# GASTROINTESTINAL NEMATODES IN TRADITIONAL CATTLE OF ZAMBIA: SEASONALITY AND RELATIONSHIP BETWEEN FAECAL EGG COUNTS AND WORM BURDEN

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**THE UNIVERSITY OF ZAMBIA**
**SCHOOL OF VETERINARY MEDICINE**
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# DECLARATION

I **DENIS PHIRI** do hereby declare that the dissertation represents my own work and that it has never been submitted before for a degree at this university or indeed any other university.

Signature: .....

Date: 24/04/98 .....

# DEDICATION

This work is dedicated to:

My beloved mother, Yustina Nchawaka Phiri, for her love, support, and encouragement throughout my academic career.

My late sisters Agness Nyirenda, Martha Patricia Ndhlovu, and Misozi Clara Ndhlovu who could have been very proud of me if they were still alive. They gave me all the love due to a young and naive brother. May their souls rest in peace.

# APPROVAL

This dissertation of **DENIS PHIRI** is approved as fulfilling the requirements for the award of the Degree of Master of Veterinary Medicine in Veterinary Helminthology of the University of Zambia.

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# ABSTRACT

Faecal egg counts, differential larval counts and post mortem total worm counts were done on seventy traditional cattle from Southern province of Zambia between February and September 1997 to establish the relationship between faecal egg counts and total worm burden and to determine the seasonality of gastrointestinal nematodes in the same traditional cattle. The animals were sampled at Turn Pike slaughter slab in Kafue district on a biweekly basis at an average of five animals per visit. During the same visits, at least 20 animals per visit were checked for gastrointestinal nematode eggs in the faeces

The highest faecal egg counts were recorded during the rainy months February to April and the lowest where in the cold dry month of July. The same was observed with total worm counts. The mean monthly temperatures were also found to be positively correlated to both the faecal egg counts and the total worm counts. Rainfall was however, only found to positively correlate with faecal egg counts and not with total worm counts. In all the eight months that the study was carried out, no inhibited larvae (hypobiotic larvae) were observed in all the animals sampled.

The genera identified by either coprocultures or post mortem worm counts were *Haemonchus spp., Cooperia spp., Oesophagostomum spp., Bunostomum spp.* and *Trichostrongylus spp.* The prevalence of these worms in the gastrointestinal contents were *Cooperia* 73 percent, *Haemonchus* 22 percent, *Oesophagostomum* two percent and *Bunostomum* and *Trichostrongylus* each at one percent. All the animals examined

were found to be infected with at least one genus of gastrointestinal nematodes and of these 84 percent were infected with Cooperia, 76 percent with Haemonchus, 74 percent with Oesophagostomum 14 percent with Bunostomum and another 14 with Trichostrongylus. The different genera were compared with the faecal egg counts to determine the relationship between the different genera and the faecal egg counts. A positive correlation was found between *Haemonchus spp* ($r = 0.5785$, $p<0.05$), *Cooperia spp* ($r = 0.7469$, $p<0.05$) and *Oesophagostomum spp* ($r = 0.5987$, $p<0.05$) and faecal egg counts. However no relationship was found between *Bunostomum, Trichostrongylus* and faecal egg counts. A positive correlation ($r^2 = 0.57$, $p< 0.05$) was found between faecal egg counts and total worm counts.

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## GENERAL INTRODUCTION AND REVIEW OF LITERATURE

### 1.1. Gastrointestinal nematodiasis in cattle

#### 1.1.1. The definition of gastrointestinal nematodiasis.

Gastrointestinal nematodiasis or verminous gastro-enteritis is a helminthosis of the digestive tract due to the presence or development of large numbers of nematodes in the abomasum, small intestines and / or large intestines (Taylor and Anders, 1992; Troncy 1981). The most common genera gastrointestinal nematodes in cattle are listed in Table 1.

**TABLE 1.** Gastrointestinal nematodes commonly found in cattle according to their location in the digestive tract.

| Parasite | Abomasum | S. Intestine | L. Intestine |
|---|---|---|---|
| *Haemonchus* | * | | |
| *Ostertagia* | * | | |
| *Trichostrongylus* | * | | |
| *Cooperia* | | * | |
| *Nematodirus* | | * | |
| *Bunostomum* | | * | |
| *Oesophagostomum* | | | * |
| *Trichuris* | | | * |

Key: * = Present
Adopted from Urquhart et al., 1992, Soulsby 1986.

### 1.1.2. The life cycles of gastrointestinal nematodes

Nematodes are either free living or parasitic. They have cylindrical and elongate bodies and, with a few exceptions, the sexes are separate. Their life cycles may either be direct or indirect (i.e. may include an intermediate host) (Urquhart *et al.*, 1992, Soulsby, 1986), though however, all Strongylida species that cause gastrointestinal strongylosis of ruminants have a direct life cycle (Troncy 1981). In the direct life cycle, the eggs are laid by the mature females harboured in the host's digestive tract and passed to the outside through faeces. Under conducive conditions (i.e. humidity and temperature), the eggs hatch into larvae which undergo two moults to develop into third stage larvae ($L_3$), the infective larvae. The infective larvae are free living for some time and entry into the host is either active (penetration through the skin) or passive (ingested). Some nematodes e.g. *Bunostomum spp*, utilise both routes. *Bunostomum spp.* can actively penetrate the host skin and muddy environments are favourable for this kind of penetration (Troncy, 1981). The larvae then migrate to the digestive tract through the heart and lungs. In the other nematodes, infection is passive with the ingestion of larvae present on the grass.

In the host the larvae undergo two further moults to develop into $L_5$ (the immature adult). These larvae may migrate in the host tissues particularly the intestinal mucosa and return into the lumen of the digestive tract to mature into adults (Urquhart *et al.*, 1992; Soulsby, 1986; Troncy, 1981) that later mate to initiate another life cycle (see Figure 1).

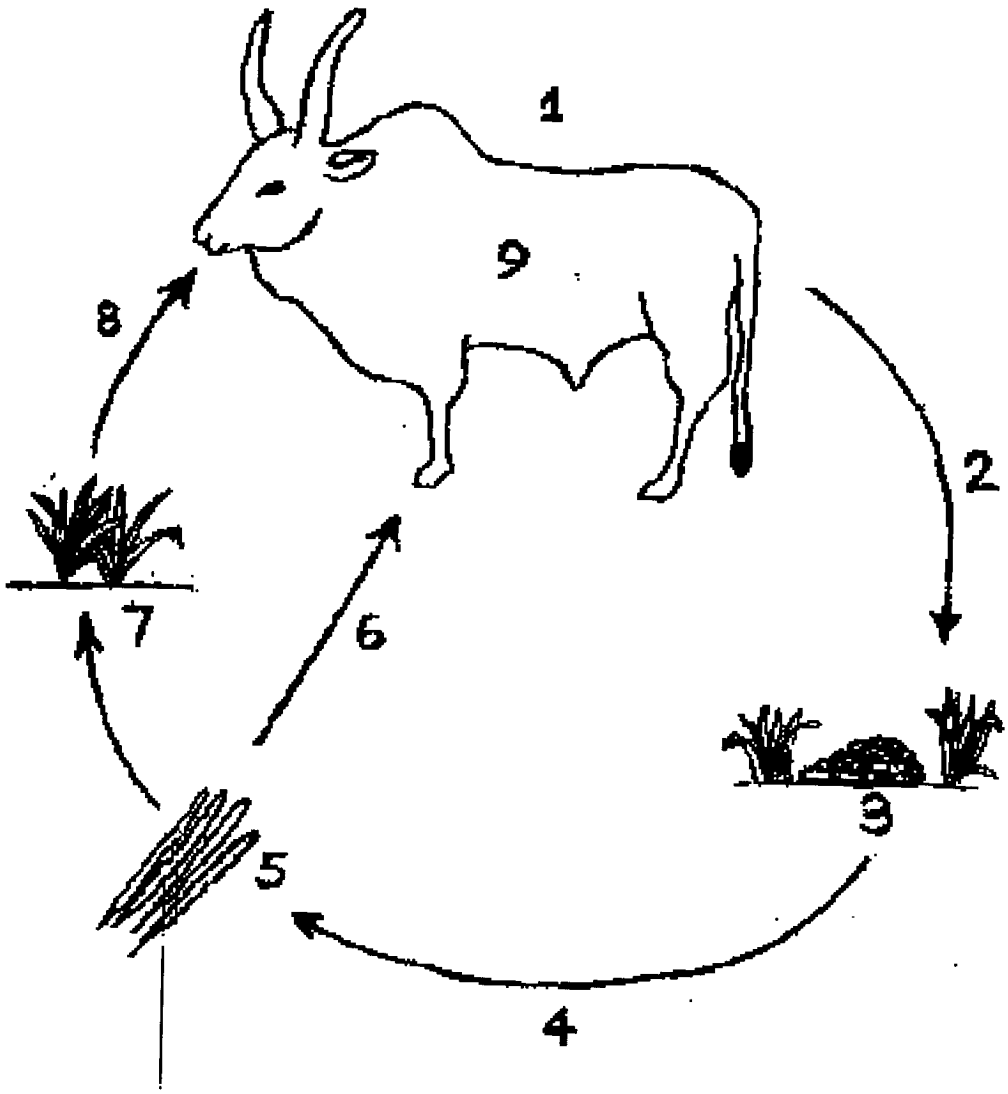


**Figure 1:** The life cycle of gastrointestinal nematodes showing the different stages of development (Hansen and Perry, 1994; Urquhart *et al.*, 1992; Soulsby, 1986; Troncy, 1981)

Key:

1. The final host
2. The eggs are passed to the outside in faeces
3. The eggs hatch under favourable conditions
4. Development of the larvae into infective larvae ($L_3$)
5. The infective larvae
6. Active entry into the host by some species of gastrointestinal nematodes e.g. *Bunostomum*
7. Some infective larvae climb onto the grass.
8. Passive entry of some infective larvae through ingestion
9. Development of the infective larvae inside the host to adult worms.

### 1.1.3. The pathogenesis of gastrointestinal nematodiasis

The pathogenesis of gastrointestinal nematodiasis is dependent on the genus of the nematode.

In *Haemonchus* infections, both the fifth stage larvae and adults are vigorous blood suckers and cause loss of blood (Radostits *et al.,* 1994). This results in anaemia and hypoproteinaemia. The migration of the larvae into the gastric glands in the abomasum interferes with the digestion of nutrients. There is also an increase in abomasal $p^H$ and plasma pepsinogen levels especially in Ostertagiosis (Radostits *et al.,* 1994; Soulsby, 1986). The increase in these two parameters also cause disturbances in the digestion of the food.

