots of egg output on worm burden showed a positive relationship (Fig. 6.2) with higher R-sq values in wet months than in dry months.

DISCUSSION

There has been controversy on whether faecal egg output could be used routinely as a reliable indicator of the intensity of nematode infection. Whitlock et al. (1972) and Roberts and Swan (1992) reported correlation coefficients between egg output and worm burden ranging from 0.49 to 0.83. In contrast, Allonby and Urquhart (1975) reported that egg output was not a reliable index of Haemonchus contortus infection in a Merino sheep flock in Kenya.

The relationship between faecal egg output and the corresponding adult worm burden may be influenced by several factors. In this study, the inverse relationship between the per capita fecundity and the worm burden demonstrated the existence of density-dependence in the reproductive biology of the parasite. Such a situation would tend to weaken the relationship between the egg output and the worm burden. However, while density dependence occurred in both the wet and the dry season, it was more intense during the dry months than the wet months. Similar findings were reported by Croll et al. (1982), Martin et al. (1983), Bundy et al. (1985) and Anderson and Schad (1985). It was therefore concluded that, under the circumstance, the relationship between the egg output and the worm burden was better during the wet periods than during the dry periods. Such a situation is biologically conceivable because it is during the wet season that it is desirable for the parasite to reproduce optimally as the probabilities of successful transmission are then high. It is not surprising therefore that, as shown in Chapter 3, the parasite undergoes such processes as hypobiosis during the dry months in an attempt to reduce its reproductive capacity at a time when transmission would be severely constrained. The lower female worm populations during the dry period gave further evidence of depressed reproduction. During the wet periods, the sex ratio was approximately 1: 1 and this, in view of lower density dependence would lead to better correlation between the egg output and worm burden.

The positive relationship between the egg output and the adult worm burden as shown by the regression lines indicated that, overall, the egg output increased with the worm burden in a linear relationship except among the goats during the dry season when no relationship was found. The reason for this result was unclear. However, in all other cases, both the correlation coefficients and the R-sq values further indicated that changes in worm burden were better reflected by changes in the faecal egg output during the wet months than during the dry months.

From the results of this experiment, it was concluded that, within the range of worm burdens in the host populations, faecal egg outputs could reliably be used as indicators of the intensity of infection during the wet months. For practical purposes, this was a desirable situation because it was during the wet season that the need to estimate the level of infection was most needed. These results were similar in both sheep and goats .

Table 6.1. Descriptive statistics of the data.

a). Means of monthly faecal egg counts (Fec) and adult worm burdens (Twc) in wet and dry months.

Season	Animal spp	n	Mean+S.E	
			Fec	Twc
WET	S	36	3664+428	3202+462
	G	36	2478+221	2443+458
DRY	S	52	841+120	300+39
	G	52	768+140	272+30

S=Sheep G=Goats

b). Monthly means of sex ratios of adult worms. The sex ratios were similar in worms recovered from both sheep and goats.

	Mean Percent ±S.E
	Males
Wet	48.8±0.8
Dry	56.6±0.9

Table 6.2. T-test comparison of monthly per capita fecundity between wet and dry months.

Season	n	Sheep (Mean±S.E)	Goats (Mean±S.E)
WET	9	2.2±0.7	2.0±0.4
DRY	13	6.1±1.1	6.6±1.2
		(t=1.45 p=0.16)	(t=1.62 p=0.11)

Table 6.3. Pearson correlation coefficients of log-transformed faecal egg count (log-fec) and log-transformed adult total worm count (log-twc) in wet and dry months.

Season	Sheep	Goats
WET	0.71 (p=0.0001)	0.58 (p=0.0001)
DRY	0.56 (p=0.0001)	0.07 (p=0.73)

Fig. 6.1. The relationship between the per capita fecundity and the worm burden in sheep and goats during wet (WT-S , WT-G) and dry (DR-S, DR-G) months. The slopes (coefficient of X term) of the plots represent density dependence.

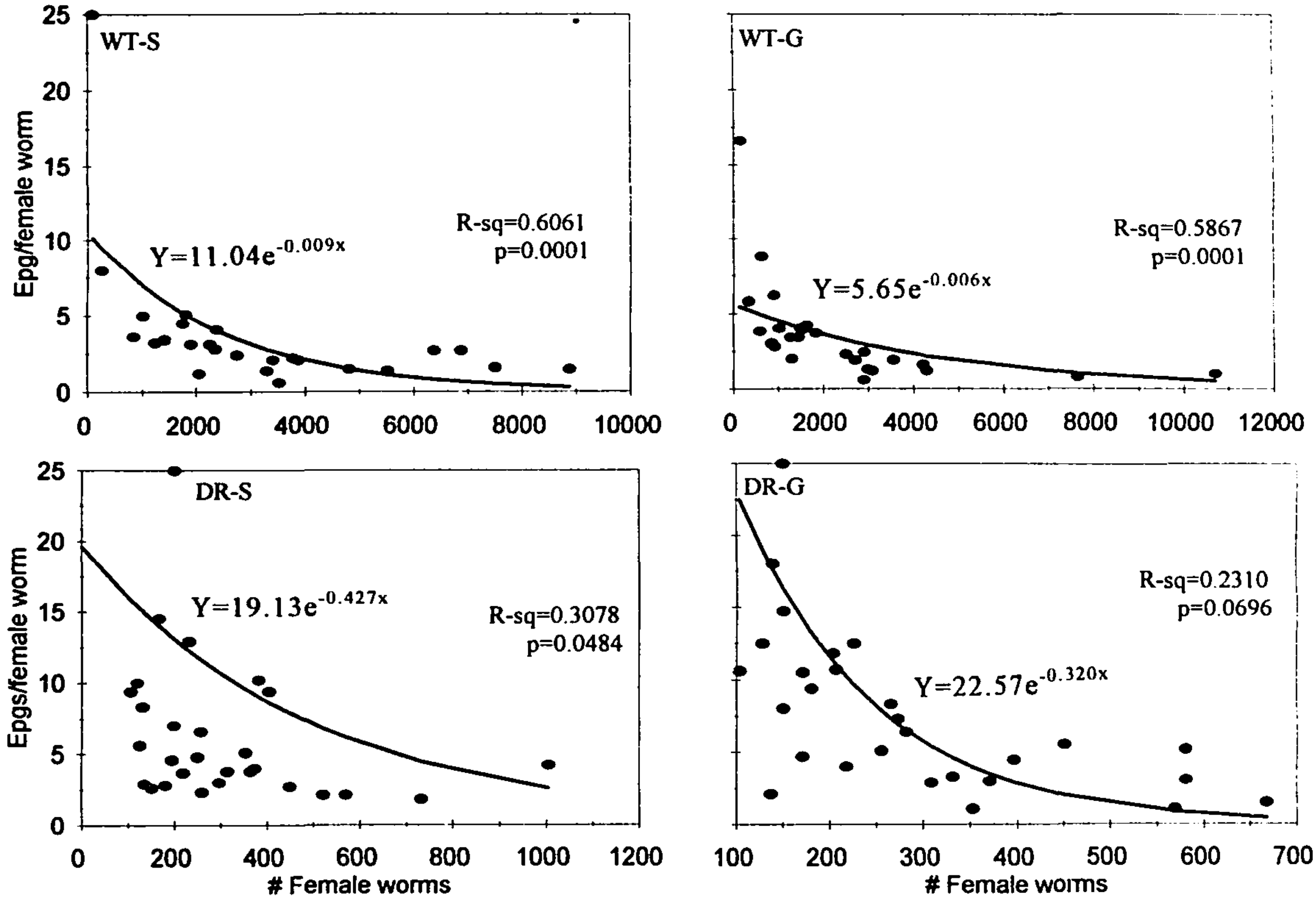
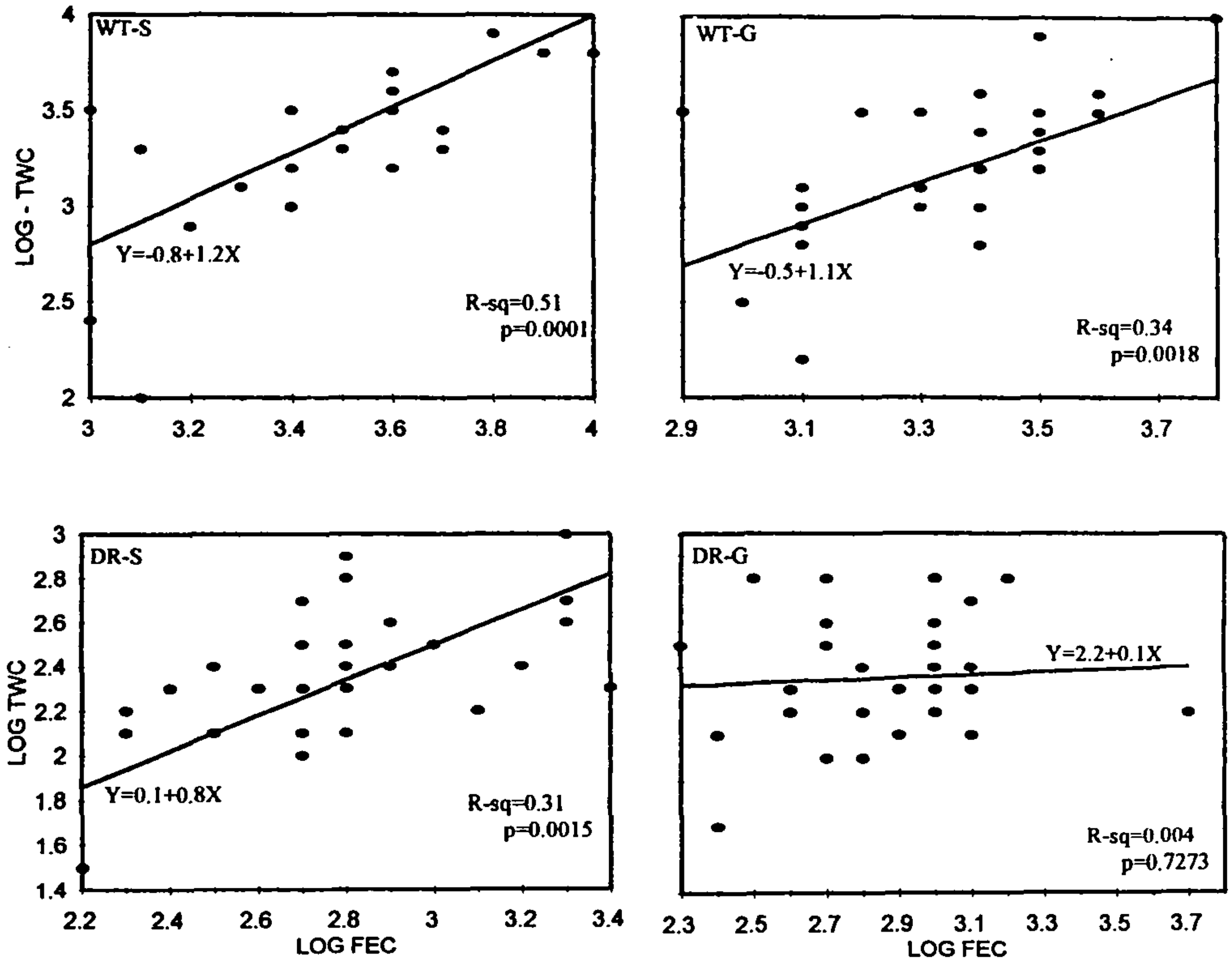

