AN EPIDEMIOLOGICAL STUDY OF GASTROINTESTINAL NEMATODES IN CATTLE WITH PARTICULAR REFERENCE TO WEATHER FACTORS IN TETU DIVISION OF NYERI DISTRICT, KENYA. //

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## PETER MAINA GATONGI, B.V.M.

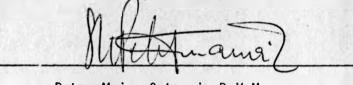
A thesis submitted in part fulfilment for the Degree of Master of Science in the Faculty of Veterinary Medicine, Department of Veterinary Pathology and Microbiology, -University of Nairobi.

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THIS THESIS HAS BEEN ACCEPTED FOR THE DROFT B. M. W. L. 1984. This thesis is my original work and has not been presented for a degree in any other University.



Peter Maina Gatongi, B.V.M.

This thesis has been submitted for examination with our approval as University Supervisors.

Milatting

J.M. Gathuma, B.V.Sc., M.Sc., Ph.D.

Aumana

W.K. Munyua, B.V.Sc., M.Sc., Ph.D., Dip. An. Husb.

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### DEDICATION

This work is dedicated to the living memory of my father, Musa Gatongi.

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### SUMMARY

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An epidemiological study of gastrointestinal nematodes of cattle was conducted in Tetu Division of Nyeri District, Kenya. This area has a tropical climate, being within the tropics. The aim of the study was to assess the influence of weather factors, stocking rate and herbage cover on the larval pasture contamination as indicated by the populations of infective larvae recovered from pasture herbage over a period of one year. Herbage was sampled at two week intervals.

The study was carried out in five farms selected by the met hod of stratified sampling to cover a representative crosssection of the farming community within the division. Three age groups of cattle (under 6 months, 6 - 12 months, and over 12 months) were used for this study. Their faecal egg counts were used as an index of their worm burdens. At any given period of time, the corresponding pasture larval contamination was considered as the source of the worm burden in the herd at the time.

Weather parameters which included rainfall, temperature, evaporation and relative humidity were recorded in a meteorological station located in the area of study. All the farms were within 10 - kilometre radius from the recording station.

A W-transect method of herbage sampling was adopted. About 500gm of herbage was collected from each paddock and thoroughly washed with water containing a small amount of detergent. Larvae were recovered by a combination of sedimentation, flotation with concentrated solution of magnesium sulphate, and finally by centrifugation to concentrate the larvae in about 0.5ml of water. Herbage was then dried to constant weight at 70°C. Infective larvae recovered were counted on a simple ordinary glass slide with calculating columns in doses of 0.1ml after adding a drop of Lugol's iodine to kill the larvae. They were recorded in numbers of infective larvae per kilogramme of dry herbage at 70°C. During the counting, larvae were also identified to genus level.

For the determination of herbage cover, foliage from three quadrants per paddock and selected at random was cut to ground level and dried separately in an oven to constant weight at 70°C. The quadrants were 30cm. by 30cm. in size. The final dry weights were recorded, their mean calculated and herbage cover of each paddock recorded as dry weight of foliage per 30cm<sup>2</sup>.

Faecal samples were collected per rectum. The modified McMaster egg counting method was adopted for the analysis of the egg counts. Faecal and herbage sampling in all the farms were done the same day.

Calves under 6 months of age had the highest egg counts and were the main source of pasture contamination followed by the 6-12 months age group. The egg counts of the over 12 months age group remained constantly low throughout the year. The highest egg counts were recorded during the dry seasons. The output went down during the rains. Larval populations were lowest during the dry periods (to a minimum of 8 L3/kg dry herbage) but went up to a maximum of 2728 L3/kg dry herbage during the rain seasons.

The highest pasture larval count was recorded in the month of February when there was a brief rainfall following a prolonged dry period. This observation was made immediately after the onset of the rains and the eggs on pasture had not had adequate time to hatch and develop to third stage larvae. It was therefore concluded that there was a ready source of infective larvae that caused the sudden rise immediately the rains came. With continued rainfall, there was a gradual decrease in the number of infective larvae recovered. This meant that either the infective larvae were carried down into the ground by percolating rain water or there was an apparent dilution effect due to increasing amount of herbage.

Rainfall and temperature were found to have a positive influence on the level of larval population in posture. A rise in rainfall was accompanied by a rise in herbage larval population while a fall was followed by a decrease in pasture larval count. A regression line of temperature drawn against pasture larval count showed a positive correlation. Similar results were obtained for herbage cover, stocking rate and evaporation. A negative correlation was obtained between relative humidity and pasture larval count.

In this study, a negative correlation was observed between rainfall and faecal egg counts in all the three age groups of cattle. During the rains the herbage became abundant and lush. Animals grazing on this herbage were passing out copious soft faeces. This was thought to dilute eggs passed in faeces and cause a false decrease in faecal egg counts. Although not investigated in this work, the possibility of self-cure taking place and causing the decrease in faecal egg-counts during the rain season was also considered a likely explanation.

Nematodes of the genus <u>Cooperia</u> were the most predominant while those of the genus <u>Strongyloides</u> were the least dominant. Other genera found were <u>Haemonchus</u>, <u>Trichostro-</u> <u>ngylus</u> and <u>Oesophagostomum</u>, in that order of prevalence; this was observed throughout the year. It was therefore concluded that no genus was more favoured or adversely affected by any seasons than others.

A wide discrepancy was always observed between the percentages of infective larvae of the genus <u>Strongyloides</u> recovered from herbage and those recovered from coproculture. It was further noted that the infective larvae of this genus did not survive as long as other larvae under laboratory conditions. This could mean that they were more susceptible to certain environmental conditions, thus leading to their short lifespan. It was suggested that under field conditions this could reduce <u>Strongyloides</u> larval population in herbage, thus leading the discrepancy between herbage cover larval recovery and coproculture recovery.

It was concluded that to control the problem of gastrointestinal parasitism, contamination of pastures that occurs during the dry period should be avoided or reduced by deworming animals during this period. Deworming during the rainy period, as is the common practice in this area, would be of temporary relief to the animals as the pastures at this time are heavily infested with infective larvae.

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Special devotion goes to my late father who passed away just prior to the beginning of this study. Despite his lowly and humble basic education, his foresightedness in education successes and achievements has been a source of great inspiration to his sons and daughters over the years.

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Deep appreciation is expressed to my beloved mother Naomi who has consistently devoted her energies, will power and love to the furtherance of the welfare of her children. She has been a source of encouragement over the period of this study.

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Last but not least, may this work be to the Glory of God to whom everything belongs.

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### INTRODUCTION

Parasitism is one of the major problems facing the animal production industry in the world today. There are ecto-and endo-parasites, each playing their significant roles in affecting domestic animals as a source of food for man. With the ever increasing world population and the subsequent decreasing amount of food per head, man can no longer afford to share his animal food resources with parasites if he has to survive. It is therefore imperative that man understands fully the epidemiology of parasites that compete against him for food in order to be able to cope with their effective control.

The gastrointestinal tract of ruminants is inhabited by a wide variety of parasites. Some of these are of little or no significance to the host animal, for example the trematode <u>Paramphistomum cervi</u> which inhabits the rumen of cattle, sheep and goats. Though the immatures of this parasites cause diarrhoea and acute enteritis in cattle (Kelly and Henderson, 1973), the host will live confortably with moderate numbers of the adult parasites. Some others like <u>Haemonchus</u> spp will sometimes cause acute syndromes or even death of the host animal particularly where young calves, sheep or goats are involved. However, chronic haemonchosis is the more important as it depresses growth rate and production of the host (Allonby and Urguhart, 1975). There are yet other parasites that will manifest their effects on the host very gradually but for a long time, thereby incapacitating the hest, for example the nematodes of the genera <u>Ascaris</u>, <u>Trichostrongylus</u> and <u>Cooperia</u> spp. Such parasites adversely affect the production of the host animal. Understanding of the factors influencing the levels of gastrointestinal nematodes will shed light on the effective means of controlling these parasites.

In temperate countries, outbreaks of gastrocnteritis caused by combined infection of <u>Ostertagia</u>, <u>Cooperia</u> and <u>Trichostrongylus</u> worms traditionally occur in lambs in late summer (Graham and Ollerenshaw, 1978). However, there is evidence to show that although most outbreaks follow this pattern, there might be a wide variation in disease levels from year to year (Graham and Ollerenshaw, 1978).

The fact that disease may arise in any class of sheep and at any time of the year, being usually associated with a wet, hot season and more rarely a very dry one, even when husbandry practices have been constant from year to year, suggests that the epidemiology of this disease is complex (Graham and Ollerenshaw, 1978). However, Borgsteede and Burg (1982) demonstrated that zero grazing significantly reduced worm burdens in cattle. Nevertheless, the problem is made more complex by the fact that different worm species are involved. Thomas and Boag (1973) and Gibson and Everett (1967, 1976 ) have shown evidence that different worm species show varying responses to environmental factors. To be able to control this

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disease, one should have the ability to predict the time of maximum infection. Thomas (1982) suggested that worm control should be aimed both at the host and the environment to be able to break parasite life cycles. At the host level, anthelmintic treatment should be used to remove the parasite and at the environmental level susceptible hosts should be segregated from the infective larval stage.

Although many aspects of the epidemiology of parasitic gastroenteritis in sheep have been investigated, the effect of one season's weather on the level of disease in the following year has attracted little attention. Most workers accept that weather plays a dominant role in determining the size of the parasite population and a high level of disease in lambs is most frequently associated with wet, hot season. However, Reid and Armour (1973) described an outbreak in housed ewes in winter following a dry late summer and autumn. Under clean grazing conditions Mitchell (1983) found that all parasitological parameters in both ewes and lambs were lower than in traditional permanent pasture flocks.

In temperate countries, lamb infection in summer results from pasture contamination earlier in the year which is largely attributed to the ewe egg output (Boag and Thomas, 1975). A sudden rise to peak pasture larval count generally occurs in July-August, resulting in a rapid build up of worm burden. The timing of this peak of pasture larval activity is therefore very important in determining the onset of severe parasitism. It is generally accepted that temperature and moisture are of major

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importance in the epidemiology of the free living stages of nematodes (Ogunsusi, 1979). Levine (1963) postulated that macro-climatic data was unsatisfactory in predicting pasture larval transmission potential and thatmicro-environment of larvae required more study. However, Graham and Ollerenshaw (1978) showed that the macro-climate could adequately be correlated with epidemiological findings.

Waller and Thomas (1978) demonstrated that there is a consistent larval population pattern which appears to be independent of the pattern of egg deposition and which shows differences in timing. Their findings suggest that the larv pattern may be due to climatic influences on the rate of development of eggs to larvae. There is need therefore for more detailed study of the relationship between egg deposition and pasture larva population. Most studies carried out on pasture larval counts have not been done in association with egg count and so the relationship between pasture contamination and animal imfection has not clearly been established (Boag and Thomas, 1971

In his study of seasonal transmission of bovine gastrointestinal nematodes in the Texas Gulf Coast, Craig (1979) showed tha weather conditions were more important than vegetation in larval transmission and that climate determines the variety of parasites in a geographical locality while weather determines time of transmission. The effect of herbage may be encountered at the micro-habitat level while moisture level determines dissemination of infective larvae to contaminate pasture.

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In the tropics, few epidemiological studies on the behaviour of nematode parasites in domestic ruminants, especially cattle, have been carried out. Henderson and Kelly (1978), working with beef cattle in the arid tropical region of north-western Australia (East Kimberley), found that arrested development of fourth stage larvae within the host was a mechanism which ands parasites to survive in areas with prolonged dry period. Ogunsusi and Eysker (1979), working with sheep in the dry region of Zaria, Nigeria, made a similar conclusion. Considerable yearl variation experienced in tropical regions between monthly average for rainfall and temperature data make detailed correlations between incidence of gastrointestinal parasites and climatic factors difficult (Henderson and Kelly, 1978). Ogunsusi (1979) concluded that moisture was the limiting weather factor for larva survival in Northern Guinea Sayanna of Nigeria.

Very little work has been done on the ecology and bionomics of helminths in Kenya (Round, 1962). This author has compiled together most of the information available on the helminth parasites in domesticated animals in Kenya and has stated that th is such a wide diversity of environmental conditions that studies carried out in one area would almost undoubtedly not be applicabl to other areas. Allonby and Urquhart (1975), working with sheep in Naivasha, a semi-arid region in Kenya, observed uniform faecal egg counts in both ewes and lambs over a long period when worm burdens fluctuated greatly. They also observed a marked decrease in worm burdens without the occurrence of classical self-cure. They therefore concluded that faecal egg counts were not a reliab

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index of <u>H. contortus</u> burdens in natural infections. In a further observation, these authors noted that despite the deposition of large numbers of eggs on pasture, only small worm burdens were established in both the "permanent" and "tracer" animals. They concluded that it was likely that fact other than rainfall and temperature were also important in th development and availability of <u>H. contortus</u> larvae on herbage.

Between January 1979 and December 1982 the Veterinary Clinical Services operating in the area of this study treated an average of 358 gastrointestinal helminthiasis cases per month in cattle as compared to 78 mastitis and 48 tick-borne disease cases in the same period. These statistics showed in a broad view the significance of gastrointestinal helminth parasites in animal health in this area. No attempt has been made to identify the helminth parasites involved or investigate their behaviour in relation to epidemiological factors. This information remains lacking.

In Kenya, ruminants are the most important animals that are a source of food for man. They mainly provide him with meat and milk. In some parts of the country, cattle are also used for tilling the land for crop production. Optimum health status of these animals is therefore of paramount importance and of great concern to us. If we can ensure effective control of the parasites affecting our animals, we shall have won a major battle in the struggle of attaining maximum production in our animal industry.

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According to the current Kenya National Food Policy (Anonymous, 1981) the country will require 2,158,000 tonnes of milk per year by the year 1989 while presently, the country is only able to produce 1,259,000 tonnes per year. To achieve the anticipated goal, a growth rate of milk production of 8.7% will have to be realised. Similarly, for meat, the country will need 314,000 tonnes per year by 1989 while the current production stands at 147,000 tonnes. This is an anticipated growth rate of 8.8%. Achieving these goals will be a difficult task. Factors influencing animal production must therefore be clearly understood and effectively controlled. Internal parasitism of ruminants is a major factor of concern in this respect which requires detailed study and investigation.

The objective of this study was to investigate the pattern of egg deposition by various age groups of cattle and the pattern of build-up of infective larval population in pasture as they relate to rainfall, ambient temperature, evaporation, humidity, stocking rate and herbage cover. The study also attempted to identify the major genera of gastrointestinal nematode parasites present in the study area and to suggest an effective method of their control.

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#### LITERATURE REVIEW

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2.1 WEATHER, ENVIRONMENT AND NEMATODE LARVAE

For a long time, it has been known that weather and climate play an important role in the survival of parasite eggs, larvae and cysts outside the bodies of the host and, therefore, in the transmission of the parasites (Levine, 1963).

Gastrointestinal nematode eggs which are passed in faeces hatch into first stage larvae (L1) in a day or more. However, Nematodirus spp. eggs hatch more slowly than other genera. The L1 feed on bacteria, moult to second stage (L2) larvae which also feed on bacteria and then moult to ensheathed non-feeding infective third stage larvae (L3). This process requires oxygen and adequate moisture. The length of time taken to reach the L3 stage depends on temperature. The higher the temperature the faster the L3 stage is reached. However, at higher temperature the L3 are extremely active and quickly exhaust their food reserve in the gut cells. Consequently, the majority of them die. At lower temperatures the L3 stage takes much longer to be reached but the larvae survive much longer due to their lowered activity. The L3 migrate out of the faeces, crawl onto herbage and rem there ready to be ingested by a new host or die.

Exposure to direct sunlight kills both developing eggs and larvae rather quickly while dessication prevents development but does not kill the ensheathed larvae rapidly. Optimum temperature for development is generally higher than the optimum temperature for survival (Levine, 1963).

2.

In a review of weather and climatic effects on nematodes, Levine (1963) stated that the roles of weather and climate in the distribution and prevalence of nematodes are different. Weather is a composite of atmospheric conditions - temperature, barometric pressure, precipitation, humidity, wind direction and velocity, sunlight, cloud cover and so forth, at a particular time. Climate determines which nematodes are generally found in a locality, while weather determines which ones can develop and infect their hosts at a particular place in a particular year.

The amount of moisture in the soil is an important determinant of larval development and survival. This depends on precipitation, evapotranspiration, soil type, the amount and type of overlying mat and the character of plants growing in it (Levine, 1963).

Berberian and Mizelle (1957) observed that when <u>Hemonchus contortus</u> eggs were smeared on slides and exposed to relative humidity of 85-92% at the optimum temperature of 33.3°C, they failed to develop. When they were exposed to relative humidity of 100% at the same temperature, infective larvae developed in 62 hours. It is therefore evident that the atmosphere immediately around the eggs must be saturated or they will not develop.

Infective larvae tend to be distributed fairly evenly on flat land but are washed downhill by rain on sloping land; the extent of this action depends on the slope. Closely related to the effect of topography is the effect of

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heavy rainfall on larval distribution. Crofton (1948) observed that heavy rainfall washed away the larvae of Trichostrongylus retortaeformis on an English pasture he was working on. It is therefore likely that a considerable number of larvae would be lost in the run-off accompanying a period of excessive rainfall. Alsagur et al.(1982) recovered infective larvae of cattle Ostertagia spp. both from the herbage and stratified soil samples to a depth of at least 15cm. Some other authors (Tripathi, 1974; Young and Trajstan, 1980; Skinner and Todd, 1980) have also demonstrated the presence of trichostrongylid third stage larvae in soil Duncan et. al. (1979) and Armour et al. (1980) have samples. independently indicated the possibility that larvae moving onto herbage from a reservoir site in the soil may have been the cause of disease in some outbreaks of dictyocauliasis and osteragiasis in cattle. Work done by Bisset (1980a) showed that infective larvae of bovine Ostertagia spp. could persist in the soil of ungrazed pasture for up to 10cm deep throughout the year.

Studying the dynamics of bovine <u>Ostertagia</u> spp. infective larval population in their environment of soil and overlying herbage, Alsaqur <u>et al</u>. (1982) found that at certain times significant differences occurred in the numbers and proportions of infective larvae present at various levels in the soil cores. They particularly noticed the disappearance of infective larvae from herbage and underlying mat during the harsh weather conditions and the reappearance in large numbers during favourable

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conditions without fresh contamination. These authors further observed that during heavy rainfall there was a reduction in the larval population from herbage. They concluded that this could be due to the percolating rain water carrying the larvae downward in the soil beyond the level that was sampled.

Recently, earthworms have been shown to act as transport hosts for infective larvae (Grøvold, 1979; Oakley, 1981). Therefore, some fluctuations of larval populations may be attributed to the activity and migration of earthworms as has been suggested by Alsaqur <u>et al</u>. (1982) that for proper understanding of seasonal patterns or infective larvae, the interaction between earthworm populations, water table, soil type and other trophic influences require detailed investigation.

Exposure to sunlight is fatal to nematode larvae; therefore the degree of shading they receive from the grass, bushes or trees will markedly affect their survival (Levine, 1963). Dinnik and Dinnik (1958, 1961) found marked differences in survival of <u>H. contortus</u> larvae under field conditions in Kenya Highlands, depending on whether they were in the shade or in the open. During the rainy season, when the air temperature varied between a mean minimum of 12°C and a mean maximum of 23°C and there was about 2.5cm of rain per period of 10 days, larvae survived for about 10 days in grass exposed to direct sunlight. In grass shaded by trees or in very thick high grass, Tarvae survived in considerable numbers up to 40 days.

The effect of predators on the survival of larvae on pasture has received little or no study at all. Millions of grass mites live on pastures and many of these may well be

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predatory on nematode larvae (Levine, 1963). Mohr (1943) and Laurence (1954) studied the fauna of cowpads on pasture and found many species of arthropods including beetles and Diptera. Faecal deposits are therefore interesting biotopes inhabited by a wide variety of both macro-and micro-biotic communities and information on the succession of events in them would be of great value to our understanding of nematode control.

Ogunsusi and Eysker (1979), working with sheep in Zaria, Nigeria observed high levels of inhibition of <u>H. contortus</u> population in very young lambs infected experimentally. This precluded host resistance as a primary cause for inhibited development as observed earlier by Dineen <u>et al.</u> (1965). From their observation, Ogunsusi and Eysker (1979) suggested that there was either existence of an obligatory type of inhibition at the end of the rainy season or existence of environmental stimuli acting on the preparasitic stage very effectively. They found out that during the dry season, adult and developing stages of <u>H. contortus</u> were replaced by inhibited larvae. They therefore concluded that this species is very prone to inhibition and survives the dry season almost exclusively as inhibited larvae in the host.

In the course of their study of helminth parasites of beef cattle, Henderson and Kelly (1978) found significant numbers of early fourth stage larvae of <u>Haemonchus placei</u> at times when conditions were unlikely to sustain the intake of infective larvae. They therefore suggested that arrested

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development of fourth stage larvae within the host was a mechanism which aids parasite survival in areas with prolonged dry periods. They also observed that by the time cattle were 16-18 months of age parasite burdens were generally down to low levels for all species of nematodes although some individuals still carried high levels of <u>H. placei</u> and <u>Cooperia</u> spp.