*Trichostrongylus, Cooperia* and *Nematodirus* spp have similar effects. The effects will depend on the species present, their location and numbers but the most common and important feature of all these is anorexia (Radostits *et al.,* 1994; Vecruysse *et al.,* 1988). *Trichostrongylus* causes mucosal and villous atrophy or flattening, sparse stunted microvilli and epithelial hyperplasia (Vercruysse *et al.,* 1988). Consequently the effective gland mass is reduced and hence levels of brush border enzymes reduced. *Cooperia* on the other hand does not tunnel into the epithelium but the worms brace and coil themselves along and among the villi. *Nematodirus* enter the deeper layers of the mucosa as third stage larvae and emerge as fourth and fifth stage larvae (Radostits *et al.,* 1994, Vecruysse *et al.,* 1988).

*Bunostomum spp* are active blood suckers and cause severe anaemia. Total worm numbers as low as 100 may cause clinical illness and numbers of 2,000 may cause death (Williams *et al.,* 1983). The worm can withdraw large quantities of blood and the site will usually remain bleeding for several minutes after the worm has moved to another site. Due to their feeding habits the worms cause severe damage to the mucosa.

*Oesophagostomum*'s life cycle involves a sojourn in the mucosa of the intestine and it is during this larval histotrophic phase that the genus has its most serious effects (Radostits *et al.,* 1994; Vecruysse *et al.,* 1988). The third stage larvae penetrate deep into the mucosa and a fibroblastic reaction develops leading to formation of nodules. The emergency of the moulted larvae from the nodules leads to colitis. The nodules eventually caseate and calcify and may cause interference with intestinal motility.

### 1.1.4. The effects of gastrointestinal nematodiasis

The effects of gastrointestinal nematode are both pathological and economical. These effects are mainly due to the parasites' feeding habits and migrations (Radostits *et al.,* 1994; Urquhart *et al.*, 1992; Merck manual, 1989; Soulsby, 1986, 1965). The economic effects cannot easily be quantified because animals usually suffer from subclinical infections and so losses of productivity may not be easily appreciated.

### 1.1.4.1. Pathological effects

The majority of gastrointestinal nematodes cause a reduction in voluntary food intake, the degree of innappentance being dependent on the level of infection. The mechanisms have not been elucidated, but are considered to be multifactorial in origin and only have correlation with changes in gastrointestinal hormones, gut motility and reduced appetite (Holmes and Coop, 1994).

General pathological changes consists of damage to the bowel by parasites such as *Nematodirus* and *Cooperia* (Soulsby, 1965) which invade the mucosa, the removal of blood by *Haemonchus spp* and competition for various minerals such as phosphorus, calcium, cobalt, copper and other minerals. Inflammatory changes of the mucosa may also cause malabsorption.

Other changes include hypoproteinaemia which is caused by deficient protein intake, impaired digestion and absorption, decreased protein synthesis and loss of protein from the body by haemorrhages or exudation from the mucous membranes (Radostits *et al.,* 1994; Urquhart, *et al.,* 1992; Merck manual, 1989; Soulsby, 1986, 1965). Exudation from the mucous membranes is likely to be an important cause of protein loss because of the marked changes which occur in infection. Leakage of plasma protein into the gastrointestinal tract may account for up to 10 percent of the total plasma volume per day (Holmes and Coop, 1994). The fate of these losses is dependent on the site of infection. In abomasal parasitism, the majority of the increased protein loss is reabsorbed in the

intestine whereas , with intestinal parasitism, they may be lost in the faeces. It seems the reason why parasitic gastro-enteritis is so debilitating is because of the combined loses of water, sodium and protein (Holmes and Coop, 1994)

It has been shown that some nematodes that inhabit the alimentary tract of animals can affect contractions of the wall of the alimentary tract and alter the movement of the gut contents along the tract. This can have important implications for the health of the animal. This can either be by increase in gut motility and hence decreased intestine transient time or inhibition of alimentary tract contractions resulting in increase in transient time through the alimentary tract (Lee and Foster, 1995).

Another pathological effect of gastrointestinal nematodiasis is the development of defensive nodules in the gut wall caused by hypersensitivity of the gut (Brander *et al.*, 1991). This is seen, for example, in *Oesophagostomum* infection where there is development of nodules leading to a condition called 'Pimply gut' (Soulsby, 1986).

In Summary gastrointestinal nematodes may

a) absorb nutrients from host's food
b) actively browse on the gut mucosal cells
c) suck blood, often causing heavy blood loss, anaemia and even death.
d) penetrate and destroy mucosal cells severely, impairing the host ability to digest and, absorb nutrients
e) physically obstruct the gut lumen,
f) cause damage to tissues during their migration.

g) activate the host immune system to cause hypersensitivity of the gut or development of defensive nodules.

### 1.1.4.2. Economical effects

It is being increasingly recognised that in the tropics as in the temperate zones, nematode infections are important causes of morbidity and motality in both small and large ruminants (Kinoti *et al.*, 1994; Hertzberg *et al.,* 1992; Aumont *et al.,* 1991). This means most farmers, both commercial and traditional, suffer high economic losses due to loss of production and deaths.

Anorexia and reduced feed intake, loss of blood and plasma proteins into the gastrointestinal tract, alteration in protein metabolism, and diarrhoea all contribute to production losses (Hawkins, 1992; Smith and Calder, 1972). It has been said that the economic losses in livestock production from nematode infections are greater than any other disease (Hall, 1988). This may be something of an exaggeration when applied to a single area, where perhaps a serious bacterial, viral or protozoan disease rages, but may be true when considered on a world scale. Bain and Urquhart, (1986) recorded a total loss of about £45.7 million (inclusive of deaths and liveweight losses) in Britain assuming an annual production of 3.5 million calves.

Subclinical infections are the most important economically since they occur in the majority of animals leading to retarded growth, reduced productivity and increased susceptibility of these animals to other infections; and they continuously contaminate the

pasture (Maingi and Gichigi, 1992). These subclinical infections may exist for the life time of the animal. Growth losses up to the age of weaning have been observed to be induced by low levels of infection even in the absence of clinical signs (Aumont *et al.*, 1991).

### 1.1.5. Gastrointestinal nematodes found in cattle in tropical Africa

A number of genera have been documented in several parts of Africa to be found in Cattle. Table 2 gives a summary of these nematodes. see also Appendix 1 for a detailed list of helminths found in cattle.

**Table 2:** A summary of cattle gastrointestinal nematodes of tropical Africa

| Country | Nematodes found | References |
|---|---|---|
| Gambia | *Haemonchus, Trichuris, Bunostomum, Cooperia, Oesophagostomum, Strongyloides, Nematodirus.* | Ndao *et al.*, 1995 Kaufmann and Pfister, 1990 |
| Guinea Bissau | *Bunostomum phlebotomum, Cooperia punctata, Haemonchus placei, Ostertagia ostertagi, Trichostrongylus colubriformis.* | Mendes, 1989 |
| Kenya | *Haemonchus, Cooperia, Oesophagostomum, Strongyloides.* | Maingi and Gichigi, 1992; Gatongi *et al.*, 1987 |
| Mali | *Haemonchus placei, Oesophagostomum radiatum, Trichostrongylus colubriformis, Bunostomum phlebotomum.* | Tembely *et al.*, 1995 |
| Senegal | *Haemonchus contortus, Oesophagostomum, Bunostomum phlebotomum, Trichuris, Cooperia, Trichostrongylus axei.* | Pangui *et al.*, 1993 |
| Nigeria | *Haemonchus, Trichostrongylus, Bunostomum phlebotomum, Cooperia, Oesophagostomum radiatum, Trichuris.* | Ogunsusi *et al.*, 1986; Chiejina and Emehelu, 1984. |
| South Africa | *Bunostomum, phlebotomum Cooperia punctata, C. pectinata, Haemonchus placei, Oesophagostomum radiatum, Ostertagia ostertagi, Trichostrongylus* | Swan *et al.*, 1985 Schroder, 1979. |
| Zambia | *Haemonchus, Cooperia punctata, C. pectinata, C. onchophora,, Bunostomum phlebotomum, Trichuris discolor,* | Tada *et al.*, 1995 |
| Zimbabwe | *Haemonchus, Cooperia, Bunostomum, Oesophagostomum, Trichostrongylus, Strongyloides, Toxocara.* | Pandey *et al.* 1993 |

### 1.1.6. Factors affecting prevalence and intensity of nematode infection

A number of factors have been implicated in influencing helminth population in the host and subsequent egg output. These include the type of food and feeding habits of the host, sex, lactation and parturition, stress, immune status, age, body size, geographical range and seasonal fluctuations (Nalubamba, 1996; Rickard and Zimmerman, 1992; Asanji and Williams, 1987; Soulsby, 1986). These factors may increase the risk of infection of the host and reduction in the host's resistance to helminth infections.

*Increase in the risk of infection*

Among the factors that increase the risk of infection are type of food, the feeding habits, and environmental factors like temperature, humidity, and rainfall. These factors affect the survival of the infective larvae in the environment and determine their availability to the final host. One important single factor among these is rainfall. It determines the availability of $L_3$ on pasture and hence consequently large numbers of $L_3$ are present on herbage during the rainy season. Under these conditions, the carrier animal is the only important means of carrying over of infection from one season to the other (Chiejina and Emehelu, 1984). It has also been observed that the profile of faecal egg counts parallels that of total rainfall and that egg counts peaks occur when the availability of moisture is optimal ( Nalubamba, 1996; Rahman, 1992). Food type and feeding habits affect infection intensity as they affect the rate of intake of larvae by the host.

*Reduction in host resistance to helminth infections*

Sex hormones, lactation status, parturition, and age reduce the host's resistance to infection. Pregnant, parturient and lactating females; acute or chronically diseased animals; stressed and immune suppressed animals are more susceptible to infection (Radostits *et al.,* 1994; Urquhart *et al.*, 1992; Merck manual, 1989; Soulsby, 1965,1986). During lactation and late pregnancy, the immune response of the host to its gastrointestinal nematodes is particularly suppressed. This increased parasitism has been observed in ewes, sows and heifers (Connan, 1976).

One other factor affecting the intensity of gastrointestinal nematode infection is the acquisition of immunity against reinfection by older hosts as a result of previous exposure (Asanji and Williams, 1987). An immune animal is usually capable of expressing antagonistic reactions which reduce the life expectancy of the parasite. The immunity may range from inhibition of larval establishment through retarded growth and development and reduced fecundity to complete elimination of the infection (Dobson, 1972). Helminthosis is thus a more serious problem in the young animals which are exposed to these infections for the first. However in certain cases such as in *Haemonchus placei*, a sudden increase in pasture contamination may break down an existing immunity and lead to clinical disease in older animals. It is also known that nutritional stress induced by poor pasture and overstocking may depress the immune status of cattle and allow an establishment of a high worm burden (Gill and Mason, 1989). According to Nalubamba (1996), immunity to gastrointestinal nematodes, may be manifested as follows:

a) An ability to expel the adult nematodes,

b) Limitations of reinfection by preventing the migration and establishment of larvae.

c) Changes in worm characteristics i.e. adult worms which do develop are stunted in size and fecundity reduced.