WT-S
Epg/female worm
Y=11.04e^-0.009x
R-sq=0.6061
p=0.0001
WT-G
Y=5.65e^-0.006x
R-sq=0.5867
p=0.0001
DR-S
Epgs/female worm
Y=19.13e^-0.427x
R-sq=0.3078
p=0.0484
# Female worms
DR-G
Y=22.57e^-0.320x
R-sq=0.2310
p=0.0696
# Female worms

Fig. 6.2. Regression plots showing the relationship between the worm burden and faecal egg output in sheep (S) and goats (G) during wet and dry months. The faecal egg output increased with increase in worm burden, a relationship that was stronger (see R-sq values) and more consistent during the wet months (WT-S, WT-G) than during the dry months (DR-S, DR-G).


WT-S
LOG - TWC
Y=-0.8+1.2X
R-sq=0.51
p=0.0001
4
3.5
3
2.5
2
3
3.2
3.4
3.6
3.8
4
WT-G
Y=-0.5+1.1X
R-sq=0.34
p=0.0018
2.9
3.1
3.3
3.5
3.7
DR-S
LOG TWC
Y=0.1+0.8X
R-sq=0.31
p=0.0015
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
2.2
2.4
2.6
2.8
3
3.2
3.4
LOG FEC
DR-G
Y=2.2+0.1X
R-sq=0.004
p=0.7273
2.3
2.5
2.7
2.9
3.1
3.3
3.5
3.7
LOG FEC

CHAPTER 7

LOSS OF SUSCEPTIBILITY TO IVERMERMECTIN BY *HAEMONCHUS CONTORTUS* POPULATION

INTRODUCTION

Development of anthelmintic resistance by gastrointestinal trichostrongylid nematodes of domestic livestock is a worrying concern on a global scale (Prichard et al., 1980, Coles 1986, Waller and Prichard, 1986 and Waller, 1957), especially when it is generally accepted that use of anthelmintics against gastrointestinal nematodes remains the most reliable method for control of these parasites.

Resistance to commonly used anthelmintics has been reported in many regions of the world, for example, Australia (Waller, 1991), New Zealand (Waller, 1986), South America (Santos and Franco, 1967), South Africa (van Wyk and Malan, 1988; van Wyk, 1990) and Western Europe (Boray et al., 1990). In the Eastern Africa region, the first documented case was reported in Kenya (Njanja et al., 1987). Subsequently, more cases were reported in Tanzania (Bjorn et al., 1991) and again in Kenya (Waruiru et al., 1991; Maingi 1991).

The majority of these cases involve the resistance of Haemonchus spp against benzimidazoles thereby suggesting that a serious problem of resistance for this group of anthelmintics exists (Lacey, 1985). However, the problem of resistance is not exclusively confined to the benzimindazoles and Haemonchus spp. Other nematode species such as Trichostrongylus spp. have been reported to have developed resistance against a wide range of anthelmintics (Waller, 1991).

With the introduction of the highly efficacious ivermectin in the early 1980s, high prospects of successful helminth control programmes in the livestock industry were anticipated. Unfortunately, this hope was shattered by the reports of resistance to the anthelmintic soon after being introduced in the market. In South Africa, for example, resistance was reported in one farm after only three treatments with ivermectin (van Wyk and Malan, 1988). To date, many other cases have been reported in many countries including New Zealand (Watson and Hasting 1990), U.S.A. (Craig and Miller, 1990) and Kenya (Mwamachi et al., 1993).

The objective of this experiment was to evaluate the susceptibility of Haemonchus contortus after exposing the parasite population to ivermectin (IVM) at the recommended dose of 200μg/kg for 2 years at the frequency of two treatments a year.