#### 2.2. POTENTIAL OF PASTURE LARVAL CONTAMINATION

The level of pasture larval contamination at any one time depends on a variety of factors. In his work on nematode larval population on different pastures, Crofton (1952) showed that microhabitats, microclimates, pasture management, types and rates of stocking, species of nematodes involved and the soil type were the most important factors.

Morrison (1956) estimated that a sheep weighing 45.5Kg will produce about 2500g of faeces per day. Levine and Clark (1961) observed that normal ewes running on pasture with their lambs shed an average of 824 eggs per gramme of faeces. Simple calculation shows that each ewe will deposit about 2,060,000 eggs per day, 14,800,000 eggs per week and 61,800,000 eggs per month. Such potential for pasture contamination is enormous. However, not all eggs hatch and the majority of larvae that hatch never survive to infect new hosts. Boag and Thomas (1975) estimated that larval mortality was as high as 99.4%-99.8%, depending on prevailing weather conditions.

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Although no figures are available, the majority of the infective larvae that develop in sheep faeces may find their way out of the faecal pellets on the vegetation. However, the situation is quite different for cattle. Roberts <u>et al</u>. (1952) observed that the hard crust that forms on dung pads stops the larvae from moving. Such larvae therefore concentrate in the faecal pad. When the rain falls and softens the pad, the larvae migrate out of it as far as 60cm horizontally in 24 hours. It is possible that dung pads protect the larvae against unfavourable conditions and permit them to survive much longer than they would if they were out on herbage.

#### 2.3. RELATION OF GRAZING HABITS TO INFECTION RATE

Social habits of animals make the distribution of faeces and consequently, of eggs and larvae irregular. Crofton (1954a) observed that sheep do not defaecate in regular or random pattern all over the field but tend to congregate in certain areas, such as hilltops and along fences. In addition, animals are selective in their grazing. The grass immediately around a faecal pad may be especially green, but it must be unappetizing since cattle ordinarily avoid it.

When pasture is lightly stocked, animals do not graze very close to the ground and therefore will not pick as many larvae as when the pasture is heavily stocked and the animals nip off every available bit of green herbage. It is therefore possible that the relation of livestock grazing habits to larval behaviour patterns may also affect the degree of parasitism.

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#### 2.4. EFFECT OF STOCKING RATE ON PARASITISM

It is generally accepted that stocking rate is an important determinant of the level of helminth infection in grazing animals. Most literature on sheep and cattle management warn of the dangers of helminthosis that result from increased stocking rate. Taylor (1939) predicted that worm burdens of grazing animals were proportional to the square of the stocking rate, thus a trebling of stocking rate would lead to a nine-fold increase in worm burdens.

The effects of an increased stocking rate on a given pasture are numerous and information available on such effects is often conflicting (Morley et al. 1978). At a higher stocking rate each individual animal will have less pasture foliage available to it while such a pasture will have received a higher level of faecal contamination. Whether or not that pasture will also be more heavily infested with nematode larvae will depend on the effects of the reduced plant cover on the hatching and development of larvae (Barger, 1979). The effects of greater faecal contamination at a higher stocking rate for species such as H. contortus whose free-living stages are vulnerable to dessication would be largely offset by increased mortality of eggs and larvae. In such a case, the incidence of parasitism would be insensitive to variation in stocking rate (Morley et al. 1978). Genera such as Ostertagia and Nematodirus which have more robust eggs and larvae, would thus increase at higher stocking rates.

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Worm burdens of grazing sheep and lambs appear to be relatively insensitive to variations over a wide range of stocking rate (Zimmerman, 1965). However, at excessively high stocking rates where sheep are malnourished, parasitism may increase (Zimmerman, 1965). Michel (1969) observed that stocking rates are not usually increased unless there is an increase in the production of herbage per unit area. Therefore, the mass of herbage available to, and contaminated by each sheep, tend to be similar at all stocking rates and the incidence of parasitism would thus be independent of stocking rate (Barger, 1979). Under these circumstances, an increase in stocking rate need not be accompanied by increased parasitism.

## 2.5. PREDICTING THE TIME OF PEAK GASTROINTESTINAL NEMATODE INFECTION

The life history of the nematode parasites of domestic ruminants usually involves the development and survival of free living stages on pasture since the animals are still largely managed in grazing systems (Thomas and Starr, 1978). Pastures are therefore the sites of deposition, development and transmission of nematode infection and meteorological factors affecting the pasture will have an influence on these parasites. However, the relationship between parasite populations and specific climatic factors is not generally known in sufficient and precise details to enable meteorological data to be used for predicting the timing of intensity of seasonal infection (Thomas and Starr, 1978). Temperature and rainfall are the major climatic factors influencing the incidence of internal parasites and in temperate areas of the world use of this data enables breaks of parasitism to be predicted with reasonable accuracy (Gordon, 1948). However, in tropical regions considerable yearly variations between monthly averages for rainfall and temperature data makes detailed correlations between incidence of gastrointestinal parasites and climatic factors difficult. Kelly (1973) and Henderson and Kelly (1978) have described the phenomenon of hypobiosis as an evolved adaptation for larval survival in adverse conditions in many trichostrongyle nematode species.

Lee <u>et al</u>. (1960) studied seasonal variation of strongyle larvae in Zebu cattle region of Nigeria with a prolonged dry season. They observed a negligible pick-up of infective larvae during the dry season with a carry-over of infective material in the host from one wet season to the next.

Successful correlations between weather conditions and the bionomics of helminth eggs and larvae have been made the basis of forecasting fascioliasis (Ollerenshaw and Rowlands, 1959; Ross, 1970) and nematodriasis (Ollerenshaw and Smith, 1966; Smith and Thomas, 1972). The potential value of such forecasts has been indicated by extensive use of these predictions in paracite control.

Thomas (1974) examined the assumption that year-to-year variation in the time of occurrence of the peak pasture larval count was influenced by temperature and rainfall. A specific

correlation with the duration of surface wetness was demonstrated. He further argued that rainfall was the factor of critical importance. Thomas and Starr (1978) examined records of larval patterns over a nine-year period in relation to meteorological data and also demonstrated a correlation between the time of the summer peak and cummulative rainfall. The effect of temperature was considerable though it was likely to be relatively constant from year to year since daily fluctuations from the mean would balance out over a period of weeks.

Abnormally high temperature has less influence than might be expected since it is usually associated with dry conditions when larval development is retarded by lack of moisture. When temperatures are very low, larval development is likewise slow and mortality high (Rose, 1963). With rising temperature, development increases, leading to maximum larval counts (Levine, 1963). Thus, temperature determines the overall pattern of larval counts rising to a peak. However, the influence of temperature is modified by the level of moisture since drying inhibits development without necessarily killing eggs and larvae (Andersen and Levine, 1968).

Sprent (1946) showed that infective larvae of <u>Bunostomum</u> <u>phlebotomum</u> of cattle could not survive during the dry season in the Vom area of Nothern Nigeria. Lee <u>et al.</u> (1960) also showed that the conditions of dry season in Northern Nigeria were unsuitable for the survival of cattle trichostrongyle larvae on pasture. Ogunsusi (1979), working in the Northern Guinea savanna of Nigeria and using tracer lambs to monitor pasture infectivity, found that the highest infectivity came during the rainy season coupled with suitable temperature and high humidity. When the rains disappeared and the dry season set in with increased temperature and lowered relative humidity, infectivity fell to a non - detectable level. Under the conditions existing in this area, the author suggested that temperature was not a limiting factor for larval survival on pasture since it ranged between 27°C and 30.6°C. He therefore concluded that lack of parasitic nematode larvae in pasture during dry seasons was definitely due to lack of moisture.

## 2.6. SEASONAL INCIDENCE OF GASTROINTESTINAL NEMATODE SPECIES

Seasonal occurrence of different species of nematode parasites in sheep and goats has been investigated and recorded by several authors. Morgan and Oldham (1934), Stewart and Douglas (1938), Parnell (1954) and Fabiyi (1973) reported an increase of infection in the wet seasons in almost all species of nematode parasites. Fabiyi (1973) also reported that environmental temperature appeared to be favourable for preparasitic development and survival.

Lee <u>et al</u>. (1950), in an investigation of the seasonal incidence of the common gastrointestinal strongyles of cattle in Nigeria, showed that only negligible infections were acquired in the dry season and animals infected during the previous rains carried infective material over to the next wet season. Hart (1964) found considerable variation in faecal egg counts, population of immature worms and adult worms between dry and wet seasons in Zebu cattle in Vom region of Nigeria.

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He reported a very sharp drop in faecal egg counts at the end of the rains leading to faeces being negative for worm eggs during the late dry season. He also reported large numbers of adult worm in young stock during the wet season which declined gradually to moderate numbers during the dry period. At the end of the dry season, he also reported very few immature stages whereas at the end of the rains there were very large numbers of immature stages. This is in contrast to the phenomenon of hypobiosis described by Henderson and Kelly (1978).

Investigating the seasonal incidence of inhibition of development in <u>H. contortus</u>, Connan (1971) found that inhibition of development followed a seasonal pattern. In a recent study, Mitchell (1983) showed that pasture larval peak corresponded with a wet spell. Seasonal fluctuation of nematode parasite population is evidently a feature of epidemiolocial significance Vercruysse (1983), working with domestic sheep and goats in Sahelian zone of Senegal, found the highest prevalence and highest mean egg counts at the end of the wet season. At the end of the dry season he found that the mean faecal egg count started to increase while hypobiosis started to terminate.

Crofton (1957) made more detailed observations of seasonal incidence of nematode parasites and showed that when the mean number of any particular species of parasite per host was low, then the frequency of occurrence of infected individuals was also low. When the flock was considered as a whole, all species of nematode parasites occurring in that flock were represented at all times of the year. On some occasions, the or

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evidence of the presence of a particular species was a low infection in one individual in the flock.

Crofton (1957) further observed that in ewes, succession of nematode parasite species was not marked, and that seasonal variation in species distribution was not very apparent. Although some variation occurred, most species were present throughout the year. Such variation appeared to be closely related to the past history of the individual sheep, and no marked seasonal changes could be seen. This does not imply that the worm burden of ewes is static. The rate of change of parasite population in ewes appeared to be much lower than in young sheep and the relatively small numbers of worms which established themselves were not sufficient to show any regular or marked species succession. In lambs, the differences in incidence of different species was well marked. At no time was there a pure infection of any one species, but at different periods of the year, different species could be regarded as being numerically dominant.

Bisset (1980b) and Eysker and Jansen (1982) demonstrated that some nematode species could be transmitted quite well between host species; for example, sheep have been found to be susceptible to <u>Ostertagia leptospicularis</u> of cattle origin. These workers also observed that <u>O. ostertagi</u> was very poorly adapted to sheep. They therefore concluded that use of alternate grazing between sheep and cattle can select against some species of helminths while enhancing the propagation of others, thus influencing the incidence of some nematode species.

#### 2.7. ANALYSIS OF WORM EGG COUNTS AND HERBAGE LARVAL COUNTS

In a study of worm egg counts, Crofton (1955) observed that the number of eggs per gram of faeces (EPG) increased slowly at first as the cold season came to an end. The rate of increase became more rapid as the warm and wet season In all his cases, the rapid increase was followed advanced. by an even more marked decrease in counts. He therefore suggested that the form of increase appeared to be logarithmic. He tested this hypothesis and found that there was a significant agreement between the observed figures and the curve plotted on the bases of logarithmic increase. He therefore concluded that the implications of a logarithmic form of EPG increase were of considerable importance. It implied that, the infection at any time, as judged by egg counts, was proportional to a previous infection and that pasture contamination must be proportional to the rate of egg production.

In other studies Roberts <u>et al</u>. (1951) and Crofton (1952, 1954a) demonstrated that a marked peak in faecal egg counts could be expected to be reflected by a peak in the number of larvae recovered from the pastures. On the basis of logarithmic increase, Crofton (1955) suggested that worm population increased as a result of increasing doses of infection while the stimulus of the host by the new infection was sufficient to cause elimination of the mature worms (self-cure). Boag and Thomas (1971), in their work on infection patterns on clean and contaminated pasture, observed that, allowing for the maturation of ingested larvae, the lamb faecal egg count and pasture larval count, were closely correlated. Their results established that in a flock on clean pasture, the post-paturient ewe faecal egg output was followed by a marked rise in infective larvae on the herbage which produced a major wave of infection in the lamb crop and a steep rise in lamb egg counts. They concluded that since the contribution of faecal deposits by grazing sheep is not random according to Donald and Leslie (1969) and neither is the distribution of infective larvae, pasture larval counts were therefore more useful in indicating major variations in the level of infection with time than in measuring absolute numbers.

Under normal circumstances, larval mortality is high and varies considerably from year to year depending on the prevailing weather conditions (Boag and Thomas, 1975). Gibson and Everett (1976 ) described a modified normal pattern of larval development as a result of an unusual pattern of rainfall and the effects of low temperatures in delaying the development of worm eggs and consequently the larval peak on the herbage. They therefore suggested that careful chservation of the weather could enable prediction when larval peaks were likely to occur, thereby allowing more exact timing of control measures.

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Michel (1969) demonstrated that in early infection 0. ostertagi worm burdens in cattle were more closely related to larval intake than faecal egg output. Similarly, studying the effect of different levels of larval intake in the output of eggs of Ostertagia circumcincta in lambs, Gibson and Everett (1978) showed that during the first six weeks, worm burdens were more closely related to larval intake than faecal egg output. After 12 weeks continuous dosing of young lambs with larvae, these workers showed evidence that resistance mechanisms were limiting worm burdens as well as faecal egg counts. Egg output by an animal, or group of animals, depended not only on the number of worms present, but also on how the worm burden was acquired. They therefore concluded that both the level of larval intake and its duration influenced the development of resistance and consequently the size of adult worms and the egg laying capacity of the females.

Pasture larval counts may be influenced by many factors, including the accuracy of estimation, the rate of deposition of eggs and their rate of development, spread and survival and the rate of herbage growth (Oakley, 1982). Worm burdens in the hosts are influenced by the number of larvae in the pasture, larval distribution over the pasture, stocking rate, grazing density, host appetite, host response to infection and selective grazing habits such as preference for the upper reaches of lush herbage and avoidance of foliage near soil level or faecal pads (Oakley, 1982). This author also demonstrated that pasture larval counts and worm burdens are measurements made at different points in the epidemiological progress of worm infection and each is the result of a complex interplay of many factors and therefore disparity between the two cannot be entirely excluded.

#### 2.8. RECOVERY OF NEMATODE INFECTIVE LARVAE FROM HERBAGE

The technique for the recovery of infective larvae from sample includes six basic steps (Burger, 1981), namely washing a representative sample of pasture herbage, collecting larvae on a series of test sieves, concentrating them in a saturated magnesium sulphate solution, counting and differentiating larvae microscopically, drying the herbage in an oven to constant weight at 70°C and calculating the number of larvae per kilogramme of dry herbage.

Donald (1967), Heath and Major (1968) and Bawden (1969) suggested that there was a relationship between the amounts of deposits and the rate of larval recovery from herbage. Lancaster (1970) demonstrated that the recovery rate was proportionally affected only when the amount of deposit was less than 6cc. After this amount, the recovery rate did not appear to be proportionally decreased by greater amount of deposit.

Lancaster (1970) compared the efficiency of the washing and screening procedure with soaking and sedimentation procedure for the recovery of larvae from herbage. He concluded that the technique of washing and screening was about 2-3 times as efficient as the soaking and sedimentation method. He however remarked that for most purposes, it is not necessary to determine the absolute numbers of larvae on herbage provided the recovery is sufficient to indicate population trends. He further investigated factors influencing recovery of larvae and concluded that the greatest volume of water and the longest washing time produced the highest recovery while saturated magnesium sulphate and zinc sulphate floation solutions gave the highest recoveries. Magnesium sulphate solution was chosen because it was cheaper.

Lennart (1974) developed a modified Baermann apparatus with screens as a technique for the assessment of larval population on herbage and manure. Tests of the technique showed that 58 - 83% of the added larvae were recovered from 200g. and 100g. of herbage for an operational time of 24 hours. They further observed that with increased amount of herbage above 200g, the efficiency of recovery drastically dropped.

In his pasture sampling technique, Burger (1981) took two herbage samples from each pasture. He pinched 100 small samples on a W - shaped transect across the pasture and collected them in a bag. He collected the second sample by walking along the transect but in the opposite direction. Each sample was processed separately. Washing of herbage was done three times in a commercial washing machine equipped with a siphon at the outlet. He concentrated the larvae by three centrifugations at 250g. for 5 , 5 and 3 minutes in a saturated solution of magnesium sulphate. For microscopic

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examination, he used a counting chamber adapted to the microscope. The number of larvae per kg. dry herbage was calculated for each sample and the arithmetic mean of the two counts was designated the "infestation of the pasture". An approximate 60% rate of larval recovery was recorded in this procedure.

#### 2.9. IDENTIFICATION OF INFECTIVE LARVAE

According to Keith (1953), Whitlock (1959), Whitlock (1960) and Borgsteede and Hendriks (1974), there are three features used in differentiating the third stage larvae from the first two non-infective stages. These include the presence of filariform oesophagus in contrast to the rhabditiform (bulbous) oesophagus of the first and second stage larvae. This feature may be confusing in the case of Bunostomum spp. where the filariform oesophagus of this genus shows a conspicuous bulb. This bulb is however a more bottle-shaped structure as compared to the nearly spherical bulb of the rhabditiform oesophagus of the first and second stage larvae. The second is the presence of a sheath around the body of the larvae commonly evidenced by corrugated surfaces and a tapering tail sheath. The sheath is however missing in Strongyloides spp. The third feature is the presence of gut (intestinal) cells. These cells are the food reserves for the third stage larvae which are nonfeeding. In some genera, for instance Cooperia, Trichostrongylus and Oesophagostomum, the gut cells are conspicuous and clearly defined whereas in others like Haemonchus, they are not as clearly defined.

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The third stage larvae are sufficiently different for genera to be identified. Burger and Stoye (1968), and Borgsteede and Hendriks (1974) used two criteria for the identification. These included measurements of lengths of larvae and observation of characteristic morphological structures typical of a genus. Three specific measurements were taken for each larva, namely, total length from head to tip of sheath, the length between anus and tip of sheath i and the length between tip of tail to tip of sheath.

Several workers have criticised the use of measurement criterion as being inconsistent and influenced by environmental and nutritional factors. Shumard <u>et al</u>. (1955) demonstrated that larvae of <u>H. contortus</u> culture from eggs of worms harboured by sheep given rations containing supplementary trace elements were significantly longer than those from sheep not given such rati Wang (1967) showed that culture temperature affected the length of larvae recovered from culture of <u>Trichostrongylus colubrifor-</u><u>mis</u>, shorter larvae being recovered from cultures kept at 30°C than from those incubated at 20°C. Wang (1970) demonstrated that the substrate upon which larvae fed influenced the length to which they grew. In faecal culture, the length of larvae of <u>T. colubriformis</u> ranged from 643/U whereas larvae grown on culture of <u>Staphylococcus aureus</u> ranged from 556/U to 685/U in length.

Studying the ecology of the free living stages of Ostertagia circumcincta, Gibson and Everett (1971) demonstrated

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that infective larvae of O. circumcincta recovered from the field were generally shorter than the lowest previously reported length of 656/U. They noted that exposed larvae on pastures are much more likely to encounter adverse climatic and nutritional conditions than larvae reared in faecal cultures. Therefore they suggested that this factor is probably responsible for the shorter length of the larvae collected from the field. They further suggested that the length was a poor criterion for the differentiation of larvae and that other features must be taken into account if accurate identification was to be achieved. The fact that length of larvae of different genera overlap is a further disadvantage of the use of this criterion. Borgsteede and Hendriks (1974) suggested that the more popular criterion would be the observation of typical characteristic morphological features which are consistent irrespective of environmental and nutritional conditions within normal limits.

#### 2.10. IDENTIFICATION OF STRONGYLE EGGS

Several authors have attempted to identify species of nematode parasites of domestic animals by examination of their eggs (Shorb, 1940; Tetley, 1941a, b; Cunliffe and Crofton 1953). The outline of a strongyle egg is approximately elliptical (Cunliffe and Crofton, 1953). If the long and short axes are measured for each of the eggs of a sample of say, 50 eggs, then these can be plotted against

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one another to make a scatter diagram. The mean dimensions of all species are reasonably well separated and thus, their scatter diagrams will occupy different loci. According to Crofton (1954b), <u>H. contortus</u> eggs are very sensitive to this method. However many of the distributions overle giving a confusing picture (Christie and Jackson, 1982). These authors developed a method for the specific identification of strongyle eggs in small samples of faeces based on dimensional and biological characteristics. According to them, more than 90% of the eggs of <u>Ostertagia</u> spp. and of <u>Trichostrongylus vitrinus</u> can be positively identified. For other species, supporting information from larval cultures may be needed. However, this need will vary according to the composition of the sample.