### 1.1.7. Survival of gastrointestinal nematodes in adverse conditions.

Weather and climate influence survival of parasite eggs and in the environment. Climate determines which nematode genera/species are generally found in a locality, while weather determines which ones can develop and infect their host at a particular place at a particular time (Gatongi *et al.,* 1988; Altaif and Yakoob, 1987). The amount of moisture in the soil is an important determinant of larval development and survival. This depends on precipitation, evaporation, soil type, the amount and type of overlaying mat and character of plants growing in it. Direct light is fatal to nematode larvae and therefore the degree of shading they receive from vegetation and faecal pads will markedly affect their survival. Hypobiosis and dung pads have been documented to be among the major factors that affect the survival of nematodes in and outside the host and are discussed below.

#### 1.1.7.1. Hypobiosis

Hypobiosis or temporarily arrested larval development, represents one of the most useful life cycle adaptations to ensure a parasite to synchronise its life cycle to changing

environmental or host conditions. It is of importance during environmental adversity when conditions for transmission are not favourable. Such adaptation occurs in cool temperate areas where the climate during a part of the year deviates to extreme cold. It also occurs in tropical and subtropical areas where the climate deviates to extreme heat and dryness. The development of the larvae is usually resumed just before the beginning of the following wet season and the result is an increased adult helminth population detectable through the increased faecal parasite egg output (Schillhorn Van Veen and Ogunsusi, 1978). It also enables parasites to have available large numbers of infective larvae at points in host reproductive cycle which coincide with production of the susceptible neonates and also for regulating populations of adult worms lessening stress on the host and favouring survival of the parasite

In many areas of the world, hypobiosis of trichostrongyles in ruminants has been reported (Al-Khalid and Al-Saeed, 1991; Capitini *et al.,* 1990; El-Azazy, 1990; Fukumoto *et al,.* 1990; Sanyal, 1988). Many explanations for this phenomenon have been proposed, but the most plausible seem to be those associated with ecological factors (Ndao *et al.,* 1995; Urquhart *et al.,* 1992; El-Azazy, 1990; Soulsby, 1986; Schillhorn Van Veen and Ogunsusi, 1978).

Despite hypobiosis being common world-wide as a phenomenon for nematode survival, it was noticed that hypobiosis seemed not to be important in the life cycle of abomasal nematodes of sheep and goats in Egypt (El-Azazy, 1990). This might have been due to the parasites not being exposed to adverse conditions during the study. In another study with

sheep and goats in Nigeria, there was no significant hypobiosis and *Haemonchus contortus* survived in the host as adults during the unfavourable dry season (Fakae, 1990)

Some nematodes genera that have been shown to exhibit hypobiosis are *Haemonchus, Cooperia* and *Oesophagostomum. Haemonchus contortus* survives almost exclusively as larvae in the abomasal mucosae, whereas *Cooperia* and *Oesophagostomum* survive partly as larvae in the lumen and also in nodules in the case of *Oesophagostomum radiatum* and partly as hypometabolic adults with much reduced fecundity (Ndao *et al.,* 1995; Fritsche *et al.,* 1993). Ndao *et al.* (1995) found that the proportion of early $L_4$ (indicating hypobiosis) of *Haemonchus contortus* was 85 - 99 percent. Horak and Louw (1978) also documented that *Haemonchus, Ostertargia, Cooperia onchophora* became inhibited in the host to survive severe winters, whereas *Haemonchus placei* a parasite of cattle in milder climate uses the same method to survive comparatively mild winters.

Kaufmann and Pfister (1990) found that pastures were helminthologically clean at the end of the dry season and later became contaminated during the rainy season. The resumed development of inhibited larvae, together with increased fecundity of the remaining adult population at the end of the dry season and at the beginning of the rain season would appear to be responsible for pasture contamination and continuity of nematode life cycle during the rainy season.

Other factors that can contribute to hypobiosis include age of host, the presence of adult worms, the host's acquired resistance and host pregnancy and lactation. Little is known

about how these factors induce and maintain larval inhibition. However, their effectiveness depends on parasite and host species and environmental conditions

### 1.1.7.2. Larval survival in dung pads

Larvae may survive for up to eight months during the dry season in faecal pads on pasture. In Zambia, survival of up to 165 days has been observed (Meeus and Ziela 1997, Personal communication). During the dry season, cattle faecal pads form a hard crust on the surface while it remains relatively moist underneath to be able to sustain developing larvae. Lack of rainfall means that the infective larvae are trapped in the dung and can not migrate onto the pasture to infest it (Hansen and Perry, 1994, Malan *et al.,* 1982). When rain eventually falls a mass release of larvae may take place, contaminating the pastures to at times very high levels. The faecal pads are well aerated by tracts made through them by burrowing beetles (Gatongi *et al.,* 1988). When the rains come and soften the hard crust, the infective larvae underneath have the opportunity to move out into the surrounding herbage and hence immediately raise the infective larvae population (Gatongi *et al.,* 1988; Soulsby, 1965).

## 1.2. Diagnostic techniques for nematodiasis

The diagnosis of gastrointestinal nematodiasis can either be on live animals (ante mortem) or dead animals (post mortem). The significance and accuracy of these techniques is discussed in the following sections.

### 1.2.1. Ante mortem techniques

These methods are conducted on live animals and involve the clinical examination of the individual animal or herd and the use of specified laboratory techniques.

#### 1.2.1.1. Clinical diagnosis

The clinical signs of gastrointestinal enteritis are several and of a general nature (see Table 3). These include progressive loss of condition, diarrhoea (which may be haemorrhagic, watery, dark green, frothy or foul smelling), anaemia especially in *Haemonchus* infections, anorexia and generalised oedema (Radostits *et al.*, 1994; Merck manual, 1989).There is also unthriftness and death in over-whelming infections (Wamae and Ihigi, 1990).

**Table 3:** A summary of clinical signs and pathology of cattle gastrointestinal nematodiasis (adopted from Vercruysse *et al.* 1988)

| PARASITE | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Haemonchus*** | +/- | +/- | ++ | | ++ | ++ | + | ++ | + | +/- |
| ***Trichostrongylus*** | + | + | | | | + | | | +/- | +/- |
| ***Cooperia*** | + | + | | | | + | | | + | |
| ***Bunostomum*** | + | + | + | | + | ++ | | ++ | + | |
| ***Nematodirus*** | + | ++ | | + | ++ | | | | + | |
| ***Oesophagostomum*** | + | + | + | | | ++ | +/- | + | ++ | |
| ***Trichuris*** | + | +/- | | | | +/- | | | +/- | |

Key:
1- Anorexia, 2- Diarrhoea, 3- Oedema, 4- Dehydration, 5- Pale mucous membranes, 6-Production loss, 7- Death, 8- Anaemia, 9- Hypoproteinaemia, 10- Blood pepsinogen increase.
+/- = Rare, + = Common, ++ = Very common

Clinical diagnosis of gastrointestinal nematodiasis is difficult since the signs are not pathognomonic. However, an animal in poor condition, anaemic and diarrhoeic should immediately be suspected of gastrointestinal nematodiasis especially if the same symptoms are observed in several animals of the same herd (Troncy, 1981). Under natural conditions, parasitic gastro-enteritis is seldom caused by a single species. Even where clinical enteritis can be attributed to a single species such as *Bunostomum phlebotomum*, a number of other nematode species are usually involved. In many cases, gastro-enteritis is due to the summation of a burden of intestinal nematodes of different species/genera.

### 1.2.1.2. Laboratory diagnosis

Laboratory diagnosis involves examination of faeces for eggs and also culture of the faeces to identify parasites to genus or species levels. Examination of faeces for the presence of eggs is not enough to confirm the diagnosis as negative faecal examination may not necessarily mean the absence of a helminth problem because some species cause disease in their larval stages. An example is oesophagostomosis where the larvae is responsible for the manifestation of the disease at a stage when no eggs are expelled (Urquhart *et al.,* 1992; Soulsby, 1986; Troncy, 1981). Therefore to determine the nematode species present in a live animal coprological examinations for nematode eggs are often applied. This is because the nematodes eggs are similar and identification to species or even genus level is difficult as they resemble in most morphological characteristics (Sommer, 1996).

#### 1.2.1.2.1. Faecal egg counts

Faecal egg counts are the most widely used method of diagnosing nematode parasites in live animals (Tarazona, 1986). The method has been used for many years as an ante mortem indicator of the intensity of nematode burdens in animals. The counts are also often used as indicators of the genus of helminths present and the rate of pasture contamination (Bryan and Kerr, 1989). They, however, do not give an indication of the availability of the infective material on the pasture that may pose a risk of infection to the host, or of the worm burden in infested animals, and their seasonal fluctuations cannot be

used as an indication of similar fluctuations in the worm burdens and /or rates of infestation (Schroder, 1979). This is because, for example in goats, increases in egg counts may not primarily be due to an increase in worm burdens but in the egg laying capacity of the worms (Wamae and Ihigi, 1990). It is true however that egg production is generally highest in summer, when conditions for infestation are also more favourable (Nalubamba, 1996; Urquhart *et al.,* 1992; Soulsby, 1986).

Many methods have been developed to examine faeces for the presence of helminth eggs but that of McMaster has been proved to be among the most consistently reliable (Rossanigo and Gruner, 1991). This floatation technique estimates the number of eggs per gram of faeces, although only 16.5 percent of the eggs are counted in the McMaster method compared to the egg extraction technique which is more sensitive (Rossanigo and Gruner, 1991). However, despite the egg extraction method being sensitive, it is both laborious and time consuming.

One problem in the use of faecal egg counts in estimation of worm burdens is that they will not be related to worm burden in any simple fashion as they are influenced by parasite density and season which have effects on parasite fecundity (Coyne *et al.*, 1991).

### 1.2.1.2.2. Coprocultures

Many nematode eggs are morphologically similar and though genera may be differentiated, the species cannot be easily differentiated from the eggs in faecal samples. For these parasites, differentiation can be achieved by the use of coprocultures which involve the hatching and development of helminth eggs into the infective larvae ($L_3$) under the correct environment (Hansen and Perry, 1994).

Addition of a little biological inert material to coprocultures significantly enhances the yield of infective larvae. The additives help to provide aeration for the culture media and to maintain proper moisture levels which are conducive for optimal larval development. Additives used include, sphagnum moss, sterile dry faeces, charcoal and vermiculite (Steffan *et al.*, 1989).Vermiculite is recommended because it is cleaner and also provides adequate moisture in the sample by absorbing excessive water (Steffan *et al.*, 1989).