MATERIALS AND METHODS

Source of infective larvae:

Infective larvae (L_3) for this experiment were acquired from flocks A and C at the end of the study in July 1993 after the parasite had been exposed to ivermectin (200µg/kg: 'Ivomec' Merck Sharp and Dohme Ltd. Switzerland) for two years in flock A but not in flock C. These infective larvae are hereafter referred to as Haemonchus strains A (A L_3) and C (C L_3). Pooled faeces from each flock (separated for sheep and goats) were cultured for 10 days at 27^0C. The infective larvae were recovered by Baermannization and standard methods of larval identification (Keith, 1953) were used to determine proportion of the major trichostrongylids present. The recovered larvae were stored at $+4^0$C for one day before artificial infection of lambs and kids.

Experimental animals:

Animals used in this experiment were helminthologically naive weaner lambs and kids (4-6 months old) from the tracer breeding flock.

EXPERIMENTAL PROTOCOL

Two experimental methods were used in this experiment to evaluate the susceptibility of Haemonchus contortus: (1) an in vivo method for estimating the percentage reduction in faecal egg count (FEC) and total worm count (TWC) and (2) an in vitro method for estimating larval migration inhibition of L_3 in a series of IVM concentrations.

(1) In vivo method:

The experimental design used for both lambs and kids is shown in Table 7.1. Forty helminth-naive lambs were randomly divided into two groups. One group was allocated to strain A L_3 and the other to strain C L_3. These were further divided into two sub-groups each, a treatment group (10 lambs) and a control group (10 lambs). On day 0, all the four sub-groups were infected (syringe-drenched) with a suspension of mixed larval inoculum containing 5000L_3s of Haemonchus contortus. On day 25, faecal samples were taken from the rectum of each animal for FEC estimate. The two treatment groups were then treated with IVM at the dose rate of 20µg/kg. This is the dose rate at which 95% of drug-naive Haemonchus contortus should be killed (Shoop, 1993). The control groups were untreated. On day 39 (14 days post treatment), faecal samples were taken a second time for FEC and the lambs in all the groups were subsequently necropsied. Abomasa were removed and processed according to common procedure (Anonymous, 1986) for the estimation of Haemonchus contortus total worm counts.

The following formula (Coles et al., 1992) was used to calculate the percentage reduction (R%) in both the FEC and the TWC.

$$\%R = \left\{ \frac{X_c - X_t}{X_c} \right\} \times 100$$

Where X_c= The arithmetic mean (FEC or TWC) in the control group at day 39.

X_t= The arithmetic mean (FEC or TWC) in the treatment group at day 39.

The same experimental design and procedure was repeated for the kids.

(2) In vitro method:

The method used in this assay was as described by Wagland et al. (1992) with a slight modification. In brief, 10 mg of pure IVM powder was dissolved in 10 ml of dimethyl sulphoxide (DMSO) to make the stock solution. Test solutions were subsequently made in phosphate buffered saline (PBS) in concentrations ranging from 0.1µg IVM/ml to 4µg IVM/ml. About 200 mixed L_3 in 750µl of larval suspension (containing 75% Haemonchus contortus) were pre-incubated in test solutions at room temperature (about 22^0C) for 3hrs and then transferred to larval migration inhibition (LMI) tubes using Pasteur pipettes. The bottom of the (LMI) tubes was made of nylon mesh with a pore diameter of 20µm. The tubes were placed in a 48-well tissue culture plate (Costar, Cambridge, MA, USA) ensuring that the bottom of the LMI tube remained above the bottom of the well. This was done by placing an O-ring around the LMI tube. Care was taken to avoid air bubbles developing at the bottom of the tubes which would prevent smooth larval migration. Positive control tubes containing 200 L_3, PBS and DMSO and negative control tubes containing 200 L_3, in 100µg IVM/ml in PBS were also set up. All the tests were performed in triplicate. The entire set-up was allowed to stand at room temperature for 17 hrs after which the Haemonchus L_3 which had migrated into each well were identified and counted. Subsequently, the number of L_3 inhibited (the dose response) was calculated. Probit analysis on this dose response was carried out to estimate the LD_{50}.

***Statistical analysis*:**

In order to estimate any differences in the rate of establishment and fecundity between the two strains of Haemonchus, total worm counts and faecal egg counts were compared by T-test.

RESULTS

Percentages of major nematode species identified:

Estimation of the percentages of the trichostrongylid genera recovered from faecal cultures showed that Haemonchus was the most prevalent genus followed by Trichostrongylus and Oesophagostomum (Table 7.2).

Comparison of egg output and establishment rates between the two strains of Haemonchus:

25 days post-infection, fecundity was similar in the two strains, in both the control and the treatment groups (Fig. 7.1a). However, marginally higher egg output of strain C was observed among the kids. On day 39, strain A showed significantly

higher fecundity ($p<0.05$) than strain C in both the control and the treated lambs. The two strains showed similar fecundity in kids.

Post-mortem results indicated that, invariably, strain A established significantly better ($p<0.05$) in both lambs and kids than strain C (Tables 7.3a & 7.3b, Fig. 7.1b).

Percent reduction in faecal egg count and total worm count:

Following treatment, neither of the strains was reduced by 95% in either the faecal egg count or the total worm count (Table 7.4, Fig. 7.2). Statistically, percent reductions were similar between the two strains in both lambs and kids. However, trends showed that these reductions were consistently higher in strain C than in strain A.

Dose response:

Results of the probit dose analysis showed that the dose inhibiting 50% of larval migration was significantly higher for strain A than strain C (Table 7.5). Doses for 90% and 95% inhibition were statistically similar in both strains. Dose response curves in Fig.7.3 showed that strain A required marginally higher doses than strain C for the same level of inhibition.

DISCUSSION

Drug resistance has been defined as a change in the gene frequency of a population that is produced by drug selection whereby more drug is required to exact some effect than was required prior to selection (Shoop 1993). This author further emphasises that the generally accepted concept that resistance to broad-spectrum anthelmintics only occurs when the therapeutic level is no longer effective, misses the defining moment of resistance development. This means that by the time resistance is formally recognised, the gene frequency of resistant strains in a particular population will have attained levels far beyond the initial stages of resistance development.

Resistance to IVM has already been reported in coastal Kenya (Mwamachi et al., 1993) in a farm where the drug had been used indiscriminately for 5 years. It is noteworthy in this case that resistance was not only observed at the therapeutic dose (200μg/kg) but at three times that dose. According to Shoop (1993), IVM is 95% efficacious against IVM-naive Haemonchus at 20μg/kg. Within this context then, resistance on the Kenyan coast property had developed thirty times over as compared to IVM-naive Haemonchus .

In a previous experiment in Chapter 3, the use of IVM at the therapeutic level was found to be 100% efficacious at the initial stages of the experiment. However, results of the last part of the experiment showed that treatment did not reduce the faecal egg count to nil. This meant two possibilities - either the faecal egg counts had dropped down to nil immediately after treatment and that faecal samples were taken at a time when egg counts were already rising again or that the efficacy of ivermectin was declining. The current experiment compared the susceptibility of this Haemonchus contortus strain with a strain that was not exposed to IVM. Susceptibility was compared at the dose rate of 20μg IVM/kg. Essentially, this protocol was expected to detect smaller changes in susceptibility than those that would be detected by the therapeutic dose.

Following treatment, neither of the strains attained 95% reduction in either faecal egg output or total worm count. This could be attributed to the low dose of IVM used. Although the percent reductions were statistically similar in both strains, examination of the data indicated that in all cases, the percent reduction in strain A was consistently lower than that in strain C. Taking into consideration the low frequency (2 times a year) at which the drug had been used, this observation suggested that differences in IVM susceptibility were taking place between the two strains.

The significantly higher LD_{50} in strain A on the Probit analysis, further suggested that susceptibility of this strain to IVM was decreasing. Although at the higher LD_{90} and LD_{95} the doses were statistically similar, the trend indicated slightly higher doses for strain A than for strain C.