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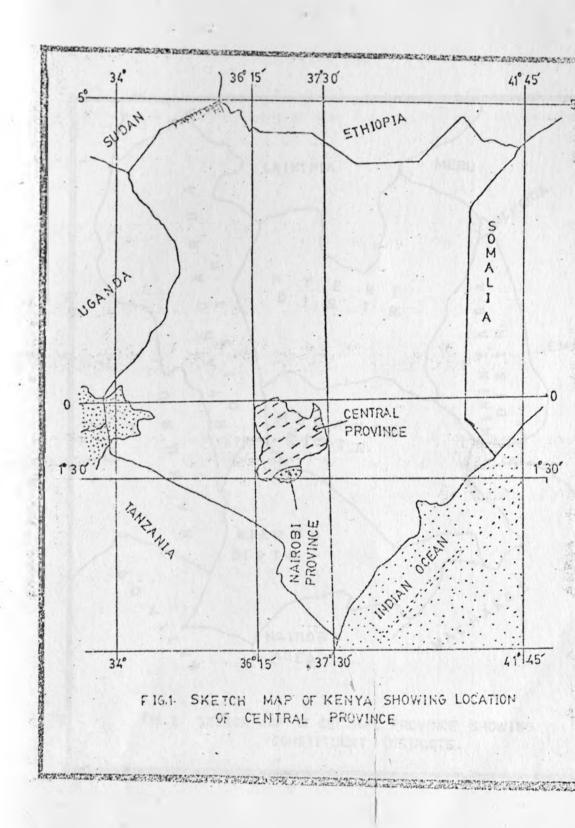
## 3. MATERIALS AND METHODS

#### 3.1. THE AREA OF STUDY

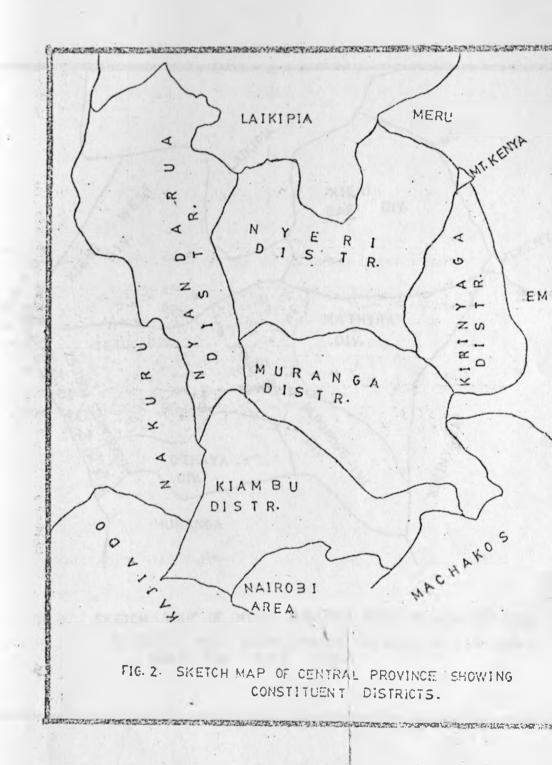
This study was conducted in Tetu Division of Nyeri District, Kenya. This area is situated at an altitude of approximately 2000 metres. The altitude is higher on the western side where the area borders the Aberdare Ranges. The mean annual rainfall ranges between 900 - 1100mm and occurs in two rain seasons, namely March to June (the long rains) and September to November (the short rains). The mean monthly minimum temperature usually varies from 10°C to 13°C and the mean monthly maximum temperature from 20°C to 25°C. Figs. 1 and 2 show the location of this area.

The land topography is characterized by hills and valleys, with streams or rivers at the bottom of the valleys. The majority of farm holdings are small with an average of 1 - 2 hectares. However, there are few large farms which vary in size from 20 - 50 hectares. Fig. 3 shows the location of farms used for this study.

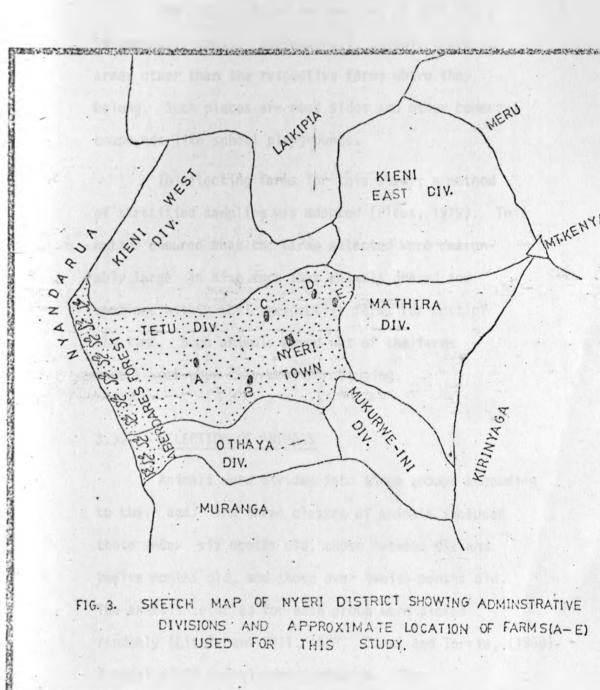
- 31 -



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SKETCH F1G. 3. DIVISIONS AND APPROXIMATE LOCATION OF FARMS(A-E) USED FOR THIS STUDY.

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## 3.2. CHOICE OF FARMS

The size of farms in the area of this study is generally small. Consequently, animals graze in areas other than the respective farms where they belong. Such places are road sides and other communal compounds like school playgrounds.

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In selecting farms for this study, a method of stratified sampling was adopted (Plews, 1979). This method ensured that the farms selected were reasonably large in size such that animals grazed and remained within their respective farms for most of . the time. Such animals moved out of the farms once a week when they went for dipping.

## 3.3. SELECTION OF ANIMALS

Animals were divided into three groups according to their age. The three classes of animals included those under six months old. those between six and twelve months old, and those over twelve months old. The animals selected for each group were picked randomly (Little and Hill, 1978 Steel and Torrie, (1980). A total of 60 animals were selected. They

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were put in groups of four animals per age group per farm. Where this number was not available at any age group in any farm, more were taken in other farms to give a total of 20 animals per age group. The initial numbers at each age group are indicated in Table 1.

# TABLE I : DISTRIBUTION OF ANIMALS BY AGE GROUPS AMONG THE FIVE FARMS STUDIED

AGE GROUPS	FARMS						
(MONTHS)	A	В	С	D	E		
< 6	5	6	6	1	2		
6-12	6	3	5	4	2		
> 12	4	4	4	4	4		

During the period of study, animals attaining the next adjacent age were immediately transferred to the new age group and their data recorded as such henceforth.

Animals were identified by their names and in one farm (Farm A) by their ear tatoo numbers.

## 3.4. COLLECTION OF FAECAL SAMPLES

Faecal samples were taken per rectum and put in faecal vials which were labelled appropriately for ease of identification. Samples were taken at intervals of two weeks.

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#### 3.5. COLLECTION OF HERBAGE SAMPLES

Kikuyu grass (Pennisetum clandestinum) was the main grass type in all the five farms. Herbage samples were collected along pre-determined W-transect (Anonymous, 1971) in the paddocks. Two technical workers were involved, starting from opposite sides and moving towards one another. Each person made a hundred stops (Burger, 1981) and collected four small lumps of grass approximately 5g. per stop. The grass was cut close to the ground and placed in bags made from cotton mosquito net. Each person collected about 500g. of grass. The top of each bag was tied with a string and placed in a plastic bag for transport to the laboratory. The plastic bag was used in order to hold free water on herbage (either due to rain or dew) which was always found to contain larvae. Collection of herbage samples started at six o'clock in the morning and was complete at about midday.

#### **3.6.** PROCESSING OF MATERIALS

#### 3.6.1. Number of Eggs per gramme of faeces (EPG)

The egg-count technique used was a modified McMaster method (Whitlock, 1948; Anon., 1971). Two grammes of faeces were weighed and put in a graduated measuring cylinder. Concentrated sodium chloride solution was added to the 30ml. mark and this was vigorously stirred to make a homogenous solution. This solution was passed through a tea strainer to re the course debris while the rest of the solution was held in a reagent bottle via a funnel. Using a teat pipette, a little of the solution was drawn after shaking the reagent bottle and quickly placed in one counting chamber of McMaster slide. The reagent bottle was shaken again and a second sample drawn and quickly placed in the second chamber of the McMaster slide. The slide was examined microscopically at a magnification of 400 times and the total number of eggs counted in the two chambers was recorded and multiplied by a factor of 50 to get the number of eggs per gramme of faeces (EPG). This was recorded for every animal. <u>Moniezia</u> eggs were observed but were not counted.

### 3.6.2. Coproculturing and Identification of Larvae

After processing faeces for egg counting, all the faecal samples from each farm were pooled together in a wide-mouthed bottle and incubated in an oven at 26°C for seven days (Roberts and O'Sullivan, 1950; Anon, 1971).

If the faeces were found to be very hard, a little water was added prior to incubation to give a fine consistency. During incubation, faeces were stirred at intervals of two days.

To recover the larvae after the incubation period was over, the wide-mouthed incubation bottle was filled with water and inverted on a petri-dish containing warm water at 37°C to a height of about 3cm (Skerman and Hillard, 1966). This set-up was left standing for about 1 hour during which larvae swam out of the incubation bottle into the petri-dish. Water containing the infective larvae was drawn from the petridish using teat pipettee and concentrated by centrifugation at 250g for 5 minutes and the supernatant decanted to leave about 0.5ml. The larvae recovered were examined microscopically under magnification of 400 times. The infective larvae were identified to the generic name according to the criteria of Keith (1953) and Borgesteede and Hendriks (1974). About 100 infective larvae were identified and percentages of various genera computed and recorded.

3.6.3. Recovery of larvae from herbage samples.

The two herbage samples collected per farm were processed separately for the recovery of infective larvae (Anon, 1971; Burger, 1981).

The bag containing the herbage sample was removed from the plastic bag and put into a 20 litre bucket. The inside of the plastic bag was washed and the washings placed in the bucket. Tap water was run in the bucket to submerge the bag containing the herbage. Five drops of detergent (Trade name Dynamo) were added to the water and thoroughly stirred. Herbage was then washed by lifting the bag up and down in the bucket. This was done for 5 minutes and the bag transfered to another bucket containing fresh water and the washing process repeated for ten minutes in the second bucket. The bag was then transfered to a third bucket containing fresh water where it was left overnight (12-24 hrs).

The following morning, all the washings from the three buckets were passed through a series of three sieves with pore diameters of 212/ $\nu$ , 63/ $\nu$  and 38  $\mu$ , respectively. After the washings of each sample were passed through the series of sieves, a strong jet of water was forced through the first sieve (diam.  $212 \mu$ ) and the debris in this sieve discarded. Using a more gentle jet of water, the contents of the second and third sieves were collected in a wide-mouthed bottle making a total of about 100ml. This was left to stand for about one hour in a refrigerator at 4°C where the deposit settled and left a clear supernatant. The supernatant was gently poured off to leave about 40ml. This was divided equally into two universal bottles and centrifuged at 300g for 5 minutes and the supernatant poured off gently. About 10ml. of concentrated magnesium sulphate solution was then added to the deposit which was agitated to give a homogenous suspension. This suspension was centrifuged at 200g for 3 minutes. The supernatant containing the larvae was placed in a wide-mouthed bottle and tap water added to lower the concentration of magnesium sulphate solution. It was made to a volume of about 100ml and left to stand for about 1 hr. at 4°C after which the supernatant was discarded to leave about 20ml. This was placed in coneshaped centrifuge bottles and centrifuged at 250g for 5 minutes and the supernatant poured off to leave a volume of about 0.5ml. A drop of Lugol's iddine was added to kill the larvae.

- 40 -

The infective larvae (L3) contained in the 0.5ml. volumes were counted and identified in aliquots of 0.1ml. The 0.1ml. volume was placed on a glass slide marked with columns 1mm wide. An ordinary coverslip was placed on top of this volume. Counting was done under a microscope at a magnification of 400 times along the columns on the glass slide. Columns made the counting process more systematic and easier.

The number of larvae was recorded for every 0.1ml. aliquot. The mean of the five aliquots was computed for every sample of herbage and finally the mean for the two herbage samples from each farm was calculated.

After the washing process, the herbage samples were first dried in the sun and then in the oven at 70°C to constant weights. The final weights were recorded and the mean for each farm calculated.

### 3.7 ESTIMATION OF HERBAGE COVER

For an approximate estimation of herbage cover, a method similar to that used by Donald <u>et al.</u> (1978) was used. Briefly, grass from three square areas of paddock was cut to ground level and labelled separately. An area of 900cm<sup>2</sup> (30cm x 30cm) was taken per sample. This grass was dried to constant weights at 70°C and the weights recorded as a measure of herbage cover. Samples for this purpose were taken randomly throughout the paddocks.

#### 3.8. COLLECTION OF METEOROLOGICAL DATA

Data for rainfall, temperature, evaporation and humidity were kindly provided by the officer in charge of the Meteorological Station at Wambugu Farmers Training Centre, Nyeri, which is located in the study area. The data was supplied at the end of every month.

#### 3.9. ASSESSMENT OF STOCKING RATE

Stocking rates were calculated in terms of livestock units per hectare of land under pasture. For calculating livestock units, the following rates were adopted (Dr. Said, Personal communication):

1 cow = 1 livestock unit 1 Heifer in calf = 0.75 livestock unit Yearlings (Over 12 months)= 0.5 livestock unit Calves 6-12 months = 0.25 livestock unit

For the purpose of this study, a calf under 6 months was taken as being equivalent to one ewe with lamb which is equal to 0.2 livestock unit.

#### 3.10. ANALYSIS OF DATA

Statistical calculations of data which included means standard deviations, correlation coefficients and regression analysis were carried out on a computer (DSC/3 PANACEA) using standard statistical methods. The method of least squares (Snedecor and Cochran, 1967; Steel and Torrie, 1980) was used for regression analysis.

## 4. RESULTS

# 4.1. THE INFLUENCE OF WEATHER PARAMETERS ON THE NUMBER OF INFECTIVE LARVAE ON PASTURE

4.1.1. Rainfall

Figure 4 shows the ditribution of rainfall as recorded during the period of this study.

The total amount of rainfall during the year was 956.6mm as compared to the calculated annual average of 935.5mm for the last thirteen years (Officer-in-charge of Wambugu Farmer's Training Centre, Personal communication) in the study area. The amounts of rainfall are recorded in terms of total rainfall between two consecutive sampling dates. Two distinct high peaks are evident, the first occurring between October and December (short rains) and the second between April and June (long rains).

During the October-December peak, there was a total of 388.2mm of rain which was 40.6% of the total annual rainfall, while the April-June peak had a total of 429.7mm of rain representing 44.9% of total annual rainfall. The month of May had the highest rainfall (245.5mm), with an average of 7.9mm. per day.

A brief, lower but distinct rainfall peak occurring between mid-February and mid-March was observed. This peak occurred between the two major peaks (Fig.4) It had a total of 74.8mm of rainfall representing 7.8% of total annual rainfall.

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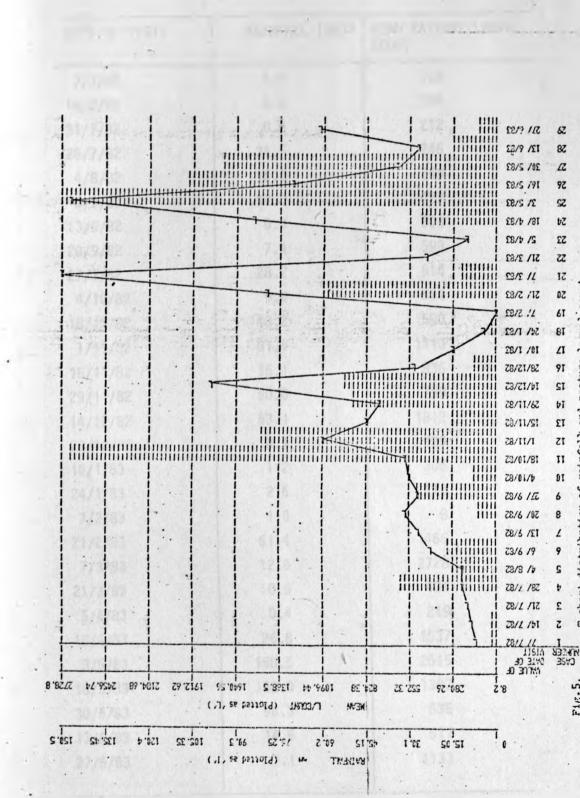
Fig. 5 shows a time-series chart (T-chart) of the distribution of pasture larval counts in relation to the distribution of rainfall. Three distinct larval peaks of 1812, 2728 and 2615 L3 per kilogramme of herbage were observed corresponding closely with the three rainfall peaks of 53.1mm, 12.6mm, and 150.5mm, respectively (Table II). The mean larval counts on Fig. 5 were the mean larval counts per kilogramme of dry herbage for the five farms included in this study. Charts for the individual farms are shown in Figs. 7-11. As in Fig. 5, the larval peaks corresponded closely with rainfall peaks.

The middle lower rainfall peaks (12.5mm and 61.4mm), coming after a dry period appeared, on the average, to correspond with the highest larval count of 2728 L3 per kilogramme of dry herbage. This observation was particularly marked in Farm A (Fig.7) and Farm C (Fig.9) as compared to other farms shown in composite charts (Figs. 12 and 13).

During the long rains (April-June), a mean larval count of 2615 L3/kg. of herbage was observed and immediately a sharp drop was then observed down to 517 L3/kg of dry herbage. During this period, there was an average of 6.9mm of rainfall per day.

The lowest larval count of 8 L3/kg of dry herbage was recorded when the amount of rainfall was 0.1mm in 14 days. The larval peak occurring in early May (2615 L3/kg of herbage) corresponded with a total amount of rainfall of 150.5mm in 14 days which was a daily average of 10.8mm. It was at about

- 45 -

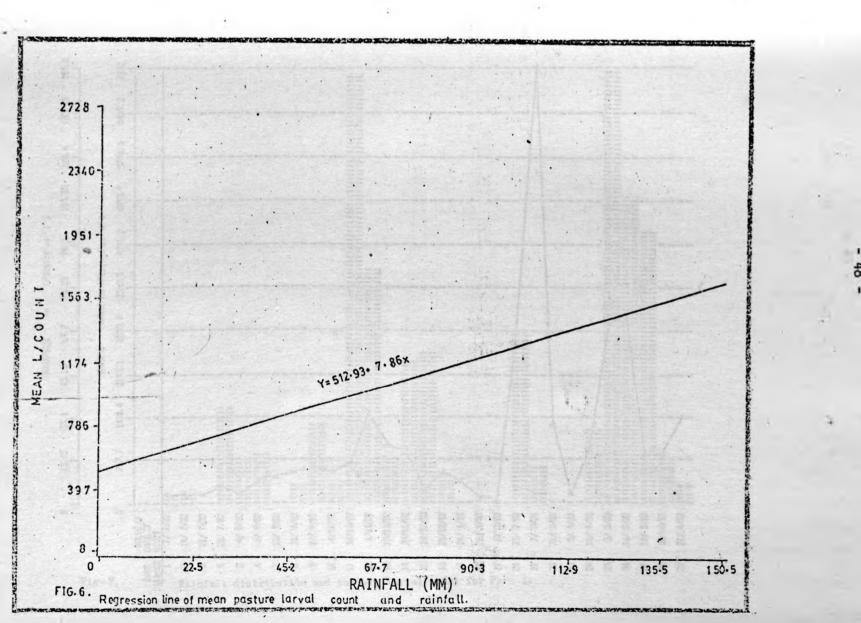


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DATE OF VISIT	RAINFALL (MM)*	MEAN PASTURE LARVAL		
7/7/82	1.7	152		
14/7/82	2.3	184		
21/7/82	0.0	212		
28/7/82	34.5	246		
4/8/82	16.2	249		
6/9/82	17.0	427		
13/9/82	0.4	503		
20/9/82	7.4	599		
27/7/82	28.2	514		
4/10/82	9.0	571		
18/10/82	148.2	590		
1/11/82	81.9	1110		
15/11/82	16.1	836		
29/11/82	50.0	759		
14/12/82	53.1	1812		
28/12/82	8.1	529		
10/1/83	1.2	306		
24/1/83	2.6	113		
7/2/83	1.0	8		
21/2/83	61.4	1466		
7/3/83	12.6	2728		
21/3/83	0.0	431		
5/4/83	0.4	219		
18/4/83	26.8	1537		
3/5/83	150.5	2615		
16/5/83	108.0	1307		
30/5/83	96.3	635		
13/6/83	14.6	517		
27/6/83	7.1	1133		

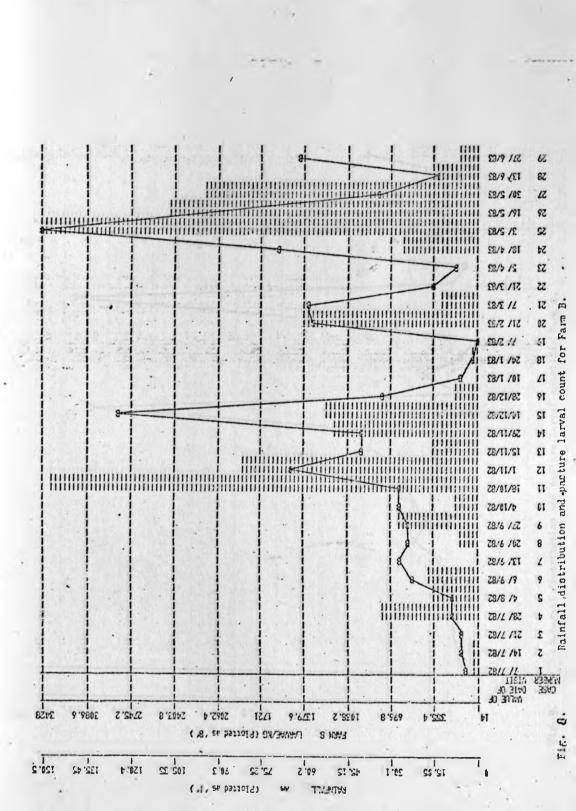
\* Rainfall means the total amount of rain falling between two consecutive sampling dates.