This method is valuable in the identification of nematode species in an infection although the larval differential counts obtained by using the method may not necessarily reflect the relative abundance of worms species in cattle because of differences in the egg laying capacities of the various species (Bryan and Kerr, 1989)

### 1.2.1.2.3. Other methods

Other methods used in clinical diagnosis include the following:

a) Combined dimensional and biological characteristics of the egg used to identify strongyle eggs, commonly found in sheep other than those of *Nematodirus* (Christie and Jackson, 1982). The eggs are identified up to genus level.

b) Blood pepsinogen which can be a more reliable diagnostic tool than the egg counts when the objective is to assess abomasal burdens in cattle (Vercruysse *et al.*, 1986). This technique is based on the principle that most abomasal worm infestations cause mucosal damage and hence release of pepsinogen into blood circulation. However, caution should be taken since blood pepsinogen levels are a non specific test because high levels in the blood result from abomasal damage due to causes other than helmithosis.

c) Estimation of the density of third stage larvae on herbage which may indicate the intensity of infection in the animals grazing on the pastures (Couvillion, 1993). The species of infective larvae will also indicate the type of species likely to be found in the animals. This method however will not indicate the infection rates of the individual animals as the results reflect upon the whole herd. It can therefore mostly be used for epidemiological and not for treatment purposes.

d) Detection of excretory/secretory products of the parasite in host faeces (coproantigens) which is a recently developed approach for the diagnosis of gastronematodiasis. The basic technique of coproantigen detection is a sandwich Enzyme Linked ImmunoSorbent Assay (ELISA). A similar technique of detecting parasite-specific DNA fragments of eggs in the host faeces is another method used. This method, combined with an amplification method such as the polymerase chain reaction (PCR) can detect the DNA of even one egg in faeces (Nonaka, 1995). Parasite species can be identified by detection of species-specific DNA fragments.

e) Digital image analysis for the identification of nematode eggs (Sommer, 1996). In this technique, images of single eggs (400 x magnification) are recorded by a CCD camera fitted onto a microscope and digitised on a PC. The positions of the egg outline are analysed by algoliths to describe size and shape.

### 1.2.2. Post mortem diagnosis and differential worm counts

Post mortem diagnosis provides a more accurate assessment of parasite burden than egg counts and the type of lesions can easily be assessed. The species of the parasite can be determined and also the numbers of parasite present known (Hansen and Perry, 1994).

The technique requires that the animal be necropsied and the entire gastrointestinal tract examined. The different organs are opened and the worms are identified and counted. The procedure is however expensive, labourious and time consuming.

In addition to the counting of the adult worms, the number of immature larvae and thus the presence of hypobiotic larvae can be assessed. This is of particular importance since some worms are more pathogenic when in their larval stages and the presence of hypobiosis may indicate the possible resumption of infection when the conditions are right.

A much cheaper method of post mortem assessment of worm burden is to collect viscera from an abattoir and do the worm counts. Though the results will not necessarily reflect the seasonal availability of viable infective larvae on the pasture, they afford an estimate of total worm burdens in the grazing cattle especially if the animals are coming from the same area (Schroder, 1979).

Another advantage of post mortem diagnosis is that in the dry season when there are a lot of hypobiotic larvae and most adult worms have reduced fecundity and hence reduced egg excretion, the method is more reliable in quantifying the worm burden of the animals (Fritsche *et al.,* 1993).

### 1.2.3. Relationship between ante and post mortem results.

Despite faecal egg counts being frequently used as a cheap and easily performed technique, the assumption that there is a relationship between the number of eggs per gram of faeces and the total number of worms has been questioned because counts are influenced by many factors including differential fecundity of species of parasites, ingesta volume, age of worms and host resistance (Tarazona, 1986).

No correlation has been reported to exist between the number of worms at necropsy and the number of eggs per gram (Ndao *et al.,* 1995; Duwel, 1990; Barth *et al.,* 1981). An accurate estimation of the number of nematodes present could not be made even after taking several approaches to data analysis. It was only the mean natural log egg count which could roughly predict the mean natural log nematode count in groups of sheep (Reinecke and Groeneveld, 1991).

Acquisition of immunity also affects the relationship in that egg production by female worms is depressed and low egg counts may be recorded even though significant numbers of adult worms are still present in the host (Winks *et al.* 1983). In *Cooperia* adults, the suppression of egg excretion in faeces is also partly due to hypometabolic state of and/or inhibition of egg laying by the females present in the digestive tract (Ndao *et al.,* 1995). Correlation of 0.74 and 0.84 between individual faecal egg counts and strongyle worm counts in lambs have been reported (Bisset *et al.,* 1996). Ploeger *et al.* (1994) reports that

faecal egg counts determined early in the first grazing season, do have the ability to point at potentially dangerous levels of exposure during the second half of the first grazing season, however, the prognostic value of egg counts will probably be less under condition other than the ones used in the experiment i.e. set stocking and no application of early season anthelmintic treatments. A negative relationship develops after the first grazing season probably due to the acquisition of immunity. Bisset *et al.* (1996) also found a significant relationship between the natural worm burdens of calves aged less than one year and the faecal egg counts and also the explanation would be the development of resistance in older animals.

It has been observed, therefore, that the egg counts are related to the worm burden in young animals especially those that are in their first grazing period. This relationship slowly fades away as the animals gain immunity against the parasites (Bisset *et al.,* 1996, Ploeger *et al.,* 1994; Aumont *et al.,* 1991).

In older animals however, faecal egg counts can be used to indicate the extent and intensity of parasites in the flock and may act as an indicator to the level of pasture contamination (Murrell *et al.,* 1989; Momin and Avasatthi, 1986; Tarazona, 1986).

## 1.3. Treatment and control of gastrointestinal nematodes

### 1.3.1. The use of anthelmintic drugs

The use of anthelmintics forms an important component in the control of parasitic infections. Many modern anthelmintics are effective against both adults and larval stages and some against arrested larvae. Currently there are seven groups of anthelmintic available on the market (Nalubamba, 1996; Brander *et al.* 1991, Merck manual 1989). These are:

a) Benzimidazoles

b) Imidazothiazoles

c) Organophosphates

d) Tetrahydropyrimidines

e) Avermectins

f) Salicylanilides, substituted phenols, aromatic amines

g) Miscellaneous e.g. Piperazine.

Control strategy based solely on periodic anthelmintic medication, as is the current practice in most farms, must involve several treatments during the rainy season in order to be effective. This is because most traditional anthelmintics are only effective for a short period of time so that grazing animals become rapidly reinfected within days of treatments (Brander *et al.,* 1991). In addition one early dry season treatment is also

necessary to prevent possible outbreaks of parasitic gastrointestinal enteritis in the dry season (Chiejina and Emehelu, 1984).

Regular use of anthelmintics, however, poses the risk of drug resistant nematode strain developing (Kinoti *et al.,* 1994). This phenomenon arises from the over reliance on anthelmintics which continually destroy susceptible parasites and leave a few individual worms with a gene of resistance which later breed to produce a flourishing resistant population. In an event of development of resistance, a new drug with a different mode of action should be used. In the evaluation of the new anthelmintic, requirements such as efficiency against adult and immature worms, degree of toxicity and cost per treatment to the user need to be considered (Ogunsusi *et al.,* 1986).

### 1.3.2. Other approaches in the control of gastrointestinal nematodes.

The principle of a helminth control programme is to keep the challenge to young livestock by the pathogenic trichostrongyle parasite at a minimum rate. This requires knowledge of the types of parasite present; herd structure and grazing management; parasite seasonal availability and survival; and the weather conditions in particular areas (Hansen and Perry, 1994).

One method of helmith control is not to keep or graze animals in one particular place. Kaufmann *et al.* (1995) established that a regular change of the corral (night holding kraals) was an effective method of reducing risk of nematode infection. This method avoids the accumulation of infective larvae in one particular place. Overstocking a

particular area also increases the risk of animals getting infected with worm parasites. Rotation of pasture is also of inestimable benefit in helminth control programmes

Control can also be by avoiding marshy areas. These marshes jeopardise control of helminths, unless they are properly managed. They are often valuable pasture but they provide an ideal environment for trematodes as well as nematodes, such as *Haemonchus spp.* which are the most important nematodes in the summer rainfall regions (Hall 1988, Van Wyk, 1990).

Many of the common gastrointestinal nematodes of sheep and cattle are also found in game animals (Van Wyk, 1990). The most common and important genera are the wire worm (*Haemonchus* spp.), nodular worm (*Oesophagostomum* spp.), hook worm (*Gaigeria* spp.), and the white bankrupt worm (*Strongyloides* spp.). These worms are sometimes maintained in the game animals in the absence of sheep or goats. Therefore in every worm control programme that is drawn up, it is essential to consider the presence of game that can serve as reservoirs of infection and may ostensibly jeopardise an otherwise effective control programme.

In the mid seventies, research was carried out to use the dung beetle, *Onthophagus gazella* to control gastrointestinal nematodes (Bryan, 1976, 1973). These beetles were released on the pastures with dung containing eggs and infective larvae of gastrointestinal nematodes. It was found that the beetles degraded the dung pads and almost completely made the pastures clean of infective larvae of gastrointestinal nematodes. This method has however not been used in tropical Africa.

## 1.4. The climate of Zambia

Climate is a combination of elements that include temperature, humidity, rainfall, air movements, radioactive conditions, barometric pressure and ionisation of which temperature and rainfall are the most important (Williamson and Payne, 1987). The two factors affect most the prevalence and distribution of gastrointestinal nematodiasis.

The country experiences three season in a year, (1) the warm and wet season from November to March/April, (2) the cold and dry season between April and August, and (3) the hot and dry season from September to November. The average temperatures range between 10°C to 25°C during the cold dry season and 18°C to 33°C in the warm/wet and hot/dry season. The rain season is however rather uncertain and there can be considerable variation from year to year. The total annual rainfall ranges from 600 to 1600 mm. There are three main rainfall zones as shown in Figure 2.