Comparison of the faecal egg count and total worm counts within the control and the treatment groups indicated that strain A had higher fecundity and better establishment. It has been shown elsewhere that higher fecundity and establishment rates may be selected for concurrently with selection for drug resistance (Kelly et al., 1978; Maingi et al., 1990). It was probable in this study that the increased fecundity and higher establishment were a result of selection and this could have occurred concurrently with selection for reduced drug susceptibility in strain A.

In a few instances in this experiment, the results in kids gave different trends from the corresponding trends in the lambs. This could be attributed to a number of factors including the dose rate, tolerance and animal species differences. The same dose rate was used in both the lambs and the kids. It is probable that the metabolism of IVM in the two animal species could be sufficiently different to cause aberrant responses especially at low doses such as the ones used in this study. Determining dose rates specific for each species would therefore be necessary.

In spite of such technical problems, results in this study suggested that the efficacy of IVM against strain A was declining.

Table 7.1. Experimental design for the study of susceptibility of Haemonchus contortus strains recovered from flocks A and C.

Group	Sub-Group	Number of animals	Source of infection	Day 25 (Treatment)	Day 39 (Necropsy)
I	Treatment	10	Flock A	20µg/kg & FEC	FEC & Kill
	Control	10	Flock A	No Trt., FEC	FEC & Kill
II	Treatment	10	Flock C	20µg/kg & FEC	FEC & Kill
	Control	10	Flock C	No Trt., FEC	FEC & Kill

Table 7.2. Abundance (%) of major trichostrongylid nematodes as estimated from pooled faecal cultures.

	Total number of larvae counted	% Haemon.	Trichos.	Oesoph.
Strain A				
Sheep	489	75.9	17.6	6.5
C.L.		(71.8-79.8)	(14.7-21.7)	(4.9-9.6)
Goats	513	69.6	19.1	11.3
C.L.		(65.8-73.9)	(15.7-22.7)	(8.5-14.1)
Strain C				
Sheep	609	73.5	19.7	6.8
C.L.		(70.4-85.7)	(16.6-23.8)	(5.2-9.3)
Goats	493	71.3	15.9	12.8
C.L.		(66.8-74.9)	(12.9-19.5)	(10.2-16.3)

Table 7. 3a. T-test comparison between strain A and strain C faecal egg count (FEC) and adult total worm count (TWC) in control groups.

Lambs

	FEC ± SE 0 dpt	FEC ± SE 14 dpt	TWC ± SE 14 dpt
Strain A	8970 ± 1457	16895 ± 3071	1589 ± 387
Strain C	6250 ± 761	10080 ± 649	543 ± 103
t	1.65	2.17	2.61
P	0.12	0.04	0.02

Kids

	FEC ± SE 0 dpt	FEC ± SE 14 dpt	TWC ± SE 14 dpt
Strain A	1728 ± 575	5700 ± 1245	1084 ± 230
Strain C	3439 ± 1868	9088 ± 3800	1717 ± 198
t	0.88	0.85	2.08
P	0.39	0.41	0.05

dpt = days post treatment

Table 7.3b. T-test comparison between strain A and strain C faecal egg count (FEC) and adult total worm count (TWC) in treatment groups.

Lambs

	FEC ± SE 0 dpt	FEC ± SE 14 dpt	TWC ± SE 14 dpt
Strain A	8360±1624	4355±1146	669±129
Strain C	7470±1169	1490±412	89±19
t	0.44	2.35	4.45
P	0.33	0.02	0.00

Kids

	FEC ± SE 0 dpt	FEC ± SE 14 dpt	TWC ± SE 14 dpt
Strain A	2550±969	1761±521	286±67
Strain C	6196±1225	2690±700	631±155
t	-2.33	-1.06	-2.04
P	0.02	0.15	0.03

dpt = days post treatment

Table 7.4. Post-treatment percent reduction in faecal egg count (FEC) and adult total worm count (TWC) between strains A and C.

		% Reduction	
		FEC	TWC
Lambs			
	Strain A	74.2	57.9
	C.L	(50.4 - 86.6)	(20.4 - 77.7)
	Strain C	85.2	83.6
	C.L	(73.5 - 91.7)	(70.5 - 90.9)
Kids			
	Strain A	69.1	63.2
	C.L	(40.6 - 73.2)	(44.2 - 82.6)
	Strain C	70.4	73.6
	C.L	(59.1 - 84.4)	(54.3 - 89.5)

Table 7.5. Probit analysis of dose response to ivermectin in a larval migration inhibition assay of strains A and C infective larvae.

	Dose (μg/ml) with confidence limits	
% Inhibition	Strain A	Strain C
50	**2.67** (2.56, 2.78)	**2.21** (2.12, 2.30)
90	**8.20** (7.48, 9.11)	**6.79** (6.24, 7.49)
95	**12.02** (10.70, 13.74)	**9.95** (8.92, 11.29)

Fig. 7.1a. T-test comparison of faecal egg output within treatment (Trt) and control (Ctr) groups.

Error bars represent egg output variations among individual animals in each group.

Level of significant difference $p<0.05$.

Key to legend:

A:Ls=Strain A in Lambs	C:Ls=Strain C in Lambs
A:Ks=Strain A in Kids	C:Ks=Strain C in Kids