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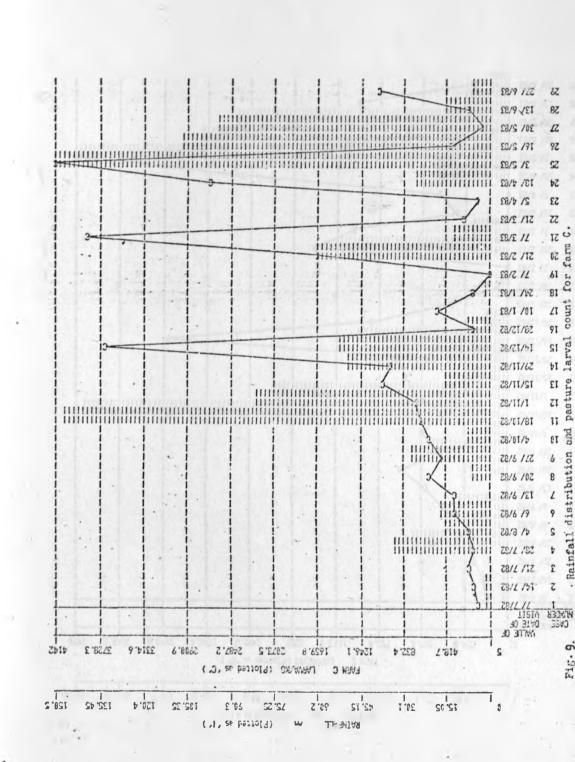


150.5 7132 135.45 6419.3 5706.5 120.4 105.35 4993.9 L'W' as batted as 'A') (Plotted as ' I' ) , 4281.2 50.3 3263.5 . 75.25 4 Ŧ. 2655.8 65.2 RADIA FARM A 12:12 2143.1 100000 1111111 1430.4 1.62 15.65 717.7 -. -S NAMER VISIT 24/ 1/33 14/ 7/82 22/1 7/22 28/ 1/82 4/ 8/32 . 61 9/32 13/ 9/82 23/ 9/62 21/ 9/32 4/10/82 18/18/82 1/11/32 15/11/82 28/11/82 14/12/92 28/17/82 10/ 1/83 11 2/23 21/ 2/83 71 3/33 21/ 3/83 5/ 4/83 18/ 4/83 28/5 /E 16/ 5/83 30/ 5/83 13/9 VET 271 6/83 1 2 51 16 13 3 5 13 3 = 14 17 15 23 24 3 26 3 3 2 Rainfall distribution and pasture larval count for Farm A. Fig. 7.

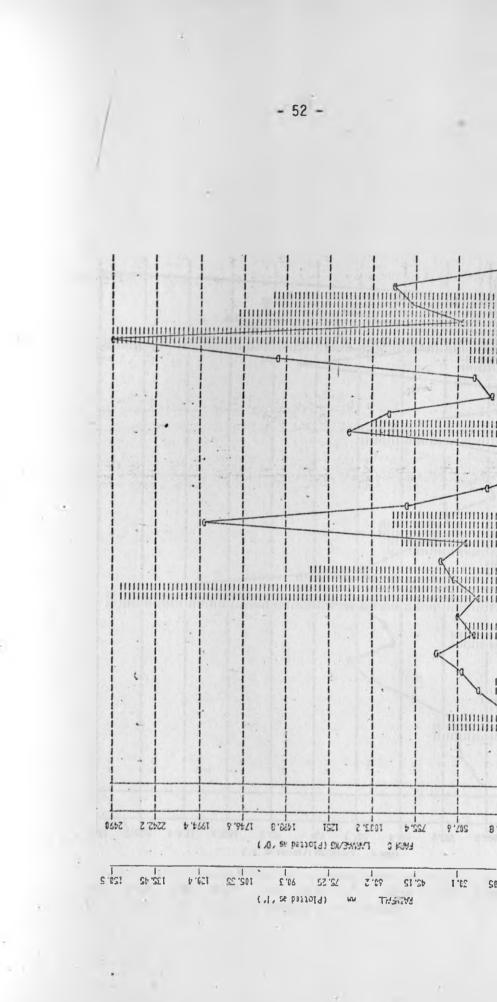
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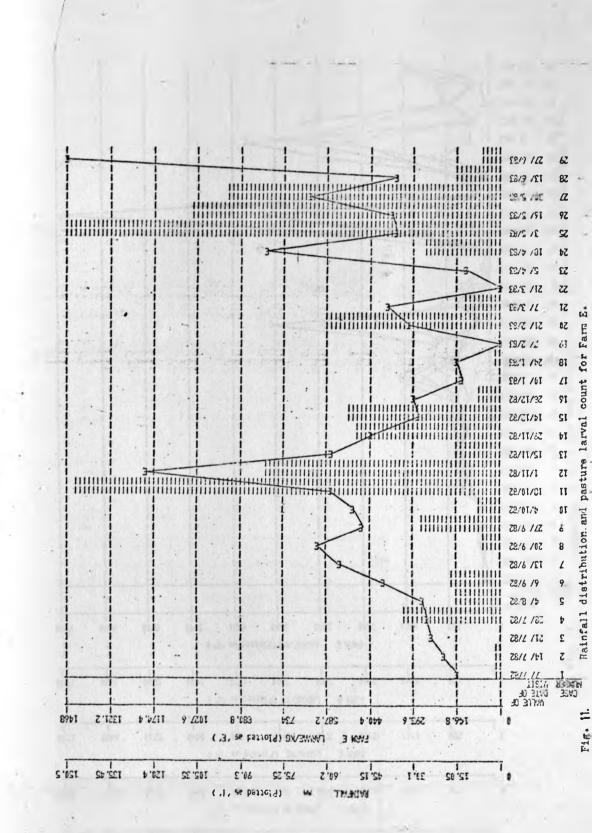


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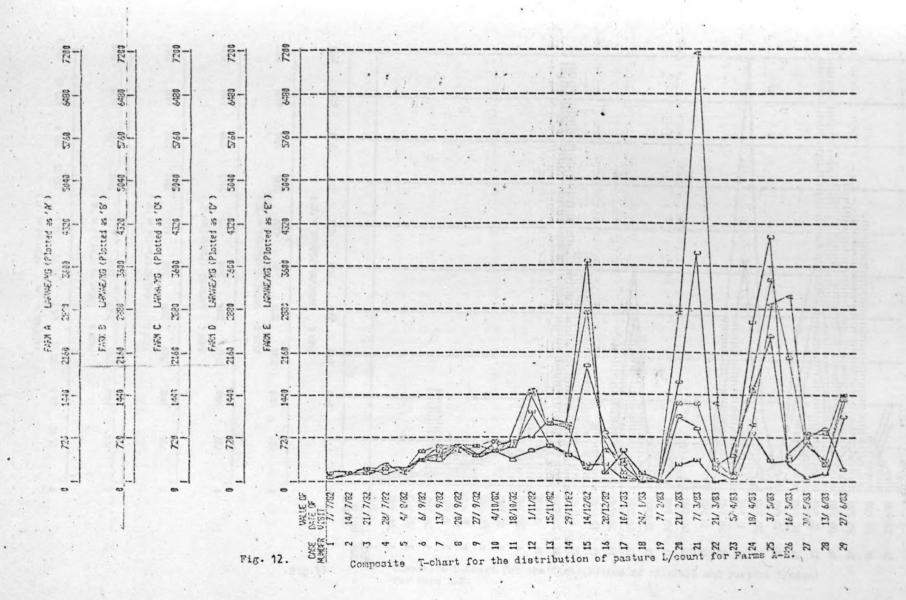


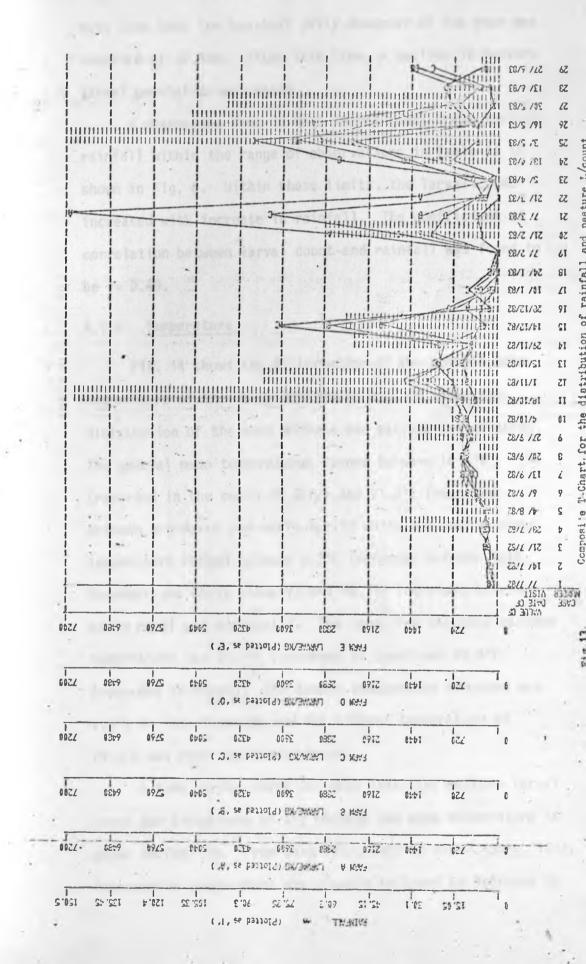
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- 53 -





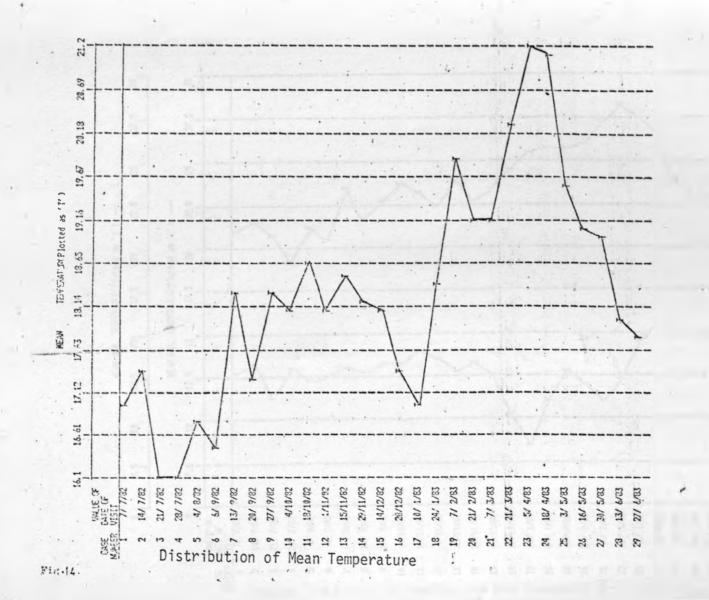
this time that the heaviest daily downpour of the year was recorded at 50.4mm. After this time, a decline in pasture larval population was noted.

A regression line of mean larval count drawn against rainfall within the range of observations in Table II is shown in Fig. 6. Within these limits, the larval count increased with increase in rainfall. The coefficient of correlation between larval count and rainfall was found to be r= 0.49.

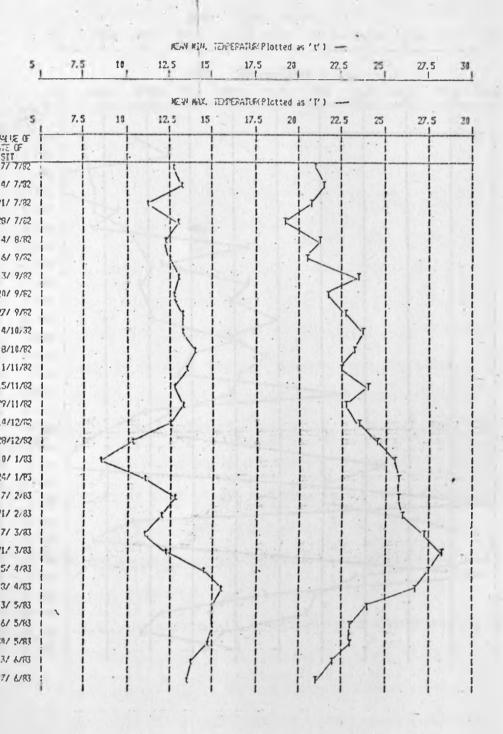
#### 4.1.2. Temperature

Fig. 14 shows the distribution of the general mean temperature of the study area while Fig. 15 shows the distribution of the mean minimum and maximum temperatures. The general mean temperatures ranged between 16.1°C (recorded in the month of July) and 21.2°C (recorded between mid-March and early April) while the mean minimum temperature ranged between 8.7°C (recorded between late December and early January) and 15.7°C (recorded between early April and mid-April). The range for the mean maximum temperature was 21.1°C (recorded in June) and 28.4°C (recorded in March). The lowest temperature recorded was 4.4°C in late December and the highest temperature of 29.6°C was recorded in mid-March.

A time series chart for mean infective pasture larval count per kilogramme of dry herbage and mean temperature is shown in Fig. 16. From late July, 1982 to mid-January, 1983, increase in temperature was closely followed by increase in

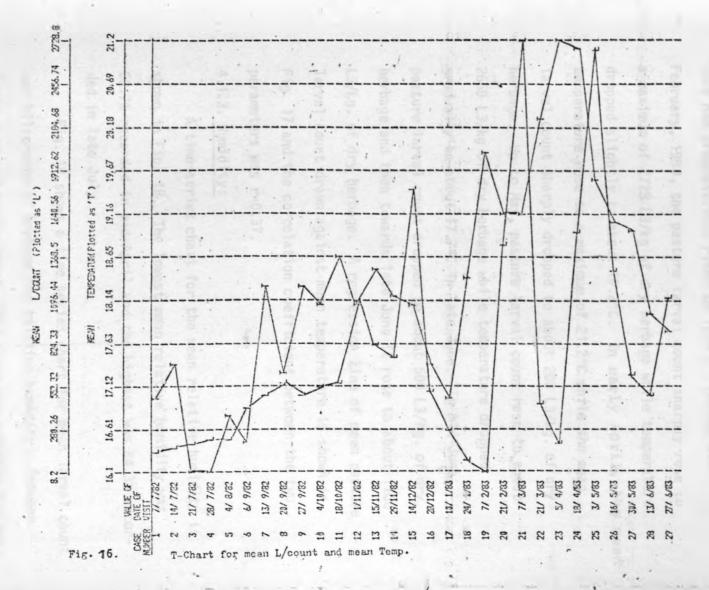


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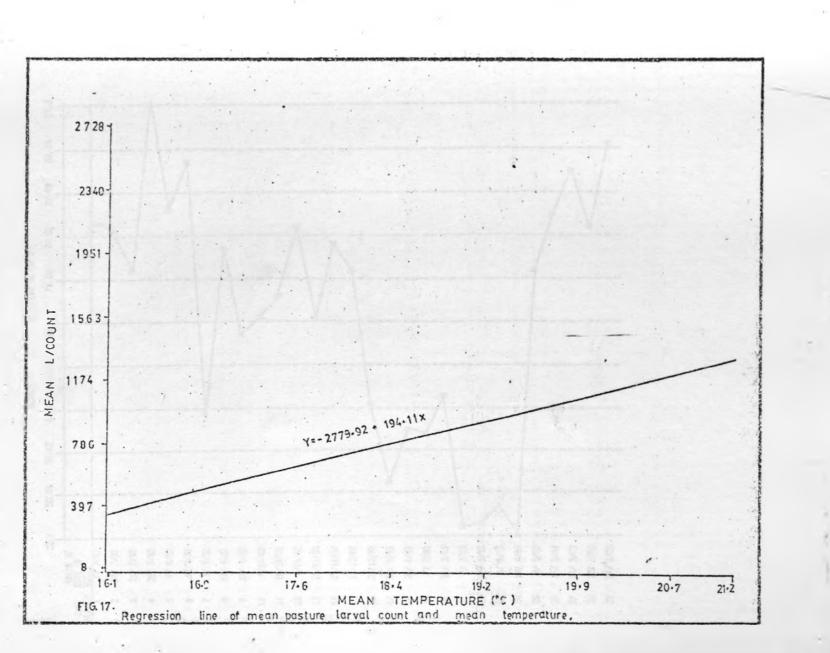
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larval counts. A break in this trend was observed in early February, 1983 when the pasture larval count was recorded at 8 L3/kg dry herbage while the mean temperature had progressively risen to 19.9°C. During mid-February, 1983, the pasture larval count sharply rose to a maximum of 2728 L3/kg of dry herbage while temperature dropped slightly to about 19.2°C. In early April, the m temperature rose to a maximum of 21.2°C while the mean larval count sharply dropped to about 200 L3/kg. of dry herbage. Up to May, pasture larval count rose to about 2650 L3/kg of dry herbage while temperature dropped gradually to about 17.7°C in late June. In mid-June, pasture larval count dropped to about 500 L3/kg. of dry herbage and then towards late June it rose to about 1100 L3/kg. of dry herbage. A regression line of mean pasture larval count drawn against mean temperature is shown in Fig. 17 and the correlation coefficient between the two parameters was r=0.37.

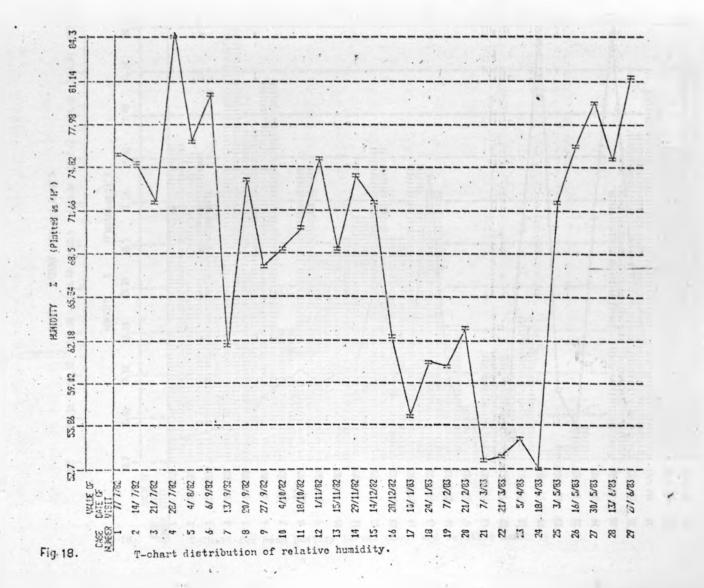
### 4.1.3. Humidity:

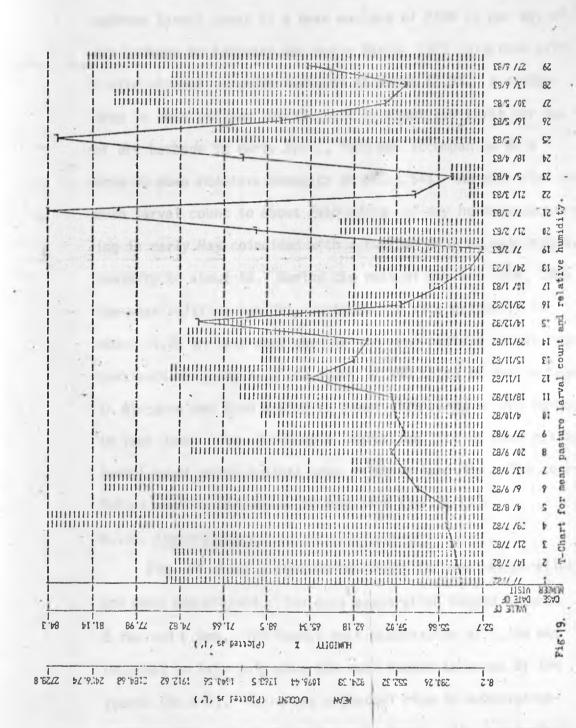
A time-series chart for the mean relative humidity is shown in Fig. 18. The lowest mean relative humidity was 52.7% recorded in mid-April and the highest was 84.3% recorded in late July.