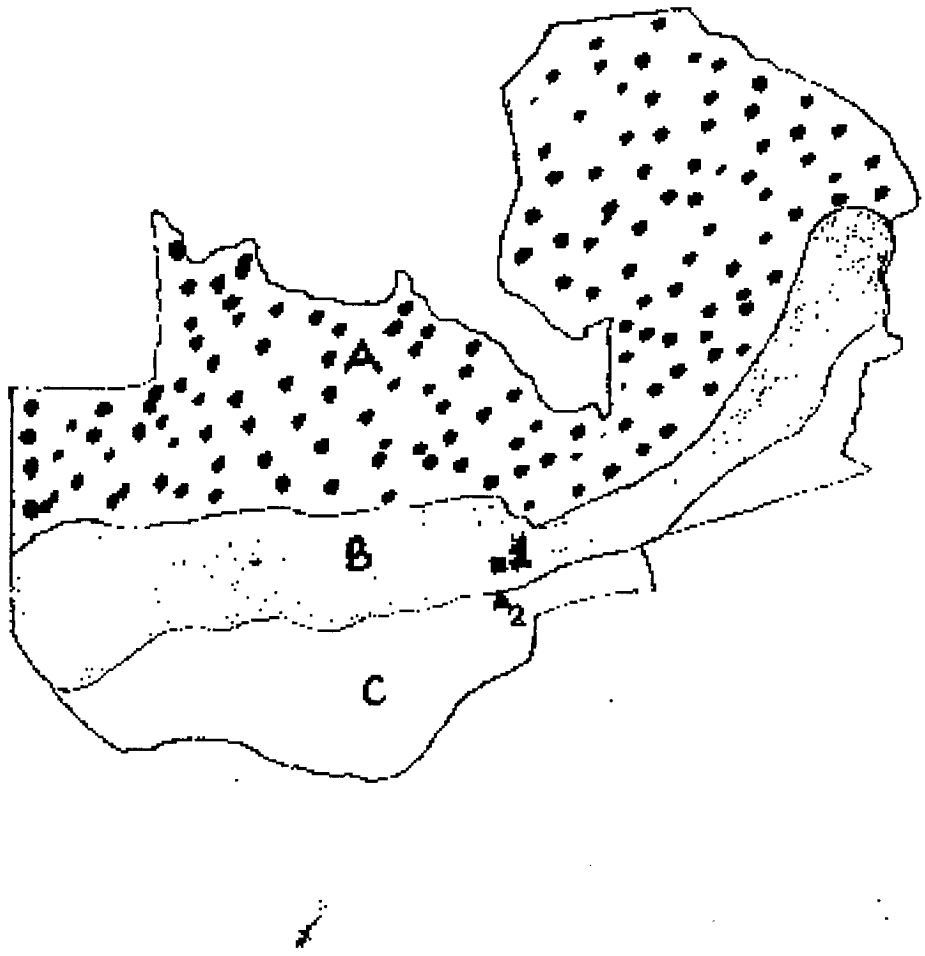


**Figure 2:** Map of Zambia showing the distribution of rainfall and the three main rainfall zones. The map also shows where the study was carried out. (Adopted from Anon. 1982)

Key:

1. Lusaka, the administrative capital of Zambia
2. Turn Pike, The location where the samples were collected.

A) 900 - 1600 mm
B) 800 - 900 mm
C) 600 - 800 mm

## 1.5. Cattle production in the traditional sector

The livestock sector is mainly based on cattle production which accounts for more than 35 percent of the total agriculture production (Kadohira *et al.,* 1996, see also Appendix 2). In Zambia 80 percent of the cattle is kept in the traditional sector (Table 4). Beef alone accounts for 29 percent of the total agriculture exports and the traditional sector contributes to half the beef consumed in the country (Chilonda, 1994). Livestock, in particular cattle, are very important in the socio-economic structure of Zambia. Cattle and other livestock sustain the employment and income of millions of Zambians especially in the rural areas, and also provide the only food and cash security available to many. They also contribute to draught oxen, dowry cattle, manure for crop production and act as indicators of social status in a community. Animals are usually killed for beef at the end of their working life (Williamson and Payne, 1987) or if they are sick or when the household need immediate cash for other purposes. Eighty percent of the total cattle population kept in the traditional sector are of the indigenous Sanga and Zebu breeds, but in this sector there is general low productivity and take off (Kadohira *et al.,* 1996, Nadaraja *et al.*, 1984, Perry *et al.* 1984).

In the traditional sector, almost all farmers practice communal grazing. The cattle graze within a few kilometres from the homestead during the rainy season and have to trek to streams and swamps during the dry season. These marshy areas are known to harbour a lot of parasites including the infective larvae of gastrointestinal nematodes. The distance covered to look for food and water may at times reach as much as 15 km (Kadohira *et al.,*

1996) and these long distances can cause stress to the already malnourished animals predisposing them to infections and infestations.

Another constraint in the traditional sector in Zambia is the lack of adequate and proper veterinary services. This has been compounded by the withdrawal of free veterinary services by the government. It has been noticed that deworming is not popular and almost non existent in the traditional sector due to the high cost of the drugs (Kadohira *et al.*, 1996), and lack of knowledge of the effects of worms on the health of the animal.

The indigenous breeds of cattle have been categorised into three, i.e. the Tonga cattle, the Barotse cattle, and the Angoni cattle. These local breeds are recognised as having excellent disease tolerance and ability to survive on low quality feed and management. They are thus well adapted to the climate and land environment in which they are developed.

**Table 4:** Cattle populations in Zambia (1993- 1997)

| SECTOR | 1993 | 1994 | 1995 | 1996* | 1997* |
|---|---|---|---|---|---|
| Traditional | 1,744,642 | 828,872 | 1,117,148 | 1,649,000 | 1,700,000 |
| Semi Traditional | 46,819 | 48,229 | 51,384 | 499,200 | 520,000 |
| Commercial | 475,636 | 209,319 | 256,870 | 494,100 | 520,000 |
| **Grand Totals** | **2,267,097** | **1,086,420** | **1,425,402** | **2,642,300** | **2,740,000** |

* = Expected cattle population
Source: Department of Animal production and Health

## 1.6. Justification and objectives of the study

Very little work has been done in Zambia as regards the epidemiology of gastrointestinal nematodes in domestic animals especially in cattle (Nalubamba, 1996, Tada Y. *et al.*, 1995, 1994). Similar studies have been carried out in other countries but due to the marked variation in the transmission patterns of the gastrointestinal nematodes, which is dependent on the geographical location in which they occur (Rickard and Zimmerman, 1992), a need was seen to carry out such a study in Zambia.

There have also been conflicting reports on the value of the use of faecal egg counts as an estimate of the size of worm burden as has been seen in the previous sections. There was thus a need to establish if there is any correlation between the two parameters in the Zambian situation.

The objectives of the study therefore were:

1. To determine the relationship between faecal egg counts and worm burden in traditional cattle of Zambia.
2. To determine the prevalence of the gastrointestinal nematodes in traditional cattle
3. To investigate seasonal variation in the prevalence of gastrointestinal nematodes in traditional cattle.

# CHAPTER TWO

## MATERIALS AND METHODS

### 2.1. Meteorological data.

Meteorological data was collected from the Zambia Meteorological Department Office - Lusaka. Data was provided for five meteorological stations in Southern Province i.e. Chipepo, Choma, Livingstone, Lusitu and Magoye. Only two of these stations i.e. Choma and Livingstone had complete data for the period August 1996 to September 1997.

### 2.2. Study animals.

The study was carried out on traditional cattle, mostly of the Tonga breed from the Southern Province of Zambia. These animals were brought for slaughter at Turn Pike slaughter slab.

### 2.3. Study area

The study was carried out at the Turn Pike slaughter slab. The location is about 45 km south of Lusaka along the Lusaka Siavonga road near the Livingstone junction (see Figure 2). The place is run by a co-operative society that apart from providing a slaughter slab for the local people, has other activities such as a restaurant, a bar and a few grocery

shops. The place is however mainly used by the local people to slaughter their animals for sale. Most of the meat is sold right at the slab whilst some is delivered to Kafue and Lusaka for sale in various butcheries.

Almost all the animals that are slaughtered are of traditional breed and come mainly from the Southern province of Zambia. These animals are usually trekked from as far as Siavonga and Gwembe (A distance of approximately 100 km from the slaughter slab) by the local people. They are brought to this place mainly on foot while those who can afford to hire transport bring them on trucks. The local people either slaughter the animals themselves or sell to middlemen who slaughter and later resale.

The reasons for bringing these animals for slaughter are varied and include old age and disease and as a mere source of generating revenue for various reasons.

**2.4. Sampling of the animals and collection of specimens**

Five animals were randomly selected during the slaughter session. The gastrointestinal tract, from the abomasum to the rectum, were collected from these animals. The junctions between the abomasum and the small intestine and between the small and large intestine were ligated immediately after removal from the animal using jute twine to avoid mixing of the contents.

The sexes of the animals sampled were noted and efforts were made to determine the age and source of the animal. The age , however, proved hard to determine because some

*animals belonged to people who were middlemen and had very little information on the age of the animals they were selling. They however were able to supply the information* about the source of the animals.

The gastrointestinal tracts were transported to the Parasitology laboratory at the School of Veterinary Medicine in cooler boxes with ice packs.

The specimens were processed on the same day of collection and the gastrointestinal contents preserved in formalin. The faecal samples for coprocultures and egg counts were collected from the rectum.

## 2.5. Parasitological examinations

### 2.5.1. Faecal egg counts

All faecal egg counts were done using the modified McMaster technique utilising fully saturated sodium chloride solution. Four grams of faeces were weighed in a beaker and 56 ml of saturated sodium chloride was added. The suspension was mixed thoroughly and filtered through a strainer into a second beaker. The filtrate was passed through the strainer from the second to the first beaker and the process repeated until only undigested material remained on the strainer.

The suspension was thoroughly mixed by pouring from one beaker into the other several times and immediately after mixing a sub sample was taken using a Pasteur pipette to fill

one side of a McMaster counting chamber (Fujihira, Tokyo, Japan). The mixing was repeated and a second sub-sample was collected to fill the other side of the McMaster counting chamber.

The counting chamber was left to stand for at least 5 minutes and then mounted on a light microscope. The examination was done at X10 magnification. All nematode eggs within the engraved area of the counting chamber were counted. The total number of eggs in both sides of the counting chamber was multiplied by 50 to get the number of eggs per gram (see Appendix 3 for details of the MacMaster technique and calculations)

### 2.5.2. Coprocultures

#### 2.5.2.1. Incubation of infective larvae

Between 5 and 10 grams of faecal sample was placed in a 50 ml glass jar and mixed with vermiculite to obtain a moist mixture. A separate culture was made for each animal. If the sample was too dry a bit of water was added and if too wet, more vermiculite was added. The jar was closed loosely with aluminium foil and placed in an incubator (Hitec Yamato Incubator model IS-61) at 27 °C for 11 days. During the incubation period, the mixture was stirred every two days to provide enough aeration and prevent fungal growth (Anon, 1981), and tap water added when necessary to maintain a moist consistency. After 11 days of incubation, the infective larvae were recovered using the Baermann apparatus (Hansen and Perry, 1994).