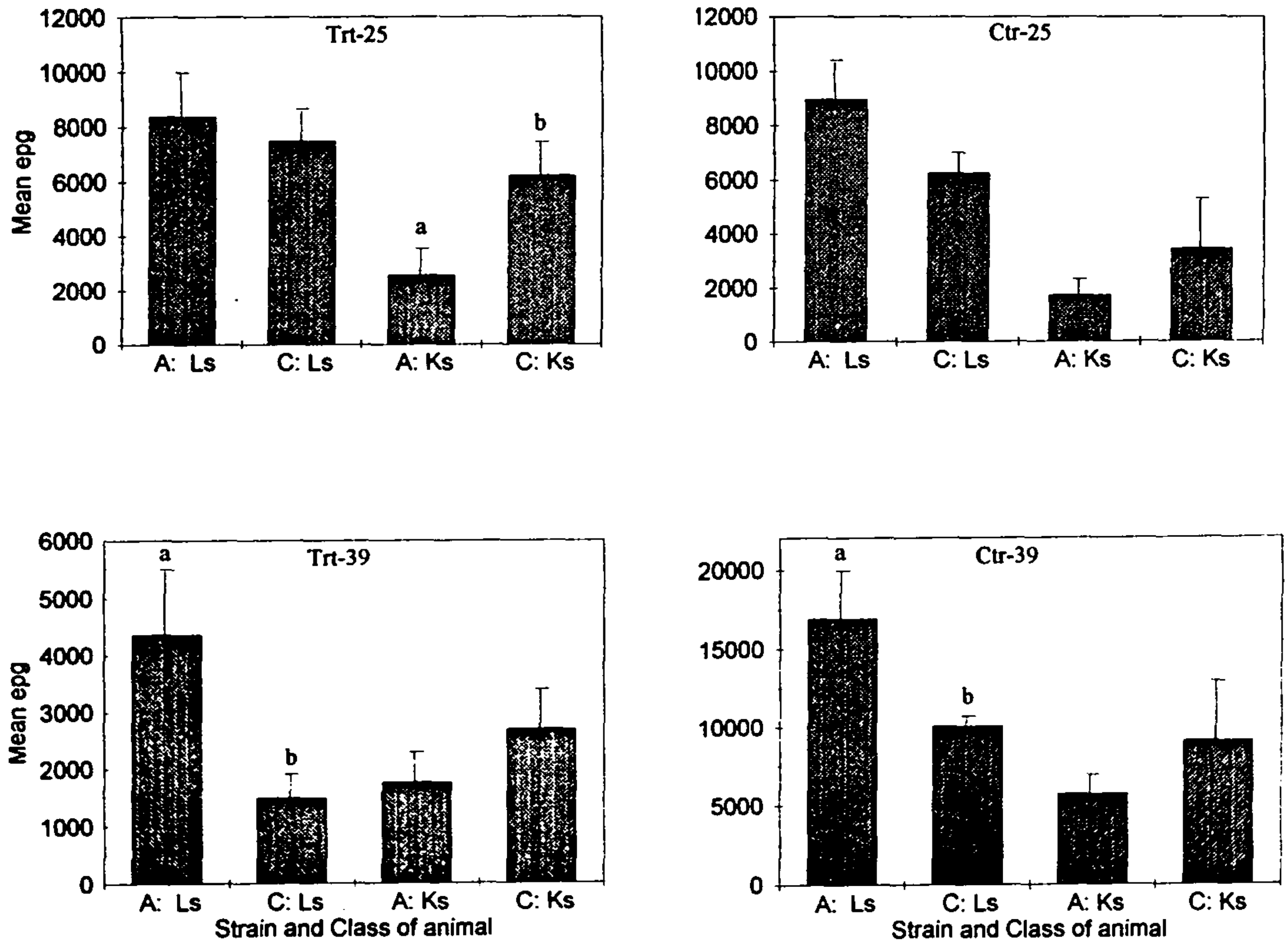

Trt-25
Mean epg
12000
10000
8000
6000
4000
2000
0
a
b
A: Ls
C: Ls
A: Ks
C: Ks
Ctr-25
12000
10000
8000
6000
4000
2000
0
A: Ls
C: Ls
A: Ks
C: Ks
Trt-39
Mean epg
6000
5000
4000
3000
2000
1000
0
a
b
A: Ls
C: Ls
A: Ks
C: Ks
Strain and Class of animal
Ctr-39
20000
15000
10000
5000
0
a
b
A: Ls
C: Ls
A: Ks
C: Ks
Strain and Class of animal

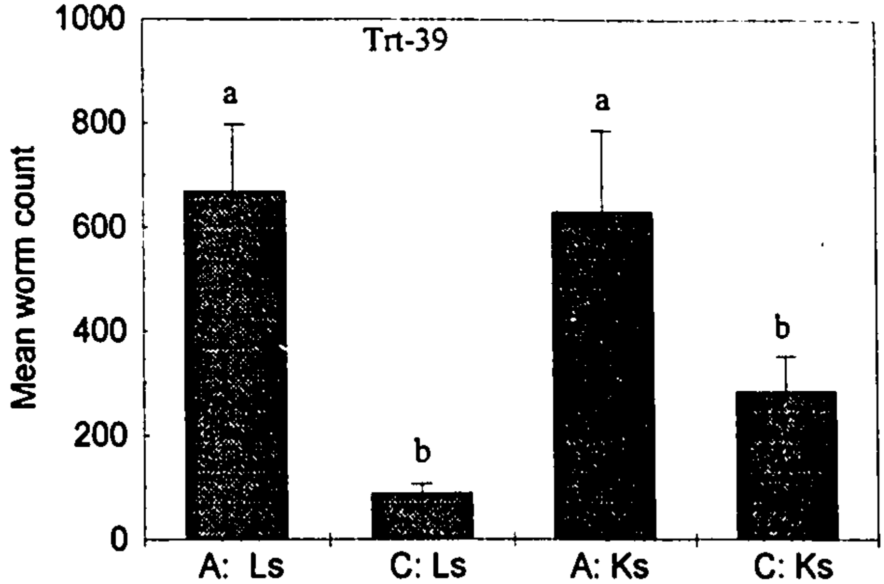
Fig. 7.1b. T-test comparison of total worm count within treatment (Trt-39) and control (Ctr-39) groups.

Error bars represent egg output variations among individual animals in each group.

Level of significant difference $p<0.05$

Key to legend:

A:Ls=Strain A in Lambs	C:Ls=Strain C in Lambs
A:Ks=Strain A in Kids	C:Ks=Strain C in Kids


Trt-39
Mean worm count
1000
800
600
400
200
0
a
b
a
b
A: Ls
C: Ls
A: Ks
C: Ks

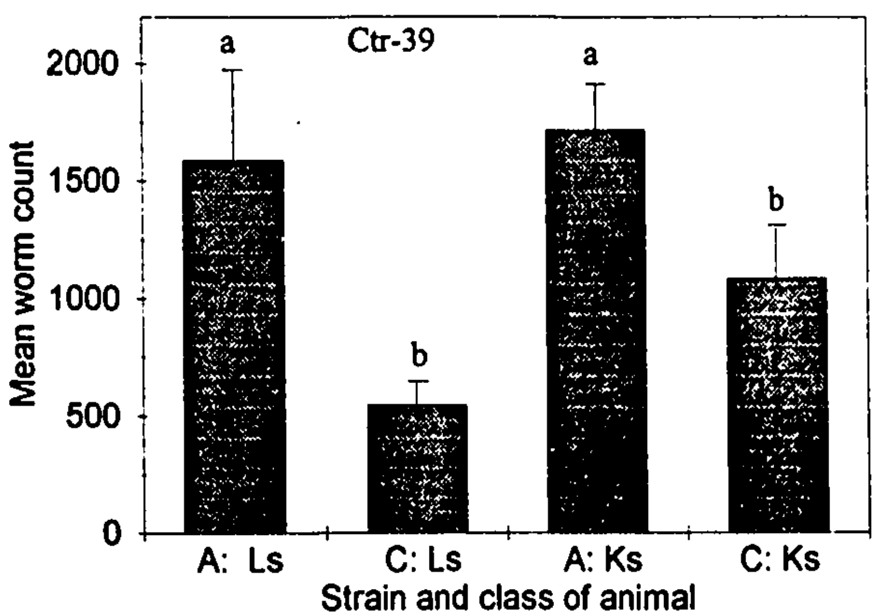

Ctr-39
Mean worm count
2000
1500
1000
500
0
a
b
a
b
A: Ls
C: Ls
A: Ks
C: Ks
Strain and class of animal

Fig. 7.2. Comparison of percent reductions in faecal egg output (L-Fec & K-Fec) and total worm count (L-Twc & K-Twc) between control and treated groups 14 days post-treatment.

Error bars represent variations in individual animals in a group.

Key to legend:

A:Cnt=Strain A control   A:Trt=Strain A treated

C:Cnt=Strain C control   C:Trt=Strain C treated

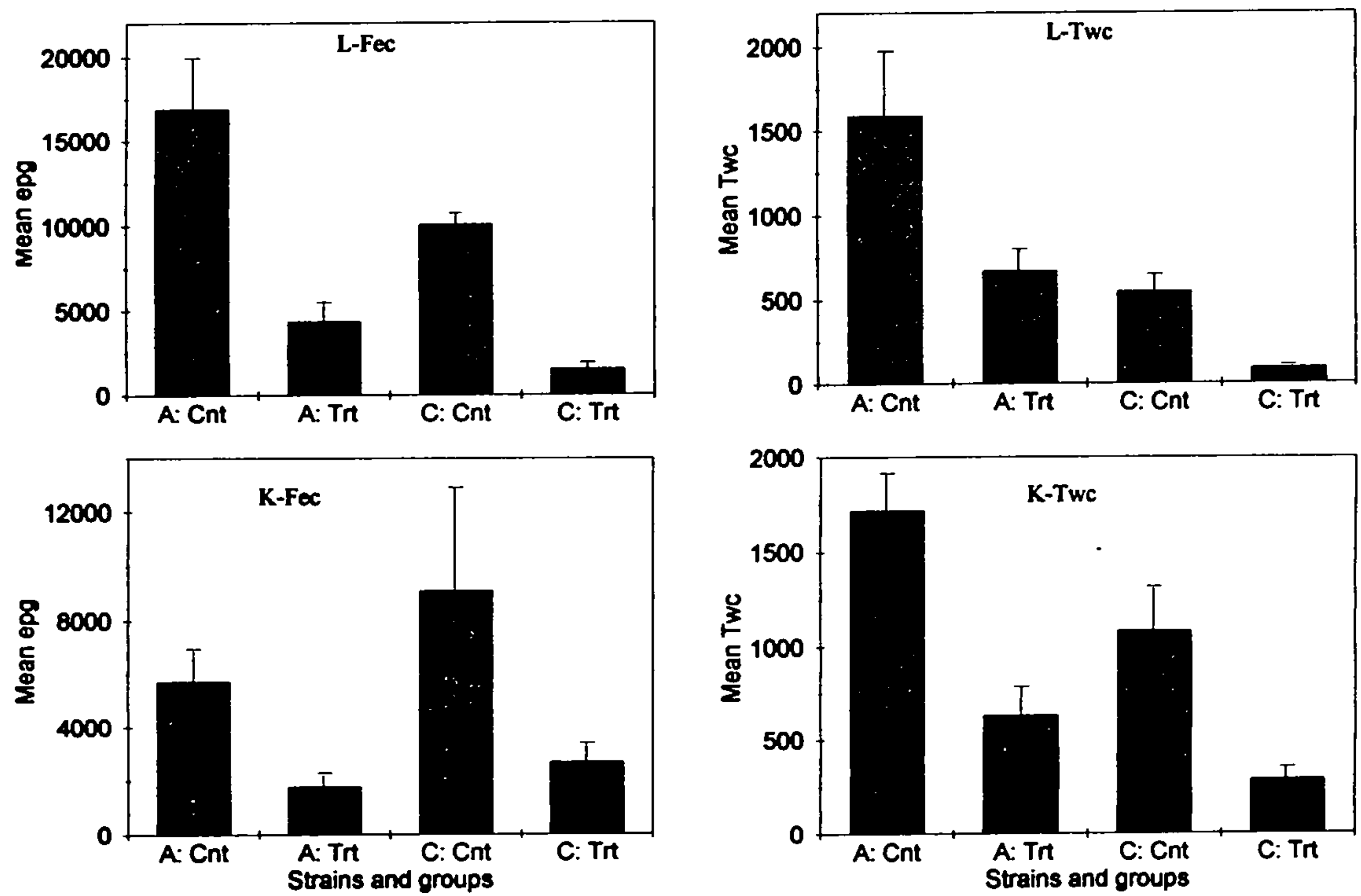
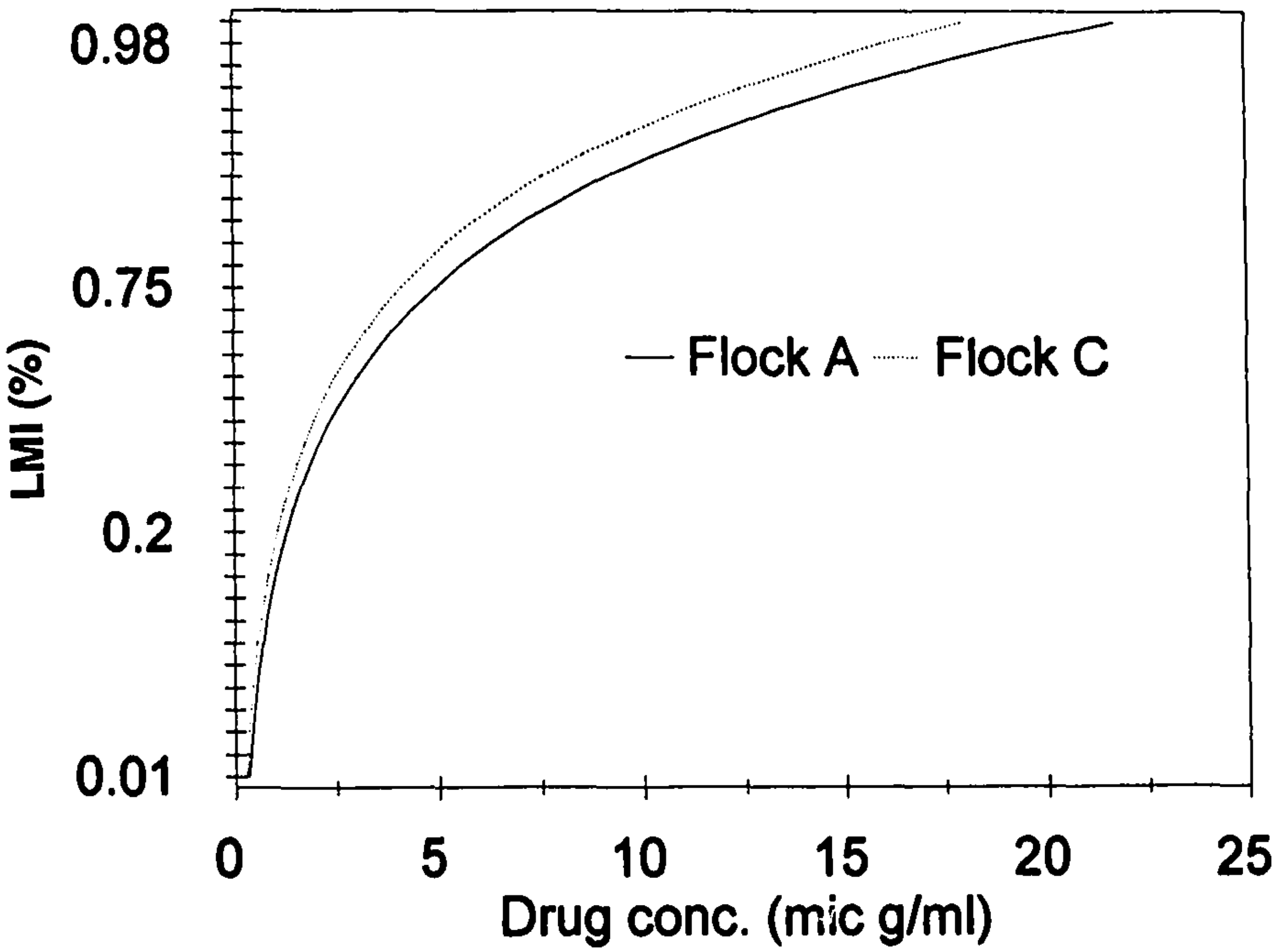

L-Fec
Mean epg
20000
15000
10000
5000
0
A: Cnt
A: Trt
C: Cnt
C: Trt
L-Twc
Mean Twc
2000
1500
1000
500
0
A: Cnt
A: Trt
C: Cnt
C: Trt
K-Fec
Mean epg
12000
8000
4000
0
A: Cnt
A: Trt
C: Cnt
C: Trt
Strains and groups
K-Twc
Mean Twc
2000
1500
1000
500
0
A: Cnt
A: Trt
C: Cnt
C: Trt
Strains and groups

Fig. 7.3. Comparison of dose response to ivermectin between strains A and C infective larvae in a larval migration inhibition assay. Throughout the plot, strain A required higher doses of ivermectin than strain C for similar levels of inhibition.


0.98
0.75
0.2
0.01
LMI (%)
Flock A
Flock C
0
5
10
15
20
25
Drug conc. (mic g/ml)

CHAPTER 8

GENERAL DISCUSSION AND CONTRIBUTIONS TO ORIGINAL KNOWLEDGE

GENERAL DISCUSSION

The main objective of this thesis was to address the following questions: Does hypobiosis significantly feature in the epidemiology of haemonchosis of small ruminants in semi-arid areas of Kenya? If so, are there seasonal variations in its extent? How do these variations influence the dynamics of pasture infectivity and the performance of the flocks that graze on these pastures? What is the most effective treatment regime that would minimise both pasture infectivity and host worm burden thereby improving performance in animal productivity?. A secondary objective was to evaluate the susceptibility of Haemonchus contortus to ivermectin at the end of the study.