Fig. 19 shows a time series chart for mean larval cour per kilogramme of herbage and relative humidity. Between early July and mid-December, 1982 there was moderate fluctua in relative humidity. During this period, the mean pasture larval count was steadily rising to a mean maximum of about



- 61





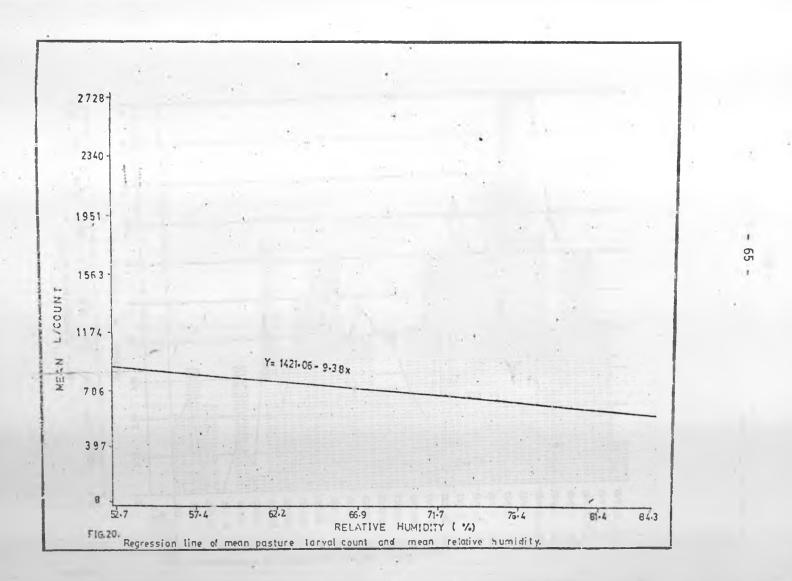
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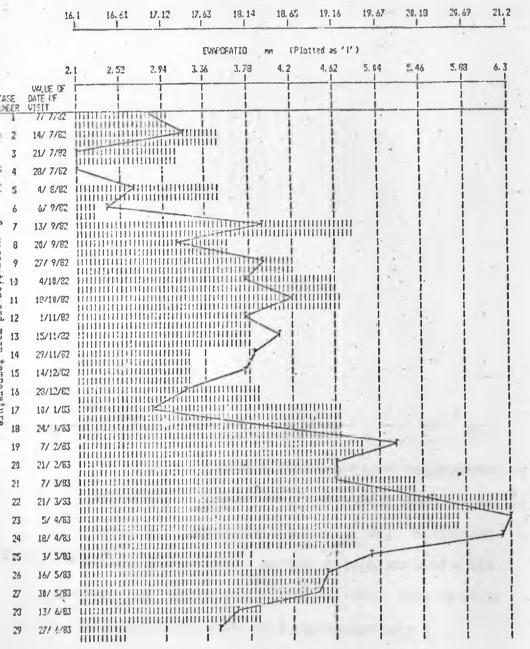
1750 L3/kg. of dry herbage in mid-December. This was followed by a drop in larval count to a mean minimum of about 8 L3/kg of dry herbage and a drop in mean relative humidity to about 50% in early January, 1983. A sharp rise in mean pasture larval count to a mean maximum of 2728 L3 per kg. of dry herbage in February and early March, 1983 coincided with a rise in mean relative humidity to about 62.3%. A sudden drop in mean pasture larval count to about 200 L3/kg per kg. of dry herbage in early April, 1983 was accompanied by a drop in mean relative humidity to about 54%. Another rise in mean larval count to about 2650 L3/kg. of dry herbage occurring in early May coincided with a furtner drop in mean relative humidity to about 5%. During the rest of May and June, 1983 the mean relative humidity continued to rise gradually to about 84.3% in late June whereas the mean pasture larval count continued dropping to a low count of 480 L3/kg of dry herbage in mid-June and then a rise to about 1120 L3/kg of dry herbage in late June. Fig. 20 shows a regression line of mean pasture larval count drawn against mean relative humidity. The correlation coefficient was a low negative (r=-0.12)

4.1.4. Evaporation:

Fig. 21 shows a time-series chart for mean evaporation and mean temperature. The mean evaporation ranged between 2.1mm and 6.3mm. The lowest mean evaporation of 2.1mm was recorded in late July when the mean temperature was at the lowest (16.1°C). There was a gradual rise in evaporation reaching the peak of 6.3mm in early March. The temperature

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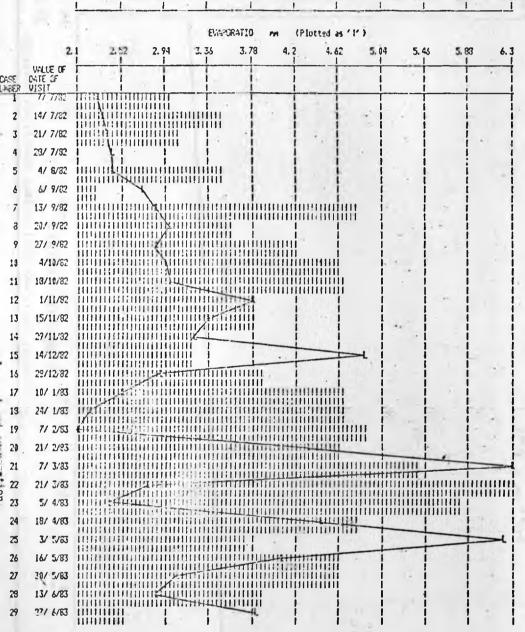
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at this time was also at the peak of 21.2°C. Thereafter, a decline in evaporation was observed as the mean temperature decreased.

- 67 -

A time-series chart for mean evaporation and mean pasture larval count is shown in Fig. 22. There was a poor correlation between the trends of the two parameters. Fig. 23 shows a regression line of mean larval count drawn against evaporation. A very low correlation coefficient was recorded (r=0.08).

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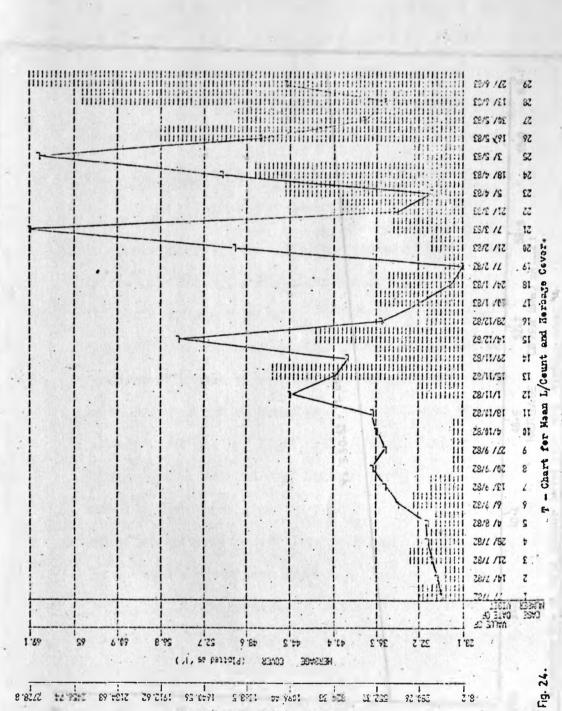


MEAN L/CUMT (Plazted 25 "L") 8.2 283.26 552.52 824.33 1076.44 1368.5 1540.56 1912.62 2134.68 2456.74 2723.8

- 89 -

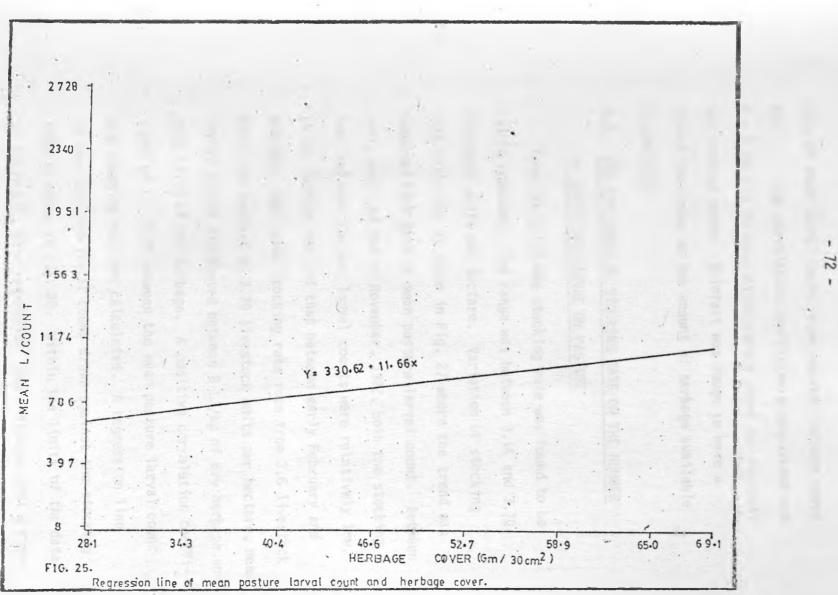
# 4.2. THE INFLUENCE OF HERBAGE COVER CN THE NUMBER OF INFECTIVE LARVAE ON PASTURE

Fig. 24 shows that there was little variation in the amount of herbage between July and mid-September. The variation at this time was between 31.8g/900 cm<sup>2</sup> and 32.6g/900 cm<sup>2</sup>. During this time, the mean pasture larval count was gradually rising from 152 1.3/kg. of dry herbage in early July to about 503 L3/kg. of dry herbage in mid-September. From mid-September to mid-October, the amount of herbage dropped to about 28.1g/900 cm<sup>2</sup> whereas larval count continued rising to about 590 L3/kg of dry herbage. From early November to mid-December, there was a significant rise in the amount of herbage to about 46.1g/900 cm<sup>2</sup> which coincided with a further rise in mean pasture larval count to about 1812 L3/kg of dry herbage. This was followed by a gradual drop in herbage cover down to 29.7g/900 cm<sup>2</sup> in early February which coincided with a minimum larval count of 8 L3/kg of dry herbage. After this there was a gradual rise in the amount of herbage to a maximum of 69.1g/900 cm<sup>2</sup> in late June. During this period there were two high larval peaks of 2728 L3/kg of dry herbage in March and 2615 L3/kg of dry herbage in early May. At the time of maximum herbage cover in June, a mean larval count of 1133 L3/kg of dry herbage was recorded. Fig. 25 shows a regression



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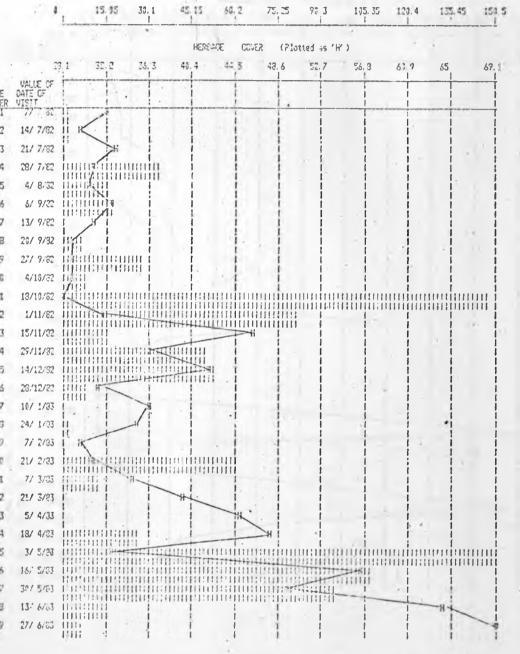
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line of mean larval count drawn against herbage cover and the correlation coefficients calculated was r = 0.18.Fig.26 shows a time-series chart for rainfall and herbage cover. Rainfall was found to have a direct influence on the amount of herbage available in pasture.

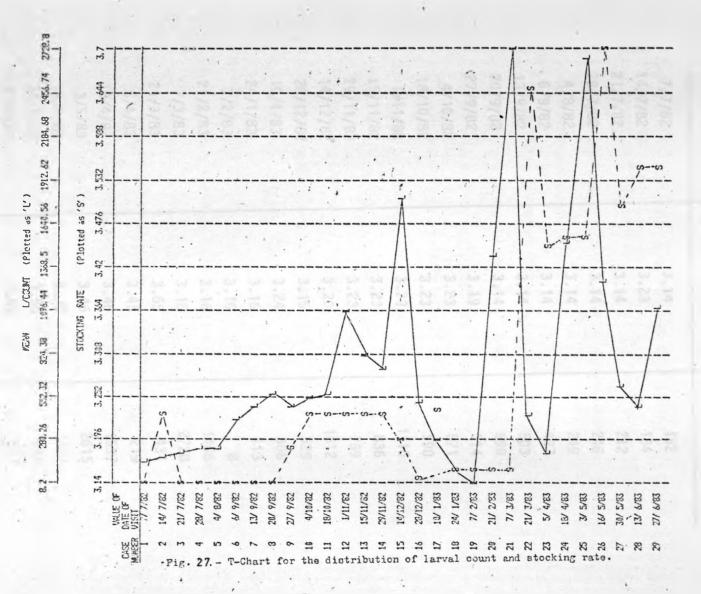
# 4.3. THE INFLUENCE OF STOCKING RATE ON THE NUMBER OF INFECTIVE LARVAE ON PASTURE

From Table III the stocking rate was found to be fairly constant. The range was between 3.14 and 3.70 livestock units per hectare. Variation of stocking rate with time is shown in Fig. 27 where the trend was compared with that of mean pasture larval count. Between early July and end of November, 1982, both the stocking rate and mean pasture larval counts were relatively low. It was further noticed that between early February and mid-May, 1983, when stocking rate rose from 3.6 livestock units per hectare to 3.70 livestock units per hectare, mean larval counts fluctuated between 8 L3/kg of dry herbage and 2728 L3/kg of dry herbage. A positive correlation coefficient of r = 0.23 between the mean pasture larval count and stocking rate was calculated. A regression line of mean pasture larval count drawn against the stocking rate is shown in Fig. 28. Within the limits of the data on Table III, this regression line indicated that a rise in stocking rate was followed by a rise in mean larval count.



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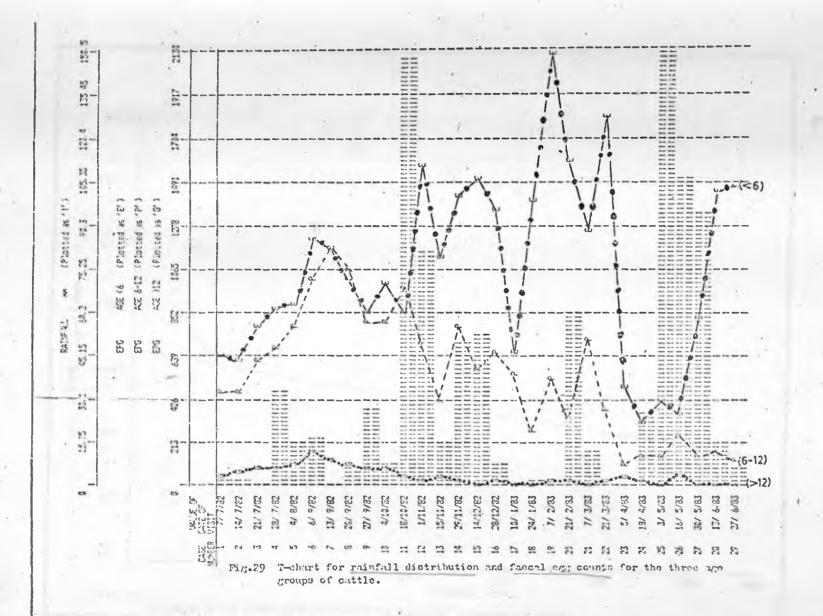
DATE OF VISIT	STOCKING RATE IN LIVESTOCK UNITS/ HECTARE	MEAN LARVAL COUNT PER KG OF HERBAGE 152			
7/7/82	3.14				
14/7/82	3.23	184			
21/7/82	3.14	212			
28/7/82	3.14	246			
4/8/82	3.14	249			
6/9/82	3.14	427			
13/9/82	3.14	503			
20/9/82	3.14	599			
27/9/82	3.19	514			
4/10/82	3.23	571			
18/10/82	3.23	590			
1/11/82	3.23	1110			
15/11/82	3.23	836			
29/11/82	3.23	759			
14/12/82	3.20	1812			
28/12/82	3.15	529			
10/1/83	3.24	306			
24/1/83	3.16	113			
7/2/83	3.16	8			
21/2/83	3.16	1466			
7/3/83	3.16	2728			
21/3/83	3.65	431			
5/4/83	3.45	219			
18/4/83	3.46	1537			
3/5/83	3.46	2615			
16/5/83	3.70	1307			
30/5/83	3.50	635			
13/6/83	3.55	517			
27/6/83	3.55	1133			

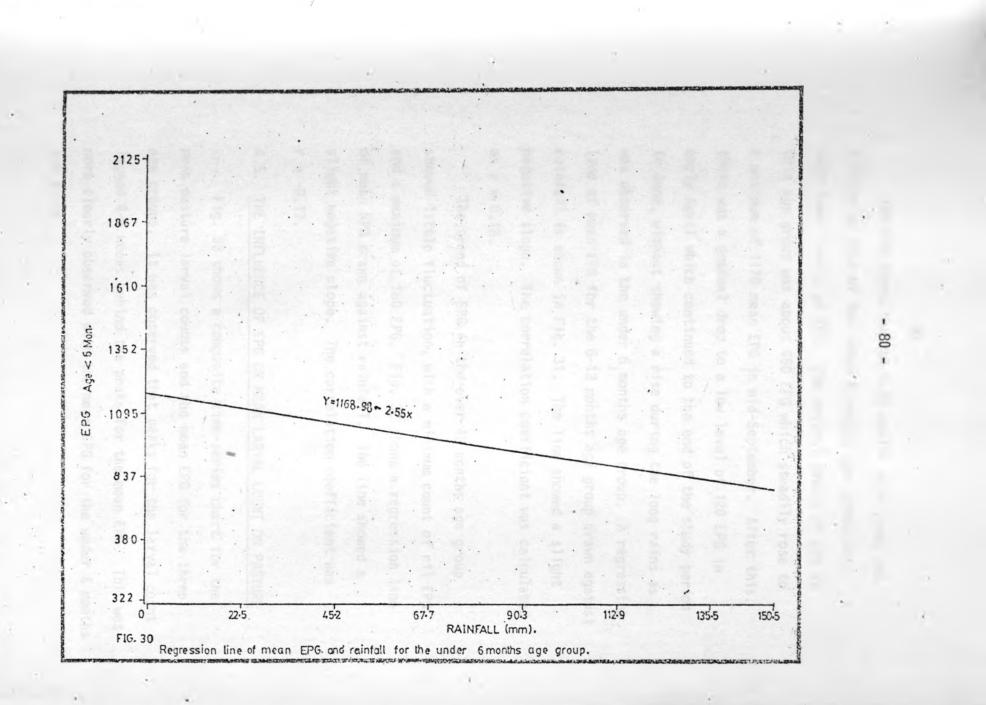
TABLE III. The influence of stocking rate on the number of infective larvae on pasture.

## 4.4. THE INFLUENCE OF AGE OF HOST ON FAECAL EGG COUNTS IN RELATION TO RAINFALL

The mean faecal egg counts per gram of faeces (EPG) for the three age groups were plotted and superimposed on a time-series chart for rainfall as shown on Fig. 29. The EPG for both the under 6 months and 6-12 months age groups increased with increase in rainfall. The EPG for the over 12 months age group remained low throughout and did not show significant response to rainfall.

In July, the mean faecal egg counts for the under 6 months age group was low at about 640 EPG. This steadily rose to 1150 EPG in early September and a decline followed to about 850 EPG in early October. All this time, rainfall fluctuated between nil and 34.5mm which was recorded in late July. A sharp rise to about 1550 EPG was recorded in early November followed by fluctuations between this number and 640 EPG in mid-January. A peak was recorded in early February at 2125 EPG. At the time of this peak, it was observed that there was little or no rain, with only 1.0mm recorded in two weeks. When the rain came in mid-April a sharp drop in EPG was recorded at about 320 EPG which continued until mid-May after which a rise was again recorded at about 1470 EPG in mid-June. Fig. 30 shows a regression line of mean number of EPG for the under 6 months age group, drawn against rainfall. The line showed a slight negative slope. A correlation coefficient of r =-0.25 was calculated.





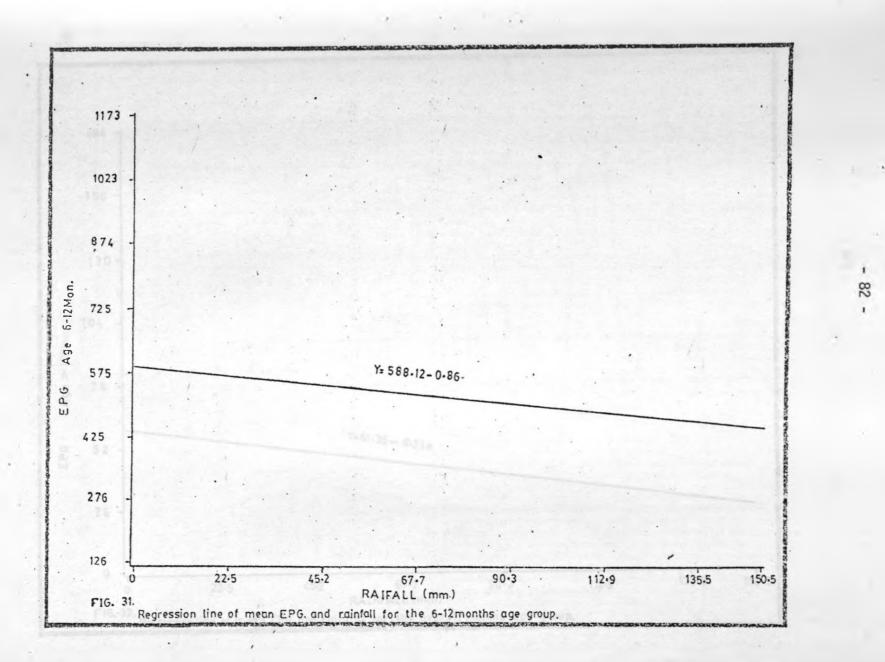
The EPG trend for the 6-12 months agre group was similar to that of the under 6 months age group but with lower levels of EPG. The initial levels of EPG in this age group was about 450 EPG which steadily rose to a maximum of 1170 mean EPG in mid-September. After this, there was a gradual drop to a low level of 120 EPG in early April which continued to the end of the study period in June, without showing a rise during the long rains as was observed in the under 6 months age group. A regression line of mean EPG for the 6-12 months age group drawn against rainfall is shown in Fig. 31. The line showed a slight negative slope. The correlation coefficient was calculated as r = -0.12.