### 2.5.2.2. Recovery of infective larvae

The recovery of the larvae was done using the Baermann technique based on the active migration of larvae from faeces suspended in water (Anon, 1981; Hansen and Perry, 1994).

The apparatus was assembled by fitting a 30cm long tubing to the stem of the large funnel. The tubing was closed with a clamp and the apparatus was supported by a stand. (See Appendix 3 for a detailed information of Baermann's apparatus).

The faecal sample was placed on a moisturised paper milk filter (Whatman®, appeture 0.15 mm). This was placed on the open end of the funnel, taking care to avoid air bubbles being trapped in the apparatus. The apparatus was allowed to stand overnight, after which time the larvae were expected to have migrated through the screen and settled in the rubber tubing. The infective larvae were then drawn into glass centrifuge tubes by opening the clamp. The suspension was kept at 4 °C for 24 hours and the supernatant aspirated using a Pasteur pipette. The larval suspension was kept at 4 °C until the differential larval counts were performed.

### 2.5.2.3. Differential larval counts

A small aliquot (1 - 2 drops) of the suspension collected from the Baermann apparatus was transferred to a microslide using a pasture pipette. A drop of iodine was added to kill

and stain the larvae and a cover slip was placed. Examination was done under X10 magnification and the keys adopted from Soulsby (1965) (Appendix 4 ) and by Hansen and Perry (1994) (Appendix 5) were used in identifying the larvae. The aliquots were examined until at least 50 larvae were identified. These larval counts were done every two weeks on all the animals sampled.

## 2.6. Post mortem differential worm counts.

### 2.6.1. Processing of different organs

#### 2.6.1.1. Abomasum

After separation from the rest of the gastrointestinal tract, the abomasum was opened along the greater curvature. The contents were collected in a bucket. The inside of the abomasum was washed with running tap water to remove adhering worms and the washings collected in the same bucket. After noting the volume of the contents collected, the contents were well mixed and 200 mls of the well mixed contents were placed in a plastic bottle. Twenty millilitres of 40 percent formalin was added for preservation and the sample stored at room temperature until the worms were counted. The samples were collected in duplicate.

After washing, the mucosa of half the abomasum was scrapped off using a hard plastic scrapper. The scrapings were placed in labelled jars for pepsin digestion. If the pepsin was

not ready, the scrapings were placed in a deep freezer till the digestion. The pepsin was prepared as described by Hansen and Perry (1994) (See Appendix 3).

### 2.6.1.2. Small intestines

The small intestines were freed from the mesentery using a scissors and placed in a plastic bucket. They were then opened along the entire length with an enterotomy scissors. The opened intestines were washed thoroughly with running tap water whilst being passed through squeezed fingers to remove adherent worms. The final volume was noted and paired samples of 200 mls were collected. Twenty milliliters of 40 percent formalin were added to plastic bottles for preservation and stored for future worm counts (Appendix 3)

### 2.6.1.3. Large intestines and caecum

The large intestine and caecum were opened lengthwise and the contents poured into a bucket. The mucosa was washed with running tap water and the washings collected in the bucket. The total volume was noted and 20 percent of the original volume was collected and put in plastic bottles. Forty percent formalin was added in such a way that the volume added was 10 percent of the sub-sample collected.

### 2.6.2. Procedure for differential adult worm counts

#### 2.6.2.1. Abomasum and small intestines

The preserved samples were poured into a glass conical flask and mixed using a magnetic stirrer. Eleven millilitre aliquots of the sample were washed in a 425μm Endcott® sieve until only undigested material remained. The washed samples were examined under a stereo microscope in a counting chamber. All the worms were removed with thumb forceps and examined under a light microscope. The females were identified to genus level while the males to species level. The examination was done until 2 percent of the original volume that was collected as stated in section **2.6.1.2.** was examined (See Appendix 3). The number of worms was multiplied by twenty to get the total worm count in the animal.

#### 2.6.2.2. Large intestines and caecum

All the collected sample was washed under running tap water in a 212μm Endcott® sieve. The washed material was poured in a large petri dish and all the worms collected and identified as for the abomasum and small intestines.

### 2.6.3. Recovery of inhibited larvae from the abomasum

Approximately 500 mls of acid pepsin (Check appendix 3 for formulation) were added to every 100 g of mucosal scrapings. The mixture was mixed thoroughly and incubated at 37°C overnight. After the digestion, the large undigested material were removed using thumb forceps and the remaining mixture topped up with tap water to 2 *l.* Paired sub-samples of 200 mls each were collected in plastic bottles and 20 mls of 40 percent formalin was added to stop the digestion and preserve the samples.

### 2.7. Statistical analysis

All the statistics and the scatter graphs were done using Microsoft C-Stat® version 1.0, a computer application for Windows®. The graphs were produced from Microsoft Excel® version 5.0a for Windows®.

# CHAPTER THREE

## RESULTS

### 3.1. Meteorological data

The monthly temperature and monthly rainfall for Choma and Livingstone are given in figures 3 and 4 respectively. These two sets of data were pooled to get an average estimate of the two variables to represent the province. The average mean monthly temperatures and mean monthly rainfall for the two stations are shown in figure 5.

The temperature pattern was, as is expected in Zambia, between 10°C and 33°C and the rainy season also fell in the expected months of November to April. The highest rainfall months were January, February and March (Figures 3, 4, and 5).

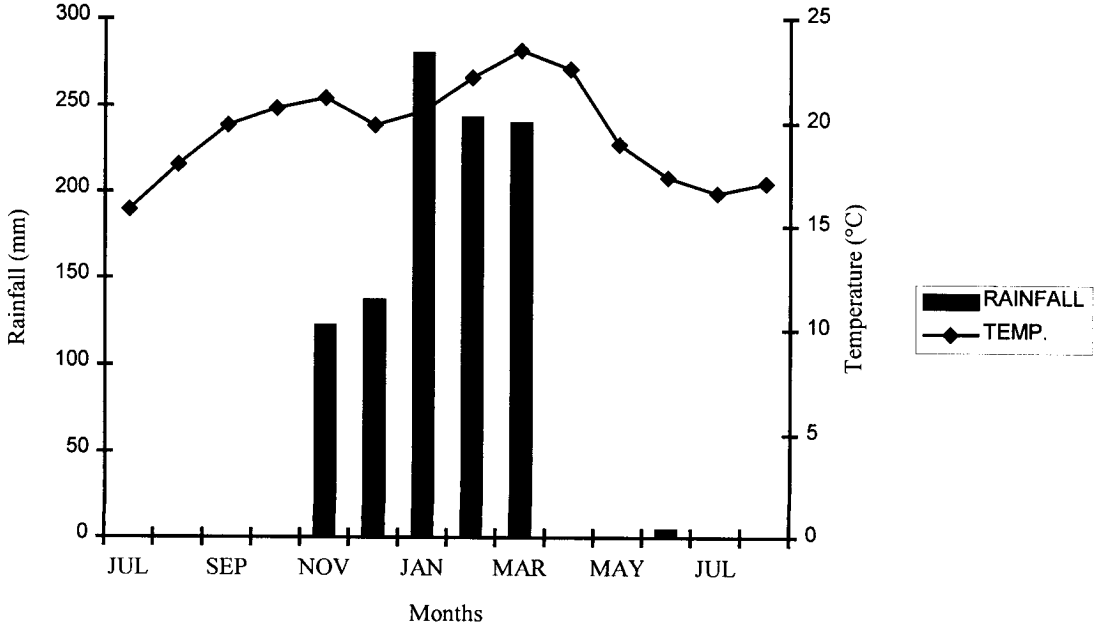


**Figure 3:** Monthly temperature and rainfall for Choma District for the period August 1996 to September 1997 (Source: Zambia Meteorological Office - Lusaka)

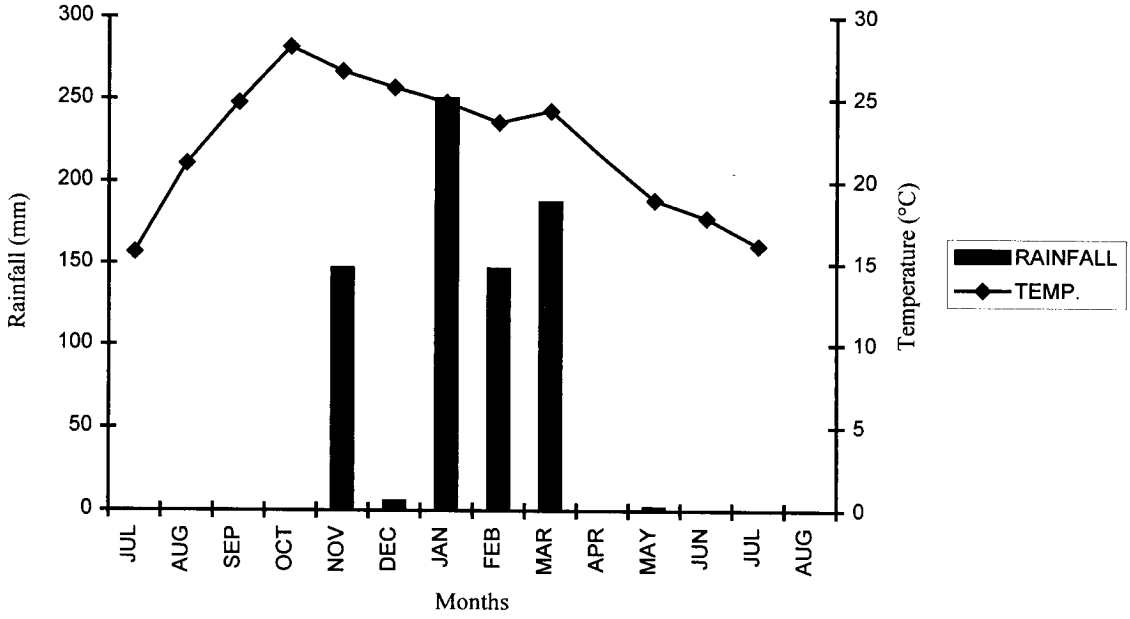


**Figure 4:** Monthly temperature and rainfall for Livingstone District for the period July 1996 to July 1997 (Source: Zambia Meteorological Office - Lusaka)