In an effort to answer these questions, a series of experiments were conducted on three flocks of mixed sheep and goats that were randomly assigned different treatment regimes. The three treatment regimes were: 1) Treatment administered 4 weeks before the onset of the rains; 2) Treatment administered 4 weeks after the onset of the rains; and 3) The control where no treatment was administered.

No detailed work on hypobiosis has been conducted in Kenya, but Allonby and Urquhart (1975) suggested that hypobiosis may not be an important factor in the epidemiology of haemonchosis in Kenya. The argument has been that, there being a bimodal rainfall pattern, the dry period between successive rain periods is too short to warrant the parasite going into the arrested stage. The results of the current work contrast this view as shown and discussed in Chapter 3 of this thesis. Hypobiosis was found at levels ranging from zero percent during the wet periods to about 80% during the dry periods. In my view, the important factor to consider with regard to suitability of parasite transmission in this area is the marked difference in the distribution of rainfall between the "long" and "short" rains. The amount of rainfall during the "long rains season" is spread out over long periods that allow growth and sustenance of substantial herbage; the short rains come in bouts that are too short to allow growth and sustenance of herbage cover. It is the state of this herbage cover that in turn influences the level of parasite transmission.

There was an average of 12-14 days of rain a month during the long rain seasons and only 3-5 days of rain during the short rains season. The implication of this was that rainfall during the short rains season was concentrated over a relatively short duration. Followed by a dry spell, this scenario does not favour vegetation growth while a similar amount of rainfall spread out over a longer period of time would favour such growth. As a result of this, the parasite would still go into or remain in a state of hypobiosis during the short rains since the chances of transmission are still severely limited. This was evident in the period Dec, 1992-Jan,1993 when the unexpected high rainfall did not influence the high level of hypobiosis already in place (Figs. 3.7, 3.9, and 3.11).

Treatment administered before the onset of the rains substantially reduced the population of both the adult worms and the hypobiotic larvae while treatment during the rains only reduced the egg output temporarily. It did not influence the population of hypobiotic larvae simply because there were virtually none by the time it was administered. The temporary reduction in the egg output implied that the animals were immediately re-infected from the already highly contaminated pastures. The effects of treatment as expressed by the changes in pasture contamination and the egg output is the subject matter of Chapter 4. About 4 weeks after the onset of the rains, rise in egg output was observed in both the control flock and the flock treated during the rains. It was concluded that this was a result of resumption of development of arrested larvae. A delayed onset of egg output was observed in the flock treated before the onset of the rains. From this observation, it was concluded that treatment at this time removed most of the hypobiotic larvae so that, at the time of resumed development, the resulting adult population contributing to the egg output was comparatively low. This was evidenced by both a lower pasture larval count and a temporal shift in the appearance of infective larvae on pasture herbage. In the long term, this treatment strategy was considered to have good prospects for reducing pasture infectivity.

The effect of different treatment regimes on the health and productivity of the flocks is discussed in chapter 5. In all the parameters measured, the performance of the flock treated before the onset of the rains was either better or equal to the performance of the flock treated during the rains. It was therefore evident that treatment before the rains offered higher benefits than treatment administered during the rains. Both of these treatment regimes produced better flock performance than the regime of no treatment.

An attempt was made in Chapter 6 to determine how reliable faecal egg output is as a measure of worm burden. Results indicated that faecal egg output was better as a measure of worm burden during the wet season than during the dry season. This was a favourable situation since it is during the wet season that livestock owners would be interested in the estimation of worm burdens in their stock. For practical purposes, this relationship was considered reasonably reliable.

Resistance against ivermectin in recent years has been a major concern as is evident from numerous reports (Prichard et al., 1980; Borgsteede, 1990; Waller, 1991; Waruiru et al., 1991; Mwamanchi et al., 1993 and Shoop, 1993). The main objective of Chapter 7 was to evaluate the susceptibility of Haemonchus contortus to ivermectin at the end of the study. By then, the animals had been exposed to the anthelmintic for 4 times at the recommended dose of 200µg/kg. The infective larvae used were acquired from the control flock and the flock treated before the rains. Larvae from the flock treated during the rains were not tested. This was because it was suspected that, of the two treated flocks, resistance would most likely show up first in the flock treated before the rains because the selection pressure was higher in this case. Both in vivo and in vitro methods of estimation were used and tested at the dose rate of 20µg/kg. Drug-naive Haemonchus contortus is known to be 95% susceptible to this dose rate. Consequently, susceptibility of the Haemonchus population was assessed at this level and not at the recommended use level. The significance of such assessment is that loss of susceptibility is detected at the moment when it first starts developing. After treatment, results indicated that the infective larvae from the exposed flock established better, had a higher fecundity and elicited a lower reduction in egg output and worm burden than the infective larvae from the unexposed flock suggesting that the drug-exposed parasite strain was losing susceptibility to the drug. This was supported by earlier results in Chapter 3 which showed failure to reduce the egg output to nil in the flock treated before the rains. This rapid loss of susceptibility was attributed to the high selection pressure exerted by the drug under the circumstances of this treatment regime. The generation of parasites in the pasture subsequent to a given treatment was virtually made up of the progeny of hypobiotic larvae surviving the treatment. With the high efficacy of ivermectin, this scenario exacerbated the selection pressure.

CONTRIBUTIONS TO ORIGINAL KNOWLEDGE.

1. This is the first detailed report of hypobiosis in Kenya. It has been established that hypobiosis is an important epidemiological factor in haemonchosis that should be taken into account in designing control programmes in semi-arid areas of Kenya. It was estimated that the level could be up to 80% during the dry seasons.

2. It has clearly been shown that the majority of hypobiotic larvae could be missed if the search for them was exclusively confined to the standard HCl-Pepsin mucosal digests. About 90% of the hypobiotic larvae in this study were found in the abomasal contents and washings.

3. Birth weights of lambs and kids are substantially improved as a result of anthelmintic treatments administered to their mothers.

4. Targeting hypobiotic larvae for treatment may accelerate loss of susceptibility of Haemonchus contortus to ivermectin.

5. Treatment before the rains delays the onset of the appearance of infective larvae on pasture thereby introducing a temporal shift on the occurrence of clinical helminthiasis. In addition, this treatment also reduces the overall level of worm burden and the subsequent pasture contamination. The consequences of this were evident in the improved performance in flock A particularly in increased birth weights.

6. Worm burdens are more accurately estimated by faecal egg output during the wet season than during the dry season.

7. Lambs and kids born of ewes and does in their first parturition experience higher mortality rates than those born of dams in their second and subsequent parturitions. In herd management therefore, special attention should be given to this class of dams that presents a potential source of losses.