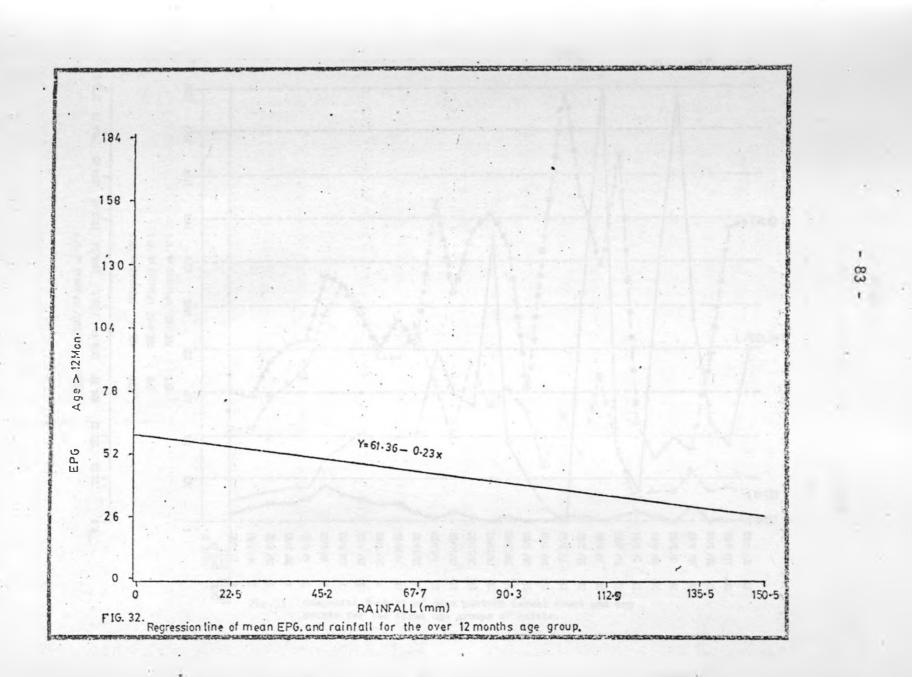
The trend of EPG in the over 12 months age group showed little fluctuation, with a minimum count of nil EPG and a maximum of 180 EPG. Fig. 32 shows a regression line of mean EPG drawn against rainfall. The line showed a slight negative slope. The correlation coefficient was r = -0.17.

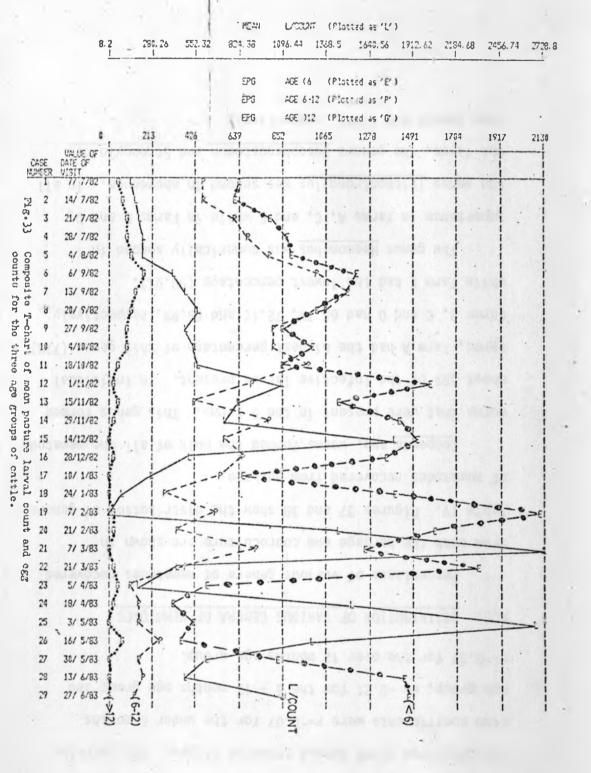
### 4.5. THE INFLUENCE OF EPG ON MEAN LARVAL COUNT ON PASTURE

Fig. 33 shows a composite time-series chart for the mean pasture larval counts and the mean EPG for the three age groups. It was observed that peaks for the larval count lagged 4-6 weeks behind the peaks for the mean EPG. This was more clearly observed with the mean EPG for the under 6 months age group.

- 81 -







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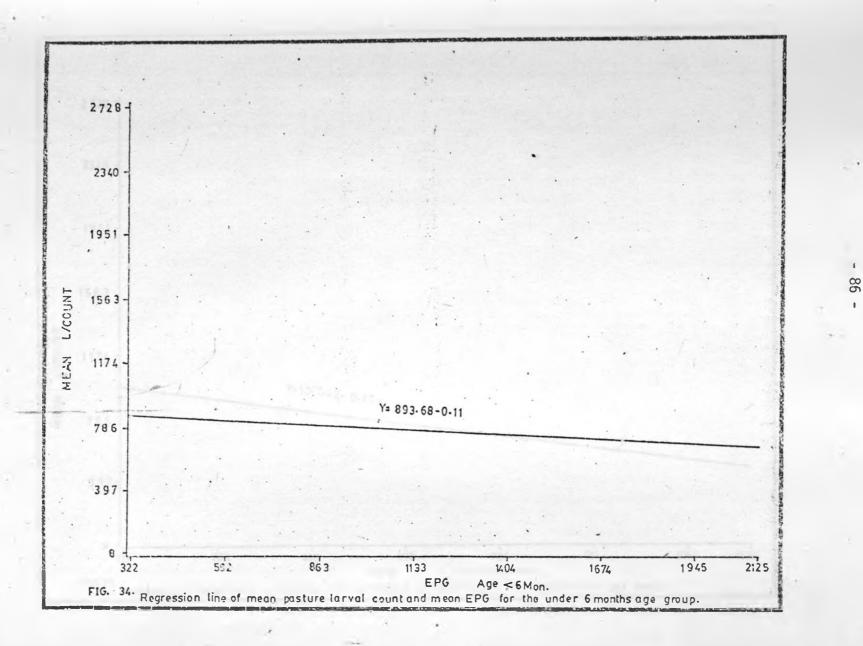
Fig. 34 - 36 show regression lines of mean larval counts drawn against the mean EPG for the three age groups. All the three lines showed negative slopes. The correlation coefficients were r=-0.07 for the under 6 months age group, r= -0.21 for the 6 - 12 months age group and r=-0.37 for the over 12 months age group.

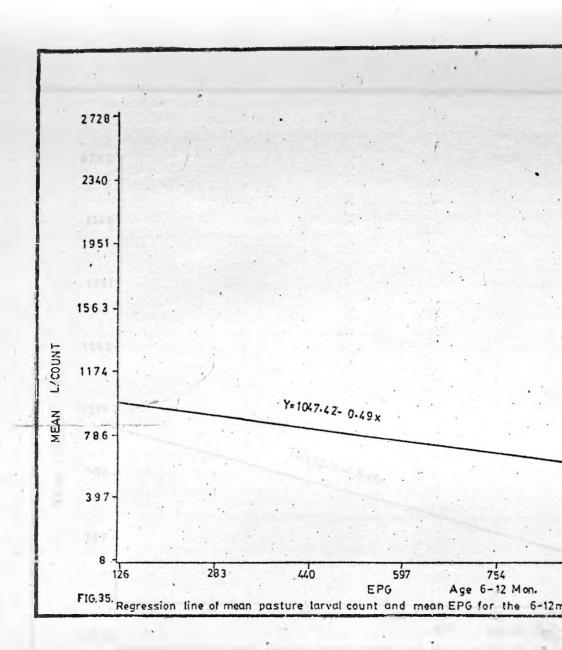
### 4.6. DISTRIBUTION OF VARIOUS GENERA OF NEMATODES

Percentages of various genera of nematodes recovered from both the herbage and coproculture are shown in Table IV. Figures 37 and 38 show the distribution of genera of nematodes recovered from herbage.

<u>Cooperia</u> spp. worms formed the bulk of all the nematode worms that were present in the 5 farms. This genus formed about 69% of the infective larvae present. In individual farms, Farm A had the highest percentage of this genus (77%). Farms B, C and D had 68.7%, 72.1% and 69.9%, respectively while Farm E had the lowest percentage (58.9%).

The genus <u>Haemonchus</u> was numerically second in importance in farms A, C, and E while in Farms B and D, the genus <u>Trichostrongylus</u> was second in abundance. In all the farms, the genera <u>Oesophagostomun</u> and <u>Strongyloides</u> came fourth and fifth, respectively.





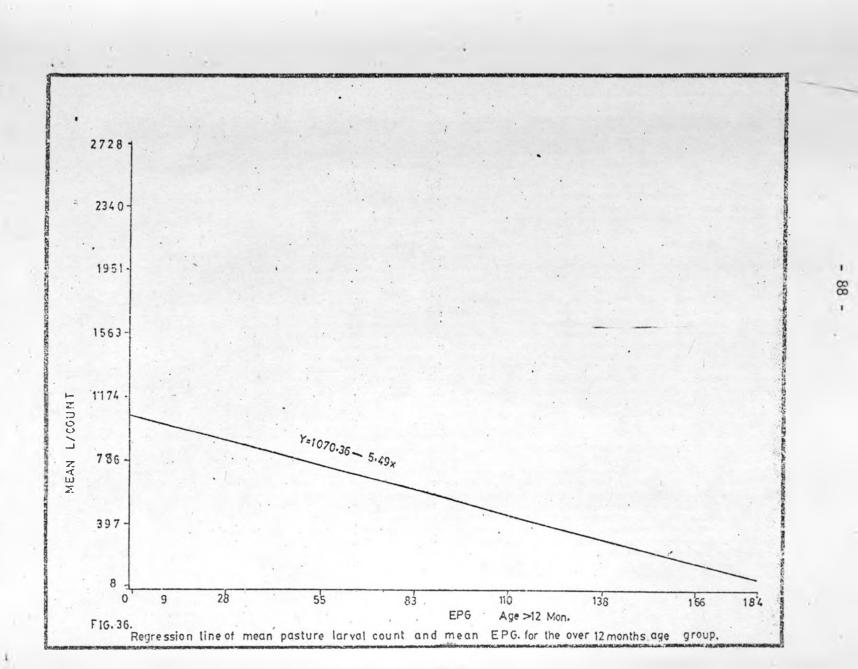


TABLE IV.	Distribution (in percentages) of various genera of gastrointestinal (GIT)	
	nematodes recovered from herbage and coproculture.	

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GENERA	COOPERIA		HAEMONCHUS		TRICHOSTR.		OESOPHAG.		STRONGYL.	
FARMS	HERB.	COPROC	HERB.	COPROC	HERB.	COPROC	HERB.	COPROC	HERB.	COPROC
A	77.0	78.3	9.4	13.9	4.0	2.9	9.5	4.9	0.0	0.0
В	68.7	65.7	9.2	17.5	12.0	9.8	10.1	5.7	0.0	1.3
С	72.1	75.2	12.5	11.5	9.6	4.2	5.9	4.0	0.0	5.1
D	69.9	73.8	9.3	11.1	12.0	7.3	8.7	7.3	0.1	0.5
E	68.9	52.9	22.1	25.4	10.3	3.2	6.4	5.4	2.3	13.1
GENERAL MEAN	69.3	69.2	12.5	15.9	9.6	5.5	8.1	5.5	0.5	4.0

KEY:

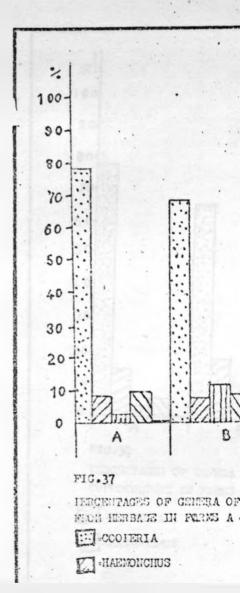
Herb.

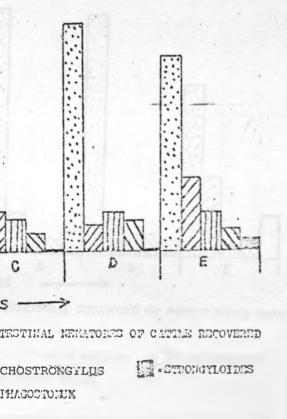
Oesophag. Strongyl.

Oesophagostomum Strongyloides

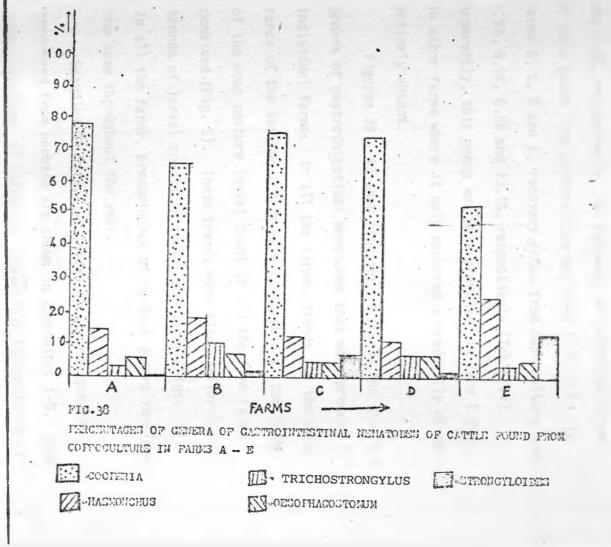
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Herbage Coproculture Trichostrongylus Coproc. -Trichostr. -





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Infective larvae of the genus <u>Strongyloides</u> were not recovered from herbage in Farms A, B and C. Recoveries of infective larvae of this genus in Farms D and E were 0.1% and 2.3%, respectively. No recovery of infective larvae of this genus from coproculture was made in Farm A. In Farms B, C, D and E, recovery rates from coproculture were 1.3%, 5.1%, 0.5% and 13.1%, respectively (Table IV). Apparently, this genus was more prevalent in Farm E than in other farms where it only occurred sporadically or was entirely absent.

Figures 39 - 43 show time-series charts for the five genera of gastrointestinal nematodes that were present in individual farms. In all the farms, trends for the occurrence of the various genera were comparable to the trend of the mean pasture larval count in all the five farms combined (Fig. 5). These trends were also similar to the trends of larval counts in individual farms (Figs. 7-11). In all the farms, predominance of various genera remained the same throughout the year.

Examples of infective larvae of various genera recovered from herbage are shown in appendices 1-5. Some common features of infective larvae like the presence of sheath, gut cells and tails were demonstrated. The infective larvae of the genus <u>Strongyloides</u> in appendix 5 had started to disintegrate. It was noted that <u>Strongyloides</u> larvae did not keep well as long as did the larvae of other genera.

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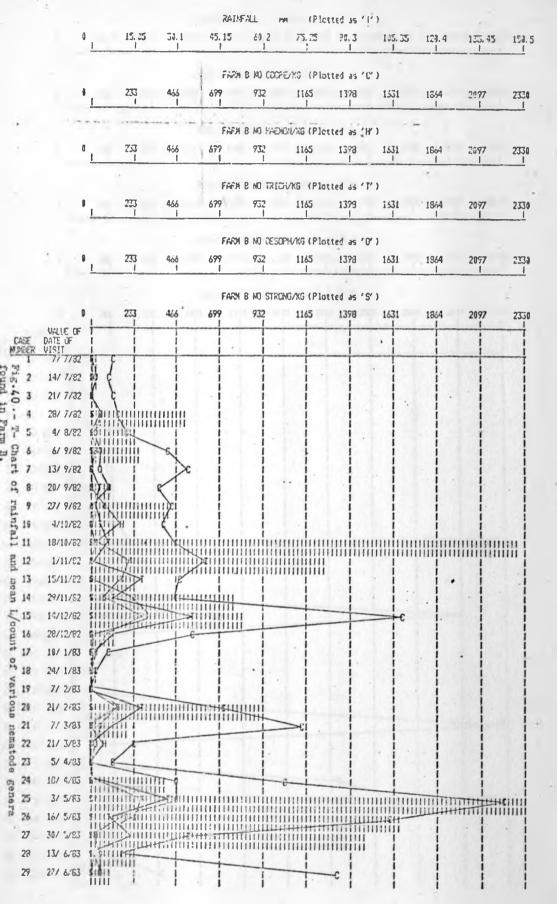
150.5 2000 10062 5062 2900 2900 S 2192 2619 2610 2610 2510 R . -1222 2320 12E 222 2320 13 12 2030 2029 2020 2030 2030 W. ~' C.t. (flotted as ''') ~ --2 ¥ ъ. s. 32.3 . \$ şe 25-17 x 12 1740 1740 1740 5 1740 15" otted A PD HEDROVKG (Plotted FARM A NO TRICHONG (Platted FACH A NO DESCRIVES (Plotted FALM A HO STROMG/KG (Plotted 13 1257 1150 1450 1450 1450 jë. IN COOLE VO ŧ 4 . 1160 1160 ... 1160 1160 1150 63. THADEAT €. \*\*\*\* FAT TEL. . 4  $^{h_{1}^{*}}_{w \rightarrow t}$ 100 575 870 878 220 12 . 13 2 5 ß 233 185 1111123 . == 33 ie. 598 298 290 3 57 ------VALLE OF DATE OF UISIT 14/ 7/82 28/1 7/82 23/ 7/82 6/ 9/82 13/ 9/82 20/ 9/32 28/6 /12 4/10/82 18/10/82 1/11/82 15/11/82 29/11/92 14/12/82 28/12/82 10/ 1/83 24/ 1/83 11 2/33 21/ 2/83 7/ 3/83 21/ 3/83 18/ 4/83 30/ 5/83 13/ 6/83 1782 8/82 4/83 5/83 5/83 6/83 4 3 3 16/ 10 CASE MUREER 2 12 5 = 13 44 16 1 18 19 20 3 23 23 3 3 8 6 8 "of rainfall and mean L/count of various nematode genera Fig. 39. - T-chart

found in Farm A.

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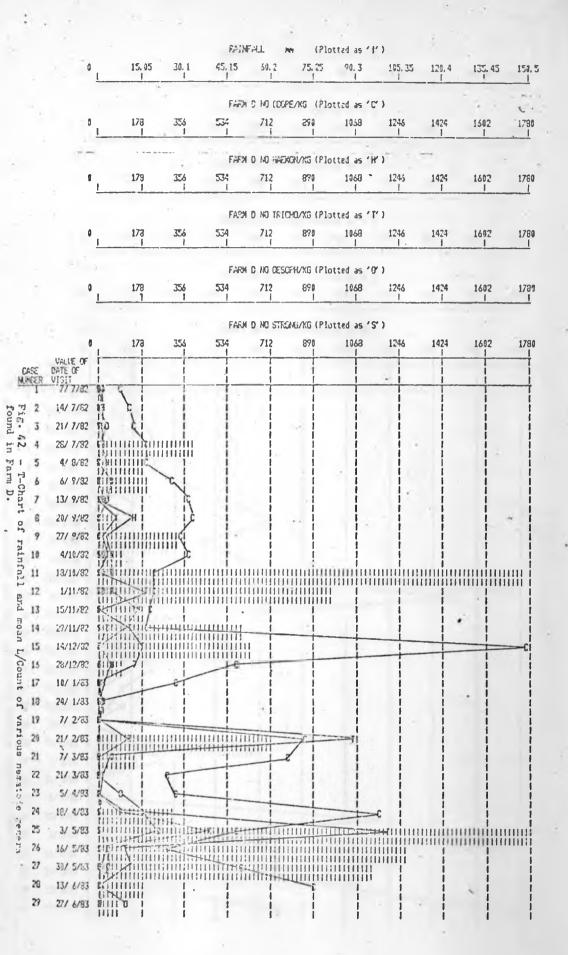


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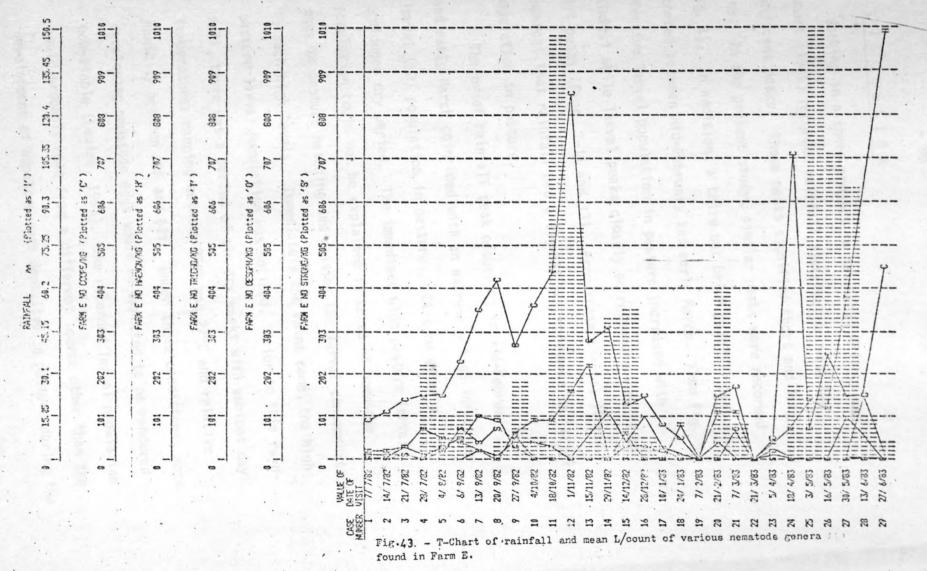
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# 5. DISCUSSION

Working in a sheep project in Naivasha, Allonby and Urquhart (1975) recorded rainfall distribution with two significant peaks. These peaks signified short and long rains. In the present study, similar peaks were recorded (Fig. 4). In addition, a third but brief rainfall peak was recorded between mid-February and early March. From Fig. 5, infective larval population in pasture increased with rainfall while larval peaks closely corresponded with rainfall peaks (Figs. 7-11 and 13). From these charts, it was apparent that rainfall had a significant influence on larval population in pasture.