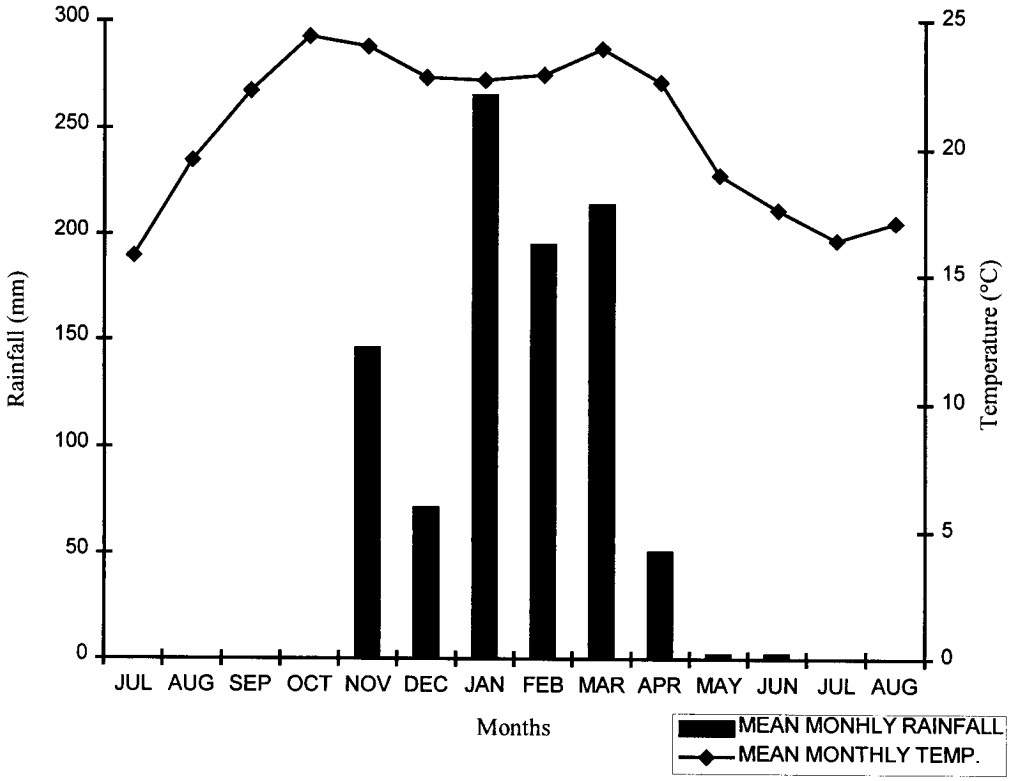


**Figure 5:** Average monthly temperatures and rainfall for Choma and Livingstone Districts for the period August 1996 to September 1997 (Source: Zambia Meteorological Office - Lusaka)

### 3.2. Gastrointestinal nematodes identified

The following genera of gastrointestinal nematodes were identified from the gastrointestinal contents: *Haemonchus spp., Cooperia spp, Oesophagostomum spp, Bunostomum spp* and *Trichostrongylus spp.* The same spectrum was observed from the coprocultures. The prevalence of these worms in the gastrointestinal contents were in the following proportions: *Cooperia spp* - 73 percent, *Haemonchus spp* - 22 percent, *Oesophagostomum spp.* - 2 percent, *Bunostomum spp.* - 1 percent, *and Trichostrongylus spp.*-1 percent. The worms were found in the different organs as shown in Table 5.

**Table 5:** Gastrointestinal nematodes of cattle found in the present study in relation to the organs of the digestive tract.

| | Abomasum | Small intestines | Large intestines |
|---|---|---|---|
| *Haemonchus* | + | -- | -- |
| *Cooperia* | + | + | -- |
| *Oesophagostomum* | -- | -- | + |
| *Trichostrongylus* | + | + | -- |
| *Bunostomum* | -- | + | + |

**Legend**: + = Present,
-- = Absent

Out of the animals examined, 84 percent were infected with *Cooperia spp.*, 76 percent with *Haemonchus spp.*, 74 percent with *Oesophagostomum spp.*, 14 percent with *Bunostomum spp.* and another 14 percent with *Trichostrongylus spp.*

All the animals examined were found to be infected with at least one genus of gastrointestinal nematodes either by faecal cultures or adult worm detection. Only 3 percent of the animals that were examined for gastrointestinal nematodes had infections that were not detected by faecal egg counts.

### 3.3. The seasonal variation of gastrointestinal nematode infection

#### 3.3.1. The seasonal variation of faecal egg counts

A total of 300 animals were examined for gastrointestinal nematode eggs during the course of the study, at an average of 40 animals per month, collected biweekly.

The highest faecal egg counts were recorded in March. There was a drastic drop in the faecal egg counts towards the end of May which remained low through the months of June, July, August and September. The lowest mean monthly faecal egg counts were recorded in July, August and September (figure 6).

The mean monthly faecal egg counts were compared with both the monthly rainfall and mean monthly temperature. The highest faecal egg counts were recorded in the rainy months and the counts dropped during the cold dry season (figure 7). A positive

correlation was highly significant between faecal egg counts and mean monthly rainfall ($r = 0.8447$, $p<0.01$) and between faecal egg counts and mean monthly temperatures ($r = 0.9762$, $p<0.05$). This means an increase in total rainfalls was accompanied with an increase in faecal egg counts.

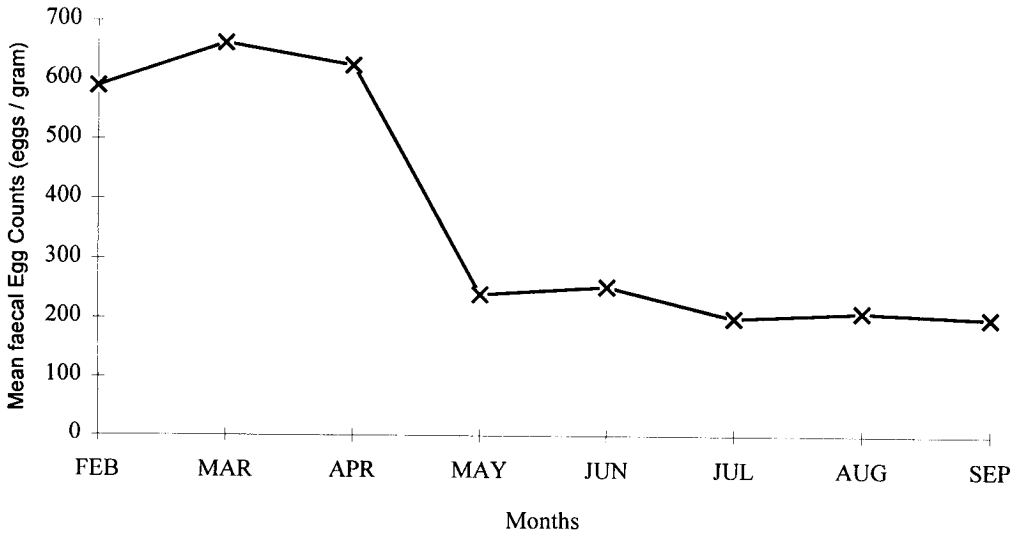


**Figure 6:** Monthly variations of faecal egg counts of traditional cattle of Southern Province of Zambia for the period February to September 1997 done using modified MacMaster technique, n ≥ 20

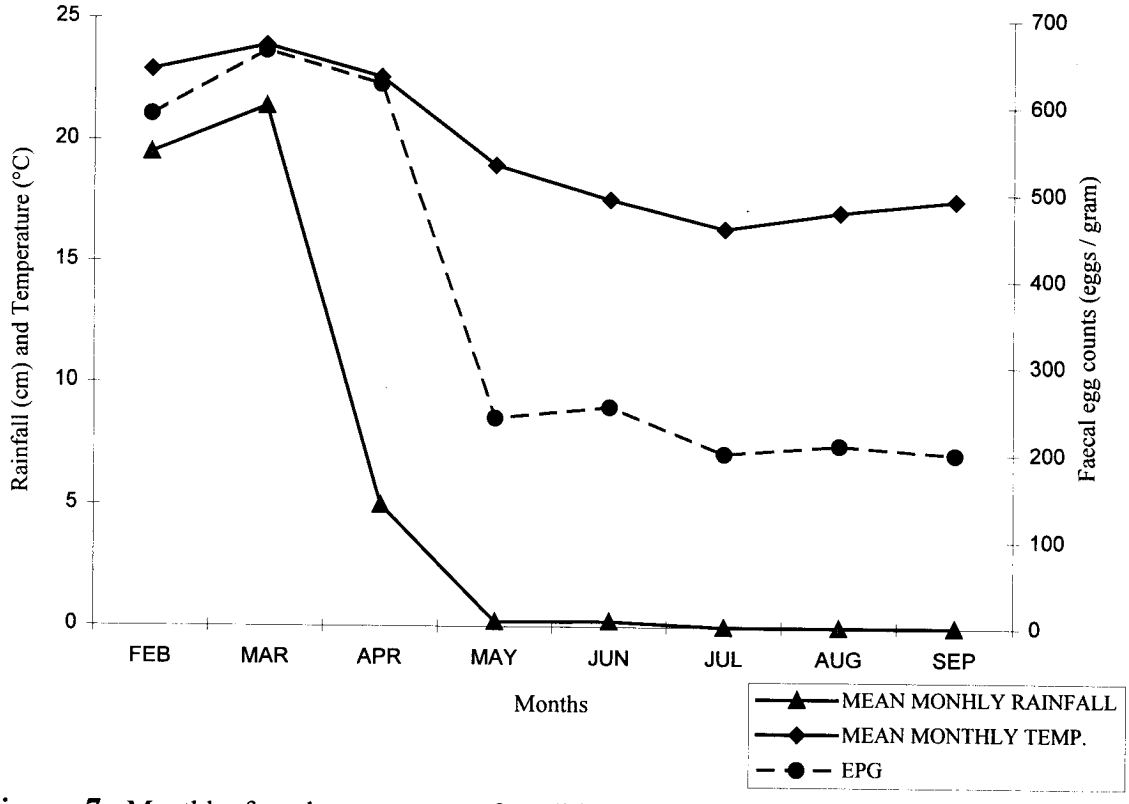


**Figure 7:** Monthly faecal egg counts of traditional cattle of Southern Province of Zambia compared with monthly rainfall and monthly temperature for the period February to September 1997.

### 3.3.2. The seasonal variation of total worm burden

A total of 70 animals were sampled during the whole study for the presence of gastrointestinal nematodes. The highest total worm burden in an animal was 30550 in an animal from Mazabuka district recorded in February, while the lowest was 50 in an animal from Siavonga in the month of August (Appendix 6). All the animals examined were found to harbour at least one genus of gastrointestinal nematode and only two of these were not detected by faecal egg counts.

On average, the highest worm burden was recorded in April and the lowest in May and August (Figure 8). In general, from the observation of the graph (Figure 9), there is no correlation between the total worm burdens and monthly temperature but the total worm burden seems to drop with mean monthly rainfall. A statistical analysis showed that an increase in total worm counts occured with an increase in temperature($r = 0.8087$, $p<0.05$) while no correlation was found between total worm counts and rainfall ($p>0.05$).