8. The short rains season is effectively a stressful period for the survival of Haemonchus contortus in this area.

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Appendix 5.1. Repeated measures analysis of variance of monthly faecal egg output of class of animals in the three flocks:

a). Ewes:

Between subject effects:

Source	DF	F value	P
Flock	2	17.87	**0.0030**
Error	166		

Comparison at $p<0.05$: Flocks A^a B^a C^b

Within subject effects:

Source	DF	F value	P
Time	22	6.46	**0.0001**
Time*Flock	44	1.85	**0.0237**
Error(Time)	3652		

b). Does:

Between subject effects:

Source	DF	F value	P
Flock	2	12.28	**0.0012**
Error	142		

Comparison at $p<0.05$: Flocks A^{a} B^{a} C^{b}

Within subject effects:

Source	DF	F value	P
Time	22	3.29	**0.0316**
Time*Flock	44	1.96	**0.0967**
Error(Time)	3124		

c). Lambs:

Between subject effects:

Source	DF	F value	P
Flock	2	29.84	**0.0008**
Error	147		

Comparison at $p<0.05$: Flocks A^a B^a C^b

Within subject effects:

Source	DF	F value	P
Time	22	3.05	**0.0241**
Time*Flock	44	0.65	**0.7598**
Error(Time)	3234		

d). Kids:

Between subject effects:

Source	DF	F value	P
Flock	2	20.19	**0.0022**
Error	114		

Comparison at $p<0.05$: Flocks A[a] B[a] C[b]

Within subject effects:

Source	DF	F value	P
Time	22	4.93	**0.0004**
Time*Flock	44	0.97	**0.5020**
Error(Time)	2508		

Appendix 5.2. Repeated measures analysis of variance of monthly packed cell volume of each class of animals in the three flocks:

a). Ewes:

Between subject effects:

Source	DF	F value	P
Flock	2	14.81	**0.0048**
Error	166		

Comparison at $p<0.05$: Flocks A[a] B[a] C[b]

Within subject effects:

Source	DF	F value	P
Time	22	2.48	**0.0713**
Time*Flock	44	1.63	**0.1675**
Error(Time)	3652		

b). Does:

Between subject effects:

Source	DF	F value	P
Flock	2	52.29	**0.0002**
Error	142		

Comparison at $p<0.05$: Flocks A^{a} B^{a} C^{b}

Within subject effects:

Source	DF	F value	P
Time	22	0.87	**0.4977**
Time*Flock	44	0.93	**0.5071**
Error(Time)	3124		

c). Lambs:

Between subject effects:

Source	DF	F value	P
Flock	2	65.72	**0.0001**
Error	147		

Comparison at $p<0.05$: Flocks A^a B^a C^b

Within subject effects:

Source	DF	F value	P
Time	22	0.59	**0.6740**
Time*Flock	44	0.82	**0.5929**
Error(Time)	3234		

d). Kids:

Between subject effects:

Source	DF	F value	P
Flock	2	7.42	**0.0238**
Error	114		

Comparison at $p<0.05$: Flocks A^a B^a C^b

Within subject effects:

Source	DF	F value	P
Time	22	1.57	**0.2139**
Time*Flock	44	0.47	**0.8629**
Error(Time)	2508		

Appendix 5.3. Repeated measures analysis of variance of monthly liveweights of sheep and goats in the three flocks.

a). Sheep:

Between subject effects:

Source	DF	F value	P
Flock	2	35.86	**0.0005**
Error	166		

Comparison at $p<0.05$: Flocks A^a B^{ab} C^b

Within subject effects:

Source	DF	F value	P
Time	22	0.91	**0.4882**
Time*Flock	44	0.35	**0.9595**
Error(Time)	3652		

b). Goats:

Between subject effects:

Source	DF	F value	P
Flock	2	13.74	**0.0058**
Error	142		

Comparison at $p<0.05$: Flocks A^{a} B^{ab} C^{b}

Within subject effects:

Source	DF	F value	P
Time	22	0.61	**0.6951**
Time*Flock	44	0.82	**0.6159**
Error(Time)	3124		

Appendix 5.4a. Repeated measures analysis of variance of body liveweight changes of lambs and kids from birth to 9 months of age.

1). Lambs:

Between-subjects effects:

Source	DF	SS	MS	F value	Pr > F
Flock	2	332.7	166.4	5.1	**0.0125**
Error	136	942.3	32.5		

Within-subjects effects:

Source	DF	SS	MS	F value	Pr > F
Age	8	847.7	1005.9	1339.2	**0.0001**
Age*Flock	16	68.5	4.3	5.7	**0.0001**
Error(Time)	1088	174.3	0.8		

Flock pair-wise comparisons at p<0.05): A^{a} B^{ab} C^{b}

2). Kids:

Between-subjects effects:

Source	DF	SS	MS	F value	Pr > F
Flock	2	239.9	119.9	8.2	**0.0023**
Error	103	306.6	14.6		

Within-subjects effects:

Source	DF	SS	MS	F value	Pr > F
Age	8	4923.1	615.4	1596.9	**0.0001**
Age*Flock	16	41.8	2.6	6.8	**0.0001**
Error(Time)	824	64.7	0.4		

Flock pair-wise comparisons at $p<0.05$: A^{a} B^{ab} C^{b}

Appendix 5.4b. One-way analysis of variance of the final weights attained by lambs and kids at 9 months of age.

1). Lambs:

Source	DF	SS	MS	F value	P
Model	2	92.8	46.4	6.2	**0.0057**
Error	134	217.1	7.5		
Corrected Total	136	309.9			

Pair-wise flock comparisons: Flocks A^{a} B^{ab} C^{b}

2). Kids:

Source	DF	SS	MS	F value	P
Model	2	50.9	25.4	9.6	**0.0011**
Error	101	55.6	2.6		
Corrected Total	103	106.5			

Pair-wise comparisons at $p<0.05$: Flocks A^{a} B^{ab} C^{b}

Appendix 5.5. One-way analysis of variance of birth weights of lambs and kids in the three flocks

1). Lambs:

Source	DF	SS	MS	F value	P
Model	2	21.5	10.8	45.4	**0.0001**
Error	326	80.0	0.2		
Corrected Total	328	101.5			

Pair-wise comparisons of flock means (Bonferroni T-test):

Flocks A[a] B[b] C[c]

2). Kids:

Source	DF	SS	MS	F value	P
Model	2	9.8	4.9	20.7	**0.0001**
Error	271	64.3	0.2		
Corrected Total	273	74.2			

Pair-wise comparisons (Bonferroni T-test):

Flocks A[a] B[b] C[c]

Appendix 5.6. Analysis of variance of age and weight at first conception in ewes and does among the three flocks.

Age:

1). Ewes

Source	DF	SS	MS	F value	P
Model	2	423.7	211.8	51.7	**0.0001**
Error	107	299.3	4.1		
Corrected Total	109	722.9			

Pair-wise comparison at $p<0.05$ Bonferroni T-test A^a B^a C^b

2). Does

Source	DF	SS	MS	F value	P
Model	2	349.5	174.8	38.6	**0.0001**
Error	95	244.5	4.5		
Corrected Total	97	594.0			

Pairwise comparison at $p<0.05$ Bonferroni T-test A^a B^a C^b **(A>C, B>C)**

Weight:

1). Ewes:

Source	DF	SS	MS	F value	P
Model	2	1.5	0.8	0.43	**0.6552**
Error	107	134.5	1.9		
Corrected Total	109	136.1			

Pairwise comparison at $p<0.05$ Bonferroni T-test A[a] B[a] C[a]

2). Does:

Source	DF	SS	MS	F value	P
Model	2	0.2	0.1	0.07	**0.9332**
Error	95	79.5	1.5		
Corrected Total	97	79.7			

Pairwise comparison at $p<0.05$ Bonferroni T-test A[a] B[a] C[a]

Appendix 5.7. Analysis of variance of lambing and kidding intervals.

1). Ewes:

Source	DF	SS	MS	F value	P
Model	2	62579.6	31289.8	516.8	**0.0001**
Error	129	11623.7	60.5		
Corrected Total	131	74203.3			

Pairwise comparison at $p<0.05$ Bonferroni T-test A^a B^a C^b

2). Does:

Source	DF	SS	MS	F value	P
Model	2	46698.5	23349.3	272.7	**0.0001**
Error	115	14811.2	85.6		
Corrected Total	117	61509.7			

Pairwise comparison at $p<0.05$ Bonferroni T-test A^a B^a C^b (A>C, B>C)