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The brief rainfall peak occurring in mid-February and early March coincided with an extremely high infective larval (L3) population in pastures. It came after a prolonged dry period. The immediate high pasture larval population could not be explained in terms of nematode eggs in pasture hatching and raising the larval contamination to such high levels. Immediately the rains came, the high pasture larval population was noticed. Prior to this rainfall, there was a period of six dry weeks with ambient day temperatures ranging between 25°C and 27°C and relative humidity between 55% and 62%. Under these conditions, most developing nematode eggs would be expected to be rendered non-viable (Levine, 1963). The recorded larval population would probably come from a different source other than the development of nematode eggs deposited in pasture during the

Infective larvae have been found to disappear from herbage into the soil during harsh conditions. They burrow down into the soil to as deep as 15 cm and reappear in large numbers during favourable conditions without fresh egg contamination (Duncan et al., 1979; Bairden et al., 1979; Armour et al., 1980). It is therefore probable that the sudden wave of high larval population during the brief rainfall was as a result of infective larvae reappearing to the grass mat after having burrowed into the soil during the preceeding dry period. Alternatively, these infective larvae might have come from the faecal pads after being softened by the rains. During the dry period, cattle faecal pads form a hard crust on the surface while it remains relatively moist underneath to be able to sustain developing larvae. The pads are also well aerated by the tracts made through them by burrowing beetles. When rain falls and softens the hard crust, the infective larvae underneath have the opportunity to move out and into the surrounding herbage and hence immediately raise the larval population. Roberts et al. (1952) estimated that such larvae moved about 60cm. away from the faecal pad in 24 hrs.

During the long rains, infective larval population initially rose to a peak (2615 L3/kg of herbage) and then declined to about 517 L3/kg of herbage as rainfall steadily continued (Fig. 5 and Table II). When the amount of rain started to decline, the infective larval population started on a gradual rise to about 1133 L3/kg of herbage at the end of

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the study period. The following explanation was suggested for this observation. As the rains came, favourable conditions ensured the hatching of nematode eggs deposited on pasture, with further development to third infective larval stage. As the rains continued, the amount of herbage increased more rapidly. Consequently, the number of infective larvae available per unit weight of herbage was gradually reduced, leading to what might be described as dilution of larval population in pasture. Grønvold (1979) and Oakley (1981) made similar observations but suggested that some fluctuations of larval activity during rain periods could be attributed to the activity and migration of earthworms. The two authors independently demonstrated that earthworms act as transport hosts for infective larvae. Alsaqur et al. (1982), on a similar observation, suggested that reduction in larval population in pasture during prolonged rainfall was due to percolating rain water carrying larvae downwards in the soil below the underlying herbage mat.

During the long rains (April - June) Farm C had the highest larval population in early May. At the end of this month, this farm had the lowest pasture larval population. The topography of this farm is one of marked slopes and it is suggested that in addition to the explanation given above on reduction of pasture larval population during prolonged heavy rainfall, washing away of larvae downhill could have accelerated the reduction of larval population in this farm. Crofton (1948), and Levine (1963)made similar suggestions.

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It has been established in this study that when the general mean temperature was at the highest peaks, the larval populations were at their lowest levels. Though during these periods the weather was dry, it was possible that the high temperatures were detrimental to larval population. According to Ogunsusi (1979) temperature is not a limiting factor in larval development in the tropics as the range of temperatures throughout the year is usually very low. However, extremely high temperatures could be detrimental to larval survival as it is apparent from the current study. The adverse effect could occur as a result of larvae being extremely active in moving about and depleting their food reserve (Levine, 1963), or could occur as a direct drying effect as suggested by Dinnik and Dinnik (1958). It was also probable that during these hot periods, infective larvae migrated below the soil level thereby creating an apparent but false reduction in herbage larval population as earlier mentioned.

During the dry period of July, 1982, there was an appreciable amount of herbage. The pasture infective larval populations were at this time sustained at moderate levels (200 L3 to 400 L3 per kg. of herbage). During the period early January, 1983 to mid-February, 1983, the amount of herbage progressively declined due to prolonged dry weather. At the same time, larval populations declined to a barely detectable level of 8 L3/kg of herbage. It was therefore concluded that herbage cover was essential for larval survival during the dry period. A similar conclusion was drawn by Dinnik and Dinnik (1961) on their determination of longevity of <u>H.</u> <u>contortus</u> on the pasture herbage in the Kenya Highlands.

Regression analyses indicated that rainfall and temperature had a direct relationship with larval population on pasture. Herbage cover and evaporation had a low positive correlation while humidity had a negative correlation. Under field conditions such as the ones under which this study was conducted, it is difficult to assess correctly the degree of influence of each of these weather parameters on pasture larval population as the parameters were not under control. The net influence was a complex interplay of all parameters involved. However, from this data analysis, it was concluded that rainfall, temperature and herbage cover had a significant influence on pasture larval population.

The observation from the regression analysis that pasture larval counts increased with increase in stocking rate was in accordance with the common\_belief. Taylor (1939) stated that worm burdens of grazing animals increased ninefold with trebling of stocking rate. However, considering the conventional definition of stocking rate (the number of livestock units per unit area of pasture land), this observation may not be explained in terms of stocking rate alone. As the stocking rate increases, there is a decrease in

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herbage cover as a result of more animals competing for the amount of herbage available in pastures. Consequently, infective larvae present are exposed to excessive sunlight and other adverse conditions. According to Dinnik and Dinnik (1961) and Barger (1979) infective larvae under such exposure would either die or disappear into the soil below levels accessible to grazing animals (Duncan <u>et ai</u>. 1979; Armour <u>et al</u>., 1980), thereby off-setting the immediate effect of increased contamination of pastures with nematode eggs by the increased herd population. This process would lead to less and less infective larvae being available to the grazing animals as a result of which worm burdens would be decreased. The stocking rate would therefore not be adequate to explain the observed increase in worm burden.

The definition of grazing intensity (the number of livestock units per unit of available foliage) takes into account the amount of herbage available at any one time. A change in grazing intensity would therefore be brought about either by a change in the number of livestock units, a change in amount of foliage or a change in both of them. The amount of foliage could be increased or decreased by the amount of rainfall available. In this respect, grazing intensity could vary widely even with a constant number of livestock units. Similarly, grazing intensity would remain constant or even decrease with increase in the number of livestock units depending on the amount of herbage available in a given pasture at a given time. Under the circumstances, the term grazing intensity would be more appropriate in explaining the numerical trend of infective larvae available to the grazing animals.

It was apparent that calves under 6 months of age shed the highest number of eggs per gram of faeces followed by the 6-12 months age group. During the period of this study, adults had a constantly low egg count, with little variation. Obviously, adults consume a lot more herbage than the younger stock and consequently ingest more infective larvae from contaminated pastures. They should therefore be expected to harbour more worms which would be laying more eggs. It was therefore probable that certain factor or factors were involved that suppressed the laying capacity of worms in adult cattle or that suppressed the establishment of infective larvae into adult worms in mature cattle. Such a factor would either be the phenomenon of arrested larvae or acquired resistance to worm infection or both combined (Blitz and Gibbs 1972, Gibbs 1982).

It was further observed that the highest egg counts in calves occurred during the dry period. These peaks in egg counts occurred about 2-4 weeks after the rains had ceased. This is contrary to the observation of Elisabeth (1982), that with the beginning of the dry season in South Mozambique the mean total egg counts in goats fell to a low level. From the data in this study it would therefore be advisable, in a worm control programme, to deworm calves

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during the dry periods to minimize pasture contamination with nematode eggs. Contamination during this time caused a flare-up of infective larval population at the onset of the rains. The practice in this area is to deworm animals when the rains come. From the information acquired from this study, such a practice would only be of temporary relief to the animal. Once the effects of the anthelmintics have waned which would be about 5-7 days, the animals would ingest more infective larvae from pasture as larval populations were at their peaks during the rain periods. These infective larvae would be stablished into adult worms and in about 2-3 weeks from the time of deworming the animal would be as heavily infected with worms as it was prior to treatment, if not more.

The results of this study showed that the most predominant genus of nematodes infesting cattle in the study area was <u>Cooperia</u> while the genus <u>Strongyloides</u> was the least predominant. Other genera present were <u>Haemonchus</u>, <u>Trichostrongylus</u> and <u>Oesophagostomum</u>. All the genera appeared to be equally influenced by prevailing weather conditions and there was no indication that a particular genus was more favoured or more adversely affected by a particular season than others.

A wide discrepancy between the percentages of infective larvae of the genus <u>Strongyloides</u> recovered from herbage and those recovered from the coproculture was noted. This observation was particularly marked in Farm E. In all cases, the percentages of larvae recovered from herbage was lower, while that recovered from coproculture was always higher. This could probably be explained by the fact that first stage larvae of the genus Strongyloides in pasture undergo development (heterogonic cycle) to sexually mature males and female (Soulsby, 1968). This process could temporarily reduce the population of recoverable infective larvae of this genus from herbage. This assumes that a similar process did not occur during coproculture under controlled and favourable temperatures, and hence favoured the homogonic cycle. This possibility was not investigated in the current study. Triantaphyllou and Moncol (1977) demonstrated that Strongyloides eggs in faeces collected from an infected animal and cultured in the laboratory developed into males, females and infective larvae in variable and relatively unpredictable numerical proportions. Varju (1966) and Galliard (1967) associated the variability in the proportion of heterogonic and homogonic individuals to certain host factors, which included the duration and density of infection, species, age and immumological status of the host. Recently, Nwaorgu (1983) indicated that temperature had a significant effect on the proportion of free-living females and infective larvae of Strongyloides papillosus developing from faecal cultures.

In the current study, it was further noted that infective larvae of the genus <u>Strongyloides</u> did not survive as long as other larvae under laboratory conditions. This may mean that they were more susceptible to certain conditions than other larvae, thus leading to their short lifespan. Under field conditions, this could reduce larvae population in pasture.

In all the three age groups of cattle studied, a poor negative correlation between the number of eggs per gram of faeces (EPG) and rainfall was demonstrated. This observation was unexpected because during the rains the infestation rate was highest as pasture larval populations rose to maximum peaks. This observation could probably be explained in terms of improved nutritional status and defense mechanisms of the animals. During the rains, pastures improved and consequently animals had adequate forage, thus leading to improved nutritional status. This could probably improve body defense mechanisms in suppressing the egg laying capacity (fecundity) of adult worms. Alternatively, during the rain seasons, pasture herbage became lush and animals grazing on such herbage voided copious amounts of loose faeces. This could result in a dilution of worm eggs and hence show a false reduction in EPG.

From the time an animal ingests infective larvae to the time these larvae mature to adult worms ready to lay eggs, complex processes take place involving both the host animal and the larvae. Such processes include exsheathing (Soulsby, 1968), and in some cases, arrested larvae (Blitz and Gibbs, 1972; Kelly and Henderson, 1973; Ogunsusi and Eysker, 1979).

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The number of infective larve ingested by an animal depends, partly on the amount of herbage the animal ingests and partly on the concentration of larvae in herbage (Cabaret et al., 1982). It has been shown that the number of EPG is a poor index of the population of nematode worms in the gastrointestinal tract of an animal (Allonby and Urguhart, 1975). It has also been established that the average age of female worms (Whitlock et al., 1972), worm population in host animal (Pradham and Johnstone, 1972), and sex ratio (Roberts and Swan, 1981) are important factors in influencing female worm fecundity. All these facts could probably explain the poor correlation observed in this study between pasture larval populations and the EPG in the three age groups of cattle examined. More data collected over a longer period of time would perhaps be required to give more conclusive results in this respect.

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# 6. CONCLUSIONS

The following observations and conclusions were drawn from this study:

- 1) The heaviest contamination of pastures with nematode eggs occurred during the dry season. Calves under six months of age were the major source of this contamination. In order to avoid this pasture contamination, grazing cattle should be dewormed during the dry period. Larval population in pasture was very low at this time and grazing animals were therefore not at risk of being heavily infected with nematode parasites.
- 2) During the rainy season, there was a significant rise in pasture larval populations. At this time, grazing animals were exposed to heavy worm infection. Pasture contamination with nematode eggs was apparently reduced. With continued rainfall there was a decrease in pasture larval population.
- 3) The sudden rise in pasture larval population during the brief period in late February and early March could suddenly raise the worm burden of grazing animals to cause clinical helminthiasis especially as it came immediately after a prolonged dry period when animals were not in good nutritional status.
- 4) Rainfall, temperature and herbage cover had significant influence on larval survival. There was an apparent rise in larval population with rise in stocking rate.

# 6. CONCLUSIONS

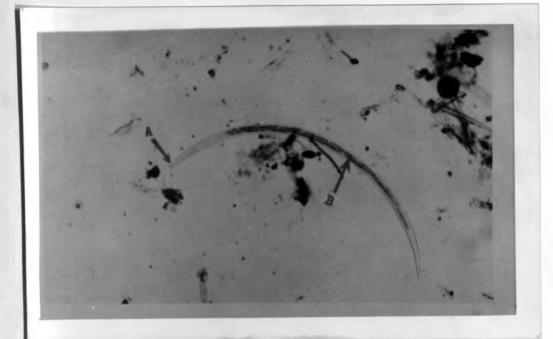
The following observations and conclusions were drawn from this study:

- 1) The heaviest contamination of pastures with nematode eggs occurred during the dry season. Calves under six months of age were the major source of this contamination. In order to avoid this pasture contamination grazing cattle should be dewormed during the dry period. Larval population in pasture was very low at this time and grazing animals were therefore not at risk of being heavily infected with nematode parasites.
- 2) During the rainy season, there was a significant rise in pasture larval populations. At this time, grazing animals were exposed to heavy worm infection. Pasture contamination with nematode eggs was apparently reduced. With continued rainfall there was a decrease in pasture larval population.
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- 4) Rainfall, temperature and herbage cover had significant influence on larval survival. There was an apparent rise in larval population with rise in stocking rate.

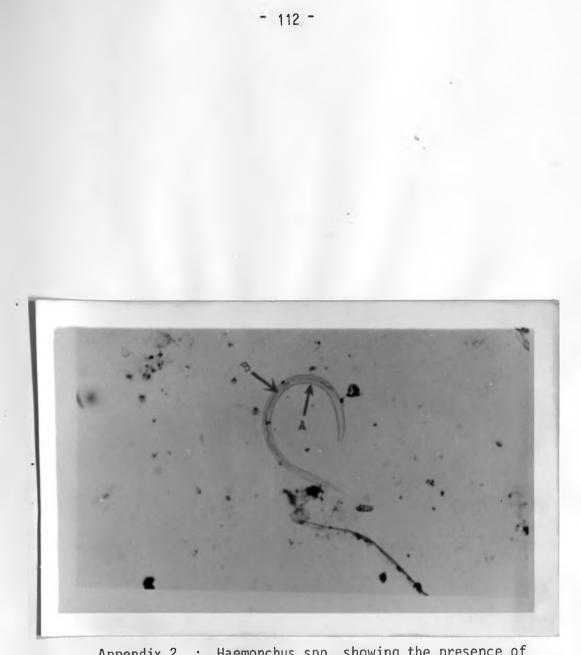
However, the definition of stocking rate was inadequate in explaining the larval dynamics that took place in the pasture. The term 'grazing pressure' takes into account the available foliage and was thus adopted as the more appropriate parameter for the study of pasture

- larval population.
- 5) Worms of genus <u>Cooperia</u> were the most predominant while those of the genus <u>Strongyloides</u> were the least predominant. Other genera present were <u>Trichostrongylus</u>, Haemonchus and Oesophagustomum.
- There was an apparent negative correlation between faecal egg counts and rainfall.

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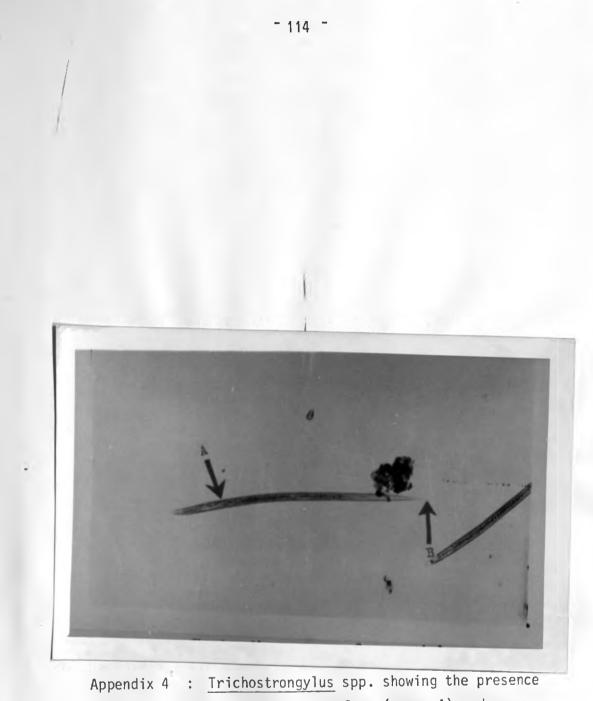
Appendix 1 : <u>Cooperia</u> spp. showing a dark spot at the head end (arrow A) and clearly defined gut cells (arrow B).



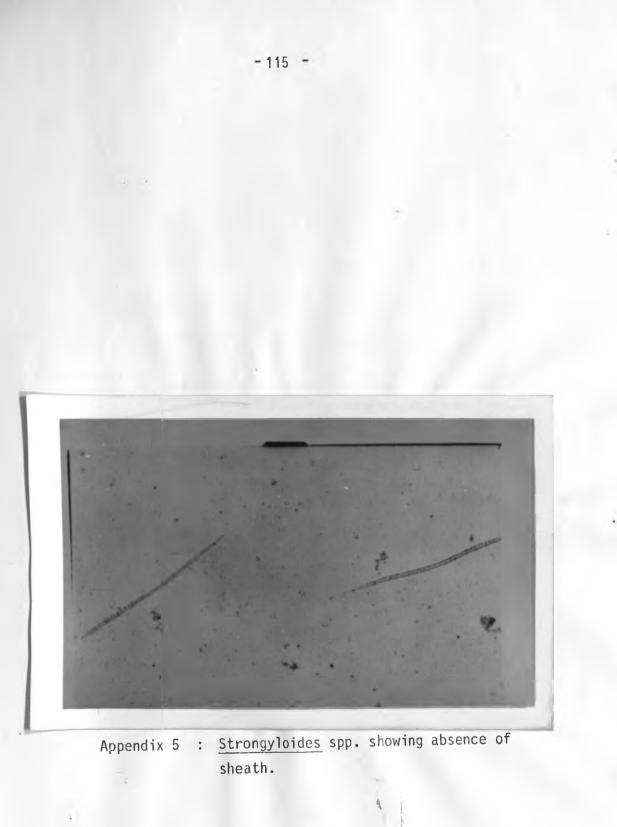
Appendix 2 : <u>Haemonchus</u> spp. showing the presence of sheath as evidenced by corrugated surface (arrow A) and gut cells that are poorly defined (arrow B).



inx 3 : <u>Desophagostomum</u> spp. showing gut cells
(arrow A), corrugated surface (arrow B)
and a long tapering tail (arrow C).



opendix 4 : <u>Trichostrongylus</u> spp. showing the presence of corrugated surface (arrow A) and very short tail end (arrow B).



# 7. <u>REFERENCES</u>

- Allonby, E.W. and Urquhart, G.M. (1975). The epidemiology and pathogenic significance of haemonchosis in a Merino flock in East Africa. Vet. Parasitol <u>1</u>: 129 - 143.
- Alsaqur, I, Bairden, K. and Armour, J. (1982). Population study of bovine <u>Ostertagia</u> spp. infective larvae on herbage and in soil. Res. Vet. Sci. <u>32</u>: 332 -337.
- Andersen, F. L. and Levine, D.N. (1968). Effect of dessication
   on survival of free-living stages of <u>Trichostrongylus</u>
   colubriformis. J. Parasitol. <u>54</u> : 117 128.
- Anonymous (1971). Manual of Veterinary Parasitological Laboratory Techniques. Tech. Bull. No. 18 of the the British Ministry of Agriculture, Fisheries and Food.
- Anonymous (1981). Kenya National Food Policy. Sessional Paper No. 4. Republic of Kenya.
- Armour, J., Saquir, I.M., Bairden, K., Duncan, J.L. and Urquhart, G.M. (1980). Parasitic bronchitis and Ostertagiasis of aftermath grazing. Vet. Rec. <u>106</u> : 184 - 185
- Bairden, K., Parkins, J.J. and Armour, J. (1979). Bovine Ostertagiasis : A changing epidemiological pattern? Vet. Rec. <u>105</u> : 33 - 35.

- Barger, I.A. (1979). Grazing management and control of parasites in sheep. Western Austr. Dept. of Agric. Bull. No. 4029 Cap. 4, Pg. 59.
- Bawden, R.J. (1969). A rapid technique for the recovery of Strongyloid larvae from pasture samples. Austr. Vet. J. <u>45</u> : 228 - 230.
- Berberian, J.F. and Mizelle, J.D. (1957). Development studies on <u>Haemonchus</u> <u>contortus</u>. Am. Midland Naturalise. <u>57</u>: 421 - 421.
- Bisset, S.A. (1980a). Species involved in Ostertagiasis in calves. N. Zealand Vet. J. <u>28</u> : 54 - 56.
- Bisset, S.A. (1980b). Goats and sheep as hosts for some common cattle trichostrongylids. Vet. Parasitol. <u>7</u>: 363 - 368.
- Blitz, N.M. and Gibbs, H.C. (1972). Studies on the arrested development of <u>Haemonchus</u> contortus in sheep. <u>I.</u> The induction of arrested development. Int. J. Parasitol. <u>2</u>: 5 - 12.
- Boag, B. and Thomas, R.J. (1971). Epidemiological studies on gastrointestinal nematode parasites of sheep. Res. Vet. Sci. <u>12</u>. 132 - 139.
- Boag, B. and Thomas, R.J. (1975). Population dynamics of nematode parasites of sheep in northern England. Res. Vet. Sci. <u>19</u> : 293 - 295.
- Borgsteede, F.H.W. and Hendriks, J. (1974). Identification of infective larvae of gastrointestinal nematodes in cattle. Tijdschr. Diergencesk. <u>99</u>: 103 - 113.

- Borgsteede, F.H.V. and Burg, W.P.J. (1982). Worm burdens in cows. <u>II</u>. An analysis of the population of nematodes in the abomasa of adult dairy cows. Vet. Parasitol. <u>10</u>: 323 - 330.
- Burger, H.J. and Stoye, M. (1968). Parasitologische. Diagnostik (Teil II) Eizahung und Larvendifferenzierung. P. 5 - 15.
- Burger, H.J. (1981). Experiences with our techniques for the recovery of nematode larvae from herbage. In: Epidemiology and control of nematodriasis in cattle. (Nansen, P., Jørgensen, R.J. and Soulsby E.J.C. Editors) P. 25 - 30.
- Cabaret, J., Raynaud, J.R. and Le Stang, J.P. (1982). Comparison between tracer calves and herbage sampling for the assessment of pasture infectivity in trichostrongylosis in cattle. Vet. Parasitol. <u>10</u>: 65 - 71.
- Christie, M. and Jackson, F. (1982). Specific identification of strongyle eggs in small samples of sheep faeces. Res. Vet. Sci. <u>32</u> : 113 - 117
- Connan, R.M. (1971). The seasonal incidence of inhibition of development in <u>Haemonchus</u> contortus. Res. Vet. Sci. <u>12</u>: 272 - 274.
- Craig, T.M. (1979). Seasonal transmission of bovine gastrointestinal nematodes in the Texas Gulf Coast. J.A.V.M.A. <u>174</u>: 844 - 846.

- Crofton, H.D. (1948). The ecology of immature phases of trichostrongyle nematodes. The effects of climatic factors on the availability of infective larvae of <u>Trichostrongylus</u> retortaeformis to the host. Parasitol. <u>39</u> : 26 - <u>38</u>
- Crofton, H.D. (1952). The ecology of immature phases of Trichostrongyle nematodes. <u>III</u> Larval populations on lowland pastures. Parasitol. <u>42</u>: 77 - 84.
- Crofton, H.D. (1954a). The ecology of immature phases of trichostrongyle nematodes. V. The estimation of pasture infestation. Parasitol. <u>44</u> : 313 - 324.
- Crofton, H.D. (1954b). Nematode parasite populations in sheep on lowland farms. I. Worm egg counts in ewes. Parasitol. <u>44</u> : 465 477.
- Crofton, H.D. (1955). Nematode parasite populations in sheep on lowland farms. <u>II</u>. Worm egg counts in lambs. Parasitol. <u>45</u> : 99 - 115.
- Crofton. H.D. (1957). Nematode parasite populations in sheep on lowland farms. <u>III</u>. The seasonal incidence of species. Parasitol. <u>47</u> : 304 - 318.
- Cunliffe, G. and Crofton, H.D. (1953). Egg sizes and differential egg counts in relation to sheep nematodes. Parasitol. <u>43</u>: 275 - 286.
- Dineen, J.K., Donald, A.D., Wagland, B.M. and Turner, J.H. (1965). The dynamics of the host - parasite relationship. The response of sheep to primary infection with <u>Haemonchus</u> <u>contortus</u>. Parasitol. <u>55</u> : 515 - 525.

- Dinnik, J.A. and Dinnik, N.N. (1958). Observations on the development of <u>Haemonchus</u> contortus larvae under field conditions in the Kenya Highlands. Bull. Epiz. Dis. Africa. 6 : 11 - 21.
- Dinnik, J.A. and Dinnik, N.N. (1961). Observations on the longevity of <u>Haemonchus</u> contortus larvae on the pasture herbage in the Kenya Highlands. Bull. Epiz. Dis. Africa. 9 : 193 - 208.
- Donald, A.D. (1967). A technique for the recovery of strongyloid infective larvae from small sample units of pasture. J. Helminth. <u>41</u> : 1 - 10.
- Donald, A.D. and Leslie, R.T. (1969). Population studies on the effective stages of some nematode parasites of sheep. <u>II</u>. The distribution of faecal deposits on fields grazed by sheep. Parasitol. <u>59</u> : 141 -157.
- Donald, A.D., Morley, F.H.W., Waller, P.J., Axelsen, A. and Donnelly, J.R. (1978). Availability to grazing sheep of gastrointestinal nematode infection arising from summer contamination of pastures. Austr. J. Agric. Res. 29 : 189 - 204.
- Duncan, J.L., Armour, J., Bairden, K., Urquhart, G.M. and Jørgensen, R.J. (1979). Studies on the epidemiology of bovine parasitic bronchitis. Vet. Rec. 104 : 274 - 278.
- Elisabeth, J.K.S. (1982). Seasonal incidence of helminths in sheep and goats in South Mozambique. Vet. Parasitol. II : 317 - 328.

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- Eysker, M. and Jansen, J. (1982). Population build-up of gastrointestinal nematode infections in ewes and lambs on pasture grazed by calves in the previous year. Res. Vet. Sci. 32 : 203 - 205.
- Fabiyi, J.P. (1973). Seasonal fluctuations of nematode infections in gcats in the Savanna Belt of Nigeria. Bull. Epiz. Dis. Africa. 21 : 277 - 286.
- Galliard, H. (1967). Pathogenesis of <u>Strongyloides</u> spp. Helminthol. Abstr. 36 : 247 - 260.
- Gibbs, H.C. (1982). Mechanisms of survival of nematode parasites with emphasis on hypobiosis. Vet. Parasitol. II : 25 - 48.
- Gibson, T.E. and Everett, G. (1967). The ecology of freeliving stages of <u>Trichostrongylus colubriformis</u>. Parasitol. 57 : 533 - 537.
- Gibson, T.E. and Everett, G. (1971). The ecology of the free-living stages of Ostertagia circumcincta. Parasitol. 64 : 451 - 460.
- Gibson, T.E. and Everett, G. (1976). The effect of weather in modifying the pattern of larval contamination with <u>Ostertagia circumcincta</u>. Int. J. Biometr. 20 : 49 - 55.
- Gibson, T.E. and Everett, G. (1978). Further observations on the effect of different levels of larval intake on the output of eggs of <u>Ostertagia circumcincta</u> in lambs. Res. Vet. Sci. 24 : 169 - 173.
- Gordon, E.G. (1948. The epidemiology of parasitic disease with special reference to studies with nematode parasites of sheep. Austr. Vet. J. <u>24</u> : 17 - 45.

- Graham, E.G. and Ollerenshaw, C.B. (1978) Forecasting
   the incidence of parasitic gastroenteritis in
   lambs in England and Wales. Vet. Rec. 103 :
   461 465.
- Grøvold, J. (1979). On the possible role of earthworms in the transmission of <u>Ostertagia ostertagi</u> third stage larvae from faeces to soil. J. Parasitol. 65 : 831 - 832.
- Hart, J.A. (1964). Observations on the dry season strongyle infestations of Zebu cattle in Northern Nigeria. Brit. Vet. J. 120 : 87 - 95.
- Heath, D.D. and Major, G.W. (1968). A technique for the recovery of strongyle larvae from masticated herbage. J. Helminthol. 42 : 299 - 304.
- , Henderson, A.W.K. and Kelly, J.D. (1978). Helminth parasites of beef cattle in the East Kimberley and Victoria River Districts of Northern Australia. Trop. Anim. Hlth. Prod. 10 : 63 - 73.
  - Keith, R.K. (1953) The differentiation of infective larvae of some common nematode parasites of cattle. Austr. J. Zool. 1 : 223 - 235.
  - Kelly, J.D. (1973). Immunity and epidemiology of helminthiasis in grazing animals. New Zealand Vet. J. <u>21</u> : 183 -194.
  - Kelly, J.D. and Henderson, A.W.K. (1973). Observations of bovine paramphistomiasis in the East Kimberley and Victoria River Districts of Northern Australia. Trop. Anim. Hlth. Prod. 5 : 192 - 195.

Lancaster, M.B. (1970). The recovery of infective nematode larvae from herbage samples. J. Helminthol. <u>44</u>: 219 - 230.

Laurence, B.R. (1954). The larval inhabitants of cowpads. J. Anim. Ecol. 23 : 234 - 260.

- Lee, R.P., Armour, J. and Ross, J.G. (1960). Seasonal variation of Strongyle infestations in Nigerian Zebu cattle. Br. Vet. J. 116 : 34 - 46.
- Lennart, P. (1974). A modified Baermann apparatus for the recovery of infective nematode larvae from herbage and manure. Zbl. Vet. Med. B. 21 : 483 - 488.
- Levine, N.D. and Clark, D.T. (1961). The relation of weekly pasture rotation to acquisition of gastrointestinal nematodes by sheep. Illinois Vet. 41 : 89 - 97.
- Levine, N.D. (1963). Weather, climate and bionomics of ruminant nematode larvae. Adv. Vet. Sci. 8 : 215 - 261.
- Little, T.M. and Hill, F.J. (1978. Randomization. In: Agriculture experimentation, design and analysis. John Wiley and Sons, New York. Cap. 4 . P. 47.
- Michel, J.F. (1969). Observations on the epidemiology of parasitic gastroenteritis in calves. J. Helminthol. 43 : 111 - 133.
- Mitchell, G.B.B. (1983). Control of ovine gastrointestinal helminthiasis by use of clean grazing and strategic dosing in the field. Res. Vet. Sci. <u>35</u> : 100 - 105

- Mohr, C.O. (1943). Cattle droppings as ecological units. Ecol. Monogr. 10 : 275 - 298.
- Morgan, D.O. and Oldham, J.N. (1934). Further observations on the effect of heavy stocking on the worm burden under a system of rotational grazing. J. Helminthol. 18 : 177 - 182.
- Morley, F.H.W., Axelsen, A., Pullen, K.G., Nadin, J.B., Dudzinski, M.L. and Donald, A.D. (1978). Growth of cattle on phalaris and lucerne pastures. <u>I</u>. Effect of pasture, stocking rate and anthelmintic treatment. Agric. Syst. <u>3</u>: 123 - 145.
- Morrison, F.B. (1956). Feeds and Feeding. A Handbook for Students and Stockman. 22nd. ed. Morrison. Ithaca, New York. Pg. 396.
- Nwaorgu, O.C. (1983). The development of the freeliving stages of <u>Strongyloides papillosus</u>. <u>I. Effect of temperature on the development</u> of the heterogonic and homogonic nematodes in faecal culture. Vet. Parasitol. <u>13</u>: 213 - 223.
- Oakley, G.A. (1981). Survival of <u>Dictyocaulus viviparus</u> infection in earthworms. Res. Vet. Sci. <u>30</u>: 255 - 256.
- Oakley, G.A. (1982). Observations on the epidemiology of <u>Dictyocaulus viviparus</u> in north west England. Res. Vet. Sci. 32 : 163 - 169.

Ogunsusi, R.A. (1979). Pasture infectivity with trichostrongylid larvae in Northern Guinea Savanna of Nigeria. Res. Vet. Sci. <u>26</u> : 320 - 323.

- Ogunsusi, R.A. and Eysker, M. (1979). Inhibited development of trichostrongylids of sheep in Northern Nigeria, Res. Vet. Sci. 26 : 108 -110.
- Ollerenshaw, C.W. and Rowlands, W.T. (1959). A method of forecasting the incidence of Fascioliasis in Anglesey. Vet. Rec. 71 : 591 - 607.
- Ollerenshaw, C.W. and Smith, L.P. (1966). An empirical approach for forecasting the incidence of nematodriasis over England and Wales. Vet. Rec. 79 : 536 - 541.
- Parnell, I.W. (1954). The sequence and the levels of the helminth infestations in Scottish hill sheep. Br. Vet. J. 110 : 499 - 507.
- Plews, A.M. (1979). The collection of statistical data. In: Introductory Statistics. Richard Clay Chancer Press, Suffolk. Great Britain. Cap. 1, P. 4.
- Pradham, S.L. and Johnstone, I.K. (1972). <u>Haemonchus</u> <u>contortus</u>. Haematological changes during prolonged exposure to daily and weekly doses of infective larvae. Parasitol. <u>64</u> : 153 - 160.
- Reid, J.F.S. and Armour, J. (1973). Type II ostertagiasis in housed sheep. Vet. Rec. 93 : 400.
- Roberts, F.H.S. and O'Sullivan, P.J. (1950). Mathods for egg counts and larval cultures for strongyles infesting the gastrointestinal tract of cattle. Austr. J. Agric. Res. <u>1</u>: 99 - 102.

- Roberts, F.H.S., O'Sullivan, P.J. and Riek, R.F. (1952). The epidemiology of parasitic gastroenteritis of cattle. Austr. J. Agric. Res. <u>3</u> : 187 - 226.
- Roberts, J.L. and Swan, R.A. (1981). Quantitative studies of ovine haemonchosis. <u>I</u>. Relationship between faecal egg counts and total worm counts. Vet. Parasitol. 8 : 165 - 171.
- Rose, J.H. (1963). Observations on the free-living stages of the stomach worm, <u>H</u>. <u>contortus</u>. Parasitol. 53 : 469 - 481.
- Ross, J.G. (1970). The Stormont 'Wet Day' forecasting system for fascioliasis. Br. Vet. J. <u>126</u> : 401 - 402.
- Round, M.C. (1962). The helminth parasites of domesticated animals in Kenya. J. Helminthol. <u>36</u>: 375 • 449.
- Shorb, D.A. (1940). A comparative study of the eggs of various species of nematodes in domestic ruminants. J. Parasitol. 26 : 223 - 231.
- Shumard, R.F., Herrick, C.A. and Pope, A.L. (1955). The effect of diet on the length of third stage larvae produced by adult <u>Haemonchus</u> <u>contortus</u> harboured by lambs. J. Parasitol. 41 : 542 - 544.

- Skerman, K.D. and Hillard, J.J. (1966). Culture of larvae from faeces of cattle. In: Handbook for studies of Helminth Parasites of Ruminants. F.A.O., N.E.A.H.I. (Near East Animal Health Institute) Handbook No. 2. P. 5.
- Skinner, W.D. and Todd, K.S. (1980). Lateral migration of <u>H. contortus</u> larvae on pasture. Am. J. Vet. Res. 41 : 395 - 398.
- Smith, L.P. and Thomas, R.J. (1972). Forecasting the spring hatch of <u>Nematodirus</u> <u>battus</u> by use of soil temperature data. Vet. Rec. 90 : 388 - 392.
- Snedecor, G.W. and Cochran , W.G. (1967). Statistical methods, 6th. ed., Iowa State University Press, Ames, Iowa. Pg. 120 - 134.
- Soulsby, E.J.L. (1968). Helminths, Athropods and Protozoa of domesticated animals. 6th.ed. Bailliere, Tindal and Cassell, London. P. 178, 223.
- Sprent, J.F.A. (1946). Some observations on the bionomics
   of Bunostomum phlebotomum, a hookworm of cattle.
   Parasitol. 37 : 202 214.
- Steel, R.G.D. and Torrie, J.H. (1980). Random sample; the collection of data. In: Principles and Procedures of Statistics. 2nd. Ed. Mc. Graw -Hill Book Company, New York. Cap. 2, P. 11.
- Stewart, M.A. and Douglas, J.R. (1938). Studies on the bionomics of <u>Trichostrongylus axei</u> and its seasonal incidence in irrigated pastures. Parasitol. 30 : 477 - 496.

- Taylor, E.L. (1939). Technique for the estimation of pasture infestation by strongyloid larvae. Parasitol. 31.: 473 - 478.
- Tetley, J.H. (1941a). <u>Haemonchus contortus</u> eggs. Comparison of those in utero with those recovered from faeces, and statistical method for identifying <u>H. contortus</u> eggs in mixed infections. J. Parasitol. 27 : 453 - 464.
- Tetley, J.H. (1941b). Differentiation of the eggs of trichostrongylid species <u>Nematodirus filicolis</u> and N. spathiger. J. Parasitol. 27 : 473 - 480.
- Thomas, R.J. and Boag, B. (1973). Epidemiological studies on gastrointestinal nematode parasites of sheep: The control of infections in lambs on contaminated pastures. Res. Vet. Sci. <u>15</u> : 238 - 242.
- Thomas, R.J. (1974). The role of climate in the epidemiology of nematode parasitism in ruminants. In: A.E.R. Taylor and R. Muller (editors): The effects of meteorological factors upon parasites. Symposium of the Brit. Soc. Parasitol. 12 : 13 - 32.
- Thomas, R.J. and Starr, J.R. (1978). Forecasting the peak of gastrointestina! nematode infection in lambs. Vet. Rec. 103 : 465 - 468.
- Thomas, R.J. (1982). The ecological basis of parasite control : Nematodes. Vet. Parasitol. <u>11</u> : 9 - 24.
- Triantaphyllou, A.C. and Moncol, D.J. (1977). Cytology, reproduction and sex determination of <u>Strongyloides</u> <u>ranisoni</u> and <u>S. papillosus</u>. J. Parasitol. <u>63</u>: 961 - 965.

- Tripathi, J.C. (1974). Longevity and migration of infective larvae of some common nematodes of goats in different types of soils. Ind. J. Anim. Sci. 44 : 104 - 208.
- Varju, L. (1966). Studies on <u>Strongyloides. VII</u>. The nature of changes in developmental course of swine <u>Strong-</u> yloides. Z. Parasitenkd. 28 : 175 - 192.
- Vercruysse, J. (1983). A survey of seasonal changes in nematode faecal egg count levels of sheep and goats in Senegal. Vet. Parasitol 13 : 239 - 244.
- Waller, P.J. and Thomas, R.J. (1978). Nematode parasitism in sheep in North-east England. The epidemiology of Ostertagia spp. Int. J. Parasitol. <u>8</u>: 427 - 433.
- Wang, G.T. (1967). The effect of temperature and cultural methods on development of the free-living stages of <u>Trichostrongylus</u> colubriformis. Am. J. Vet. Res. 28 : 1085 - 1090.
- Wang, G.T. (1970). Suitability of various species of microorganisms as food for the free-living stages of <u>Trichostrongylus colubriformis</u>. J. Parasitol. 56 : 753 - 758.
- Whitlock, H.V. (1948). Some modification of the McMaster helminth egg-counting technique and apparatus. Austr. Council. Sci. and Industr. Res. J. 21 : 77 - 80.
- Whitlock, H.V. (1959). The recovery and identification of first stage larvae of sheep nematodes. Austr. Vet. J. 35 : 310 - 316.

Whitlock, J.D. (1960). The diagnosis of Veterinary Parasitisms. Kempton, London. P. 157.

Whitlock, J.H., Crofton, H.D. and Georgi, J.R. (1972). Characteristics of parasite populations in endemic trichostrongylidosis. Parasitol. 64 : 413 - 427.

- Young, R.R. and Trajstan, A. (1980). A rapid technique for the recovery of Strongyloid infective larvae from pasture and soil samples. Parasitol. <u>80</u>: 425 - 431.
- Zimmerman, W.J. (1965). The role of selected management systems in the control of sheep parasites. J.A.V.M.A. 147 : 499 - 505.