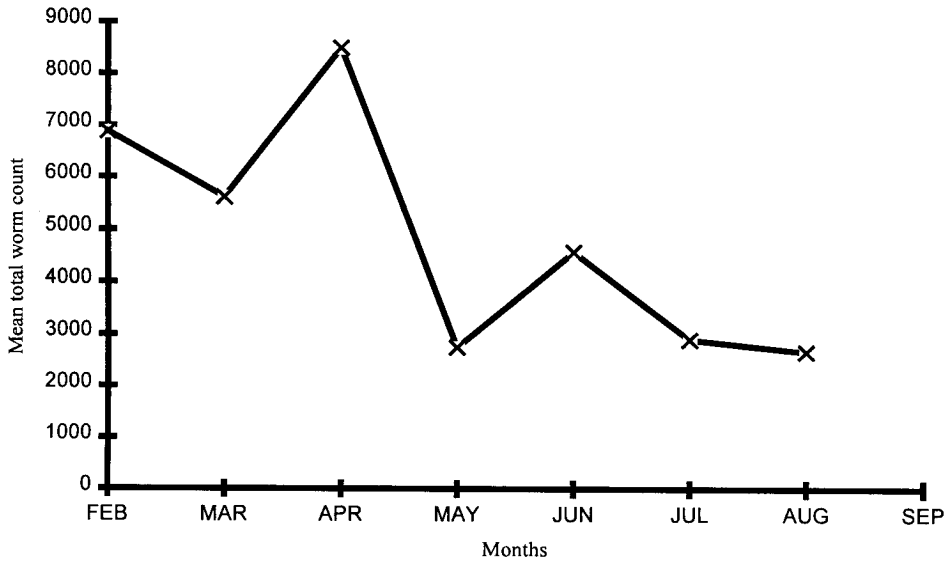


**Figure 8:** Mean monthly variation of total worm counts ($n \geq 10$) performed by post mortem worm counts (Appendix 3) of traditional cattle of Southern Province of Zambia for the period February to August 1997.

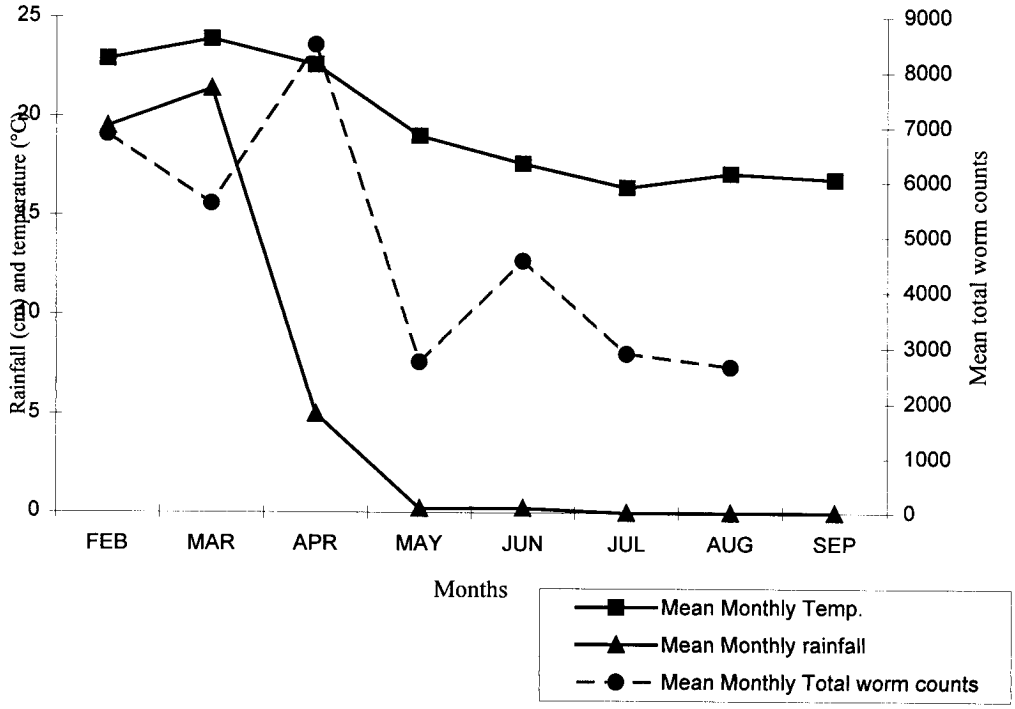


**Figure 9:** Mean monthly total counts of traditional cattle of Southern Province of Zambia ($n \geq 10$) compared with rainfall and temperature for the period February to September 1997.

### 3.4. The relationship between faecal egg counts and worm burden

#### 3.4.1. The relationship between faecal egg counts and total *Haemonchus* worms

The scatter graph and regression line at 95 percent confidence interval are shown in figures 10 and 11, respectively. There was a significant positive correlation ($r = 0.5785$), between the faecal egg counts and the number of *Haemonchus* worms at $p<0.05$.

#### 3.4.2. The relationship between faecal egg counts and total *Cooperia* worms

A stronger positive correlation that was significant was observed between *Cooperia* worms and faecal egg counts compared to the correlation between *Haemonchus* worms and faecal egg counts (Figure 12 and 13, $r = 0.7469$, $p<0.05$).

#### 3.4.3. The relationship between faecal egg counts and total *Oesophagostomum* worms

The observed relationship between *Oesophagostomum* worms and faecal egg counts was similar to that for *Cooperia* and *Haemonchus* the (Figure 14 and 15) showing a significant modest correlation ($r = 0.5987$, $p<0.05$).

### 3.4.4. The relationship between faecal egg counts and total *Bunostomum* worms

Only ten animals were found with *Bunostomum* worms out of all the seventy animals studied. The scatter graph and best fitting line (Figure 16 and 17) both show lack of correlation between worm burden and egg counts ($r = 0.0875$, $p>0.05$)

### 3.4.5. The relationship between faecal egg counts and total *Trichostrongylus* worms

Only ten animals were found to harbour *Trichostrongylus* worms. The regression line of worm burden and faecal egg counts showed a very weak correlation (figure 19). Statistical analysis showed that there was no correlation at $p>0.05$ between the faecal egg counts and *Trichostrongylus* worms.

### 3.4.6. The relationship between faecal egg counts and total worm burden

The scatter graph (Figure 20) and the regression line (Figure 21) between the total worm counts and faecal egg counts showed a positive correlation. There was a strong statistical positive correlation ($r = 0.7564$, $p<0.05$) between these two variables

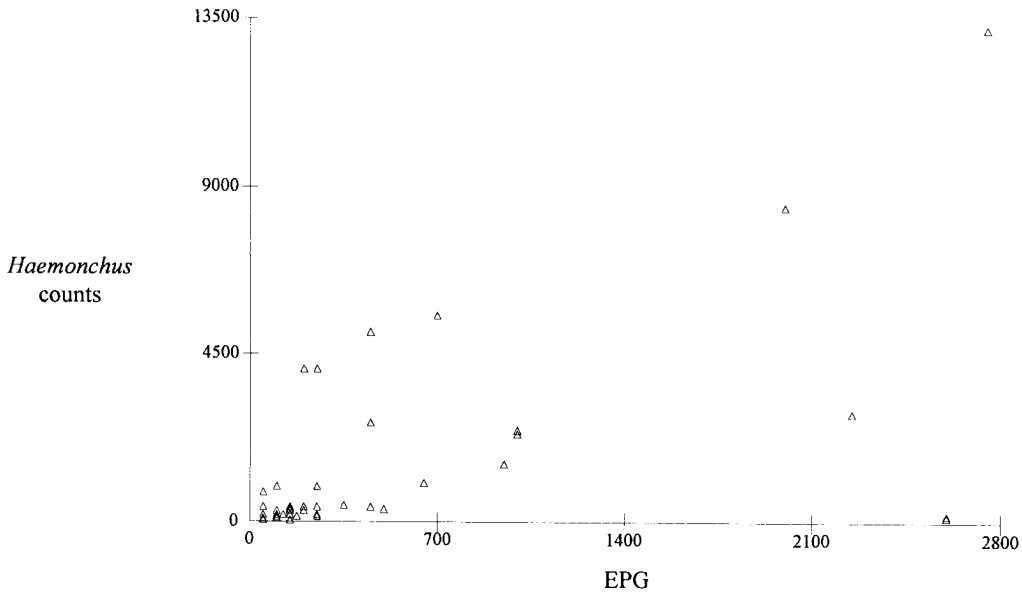


**Figure 10:** Scatter graph for *Haemonchus* worm counts compared with faecal egg counts in Traditional cattle of Southern Province of Zambia

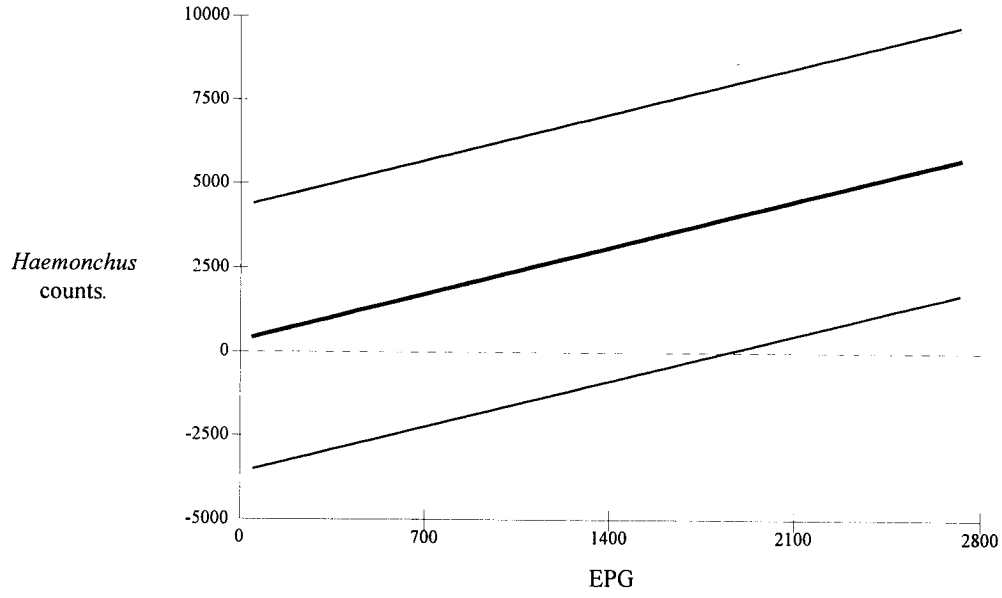


**Figure 11:** Regression line and 95% CI of *Haemonchus* worm counts compared with faecal egg counts in Traditional cattle of Southern Province of Zambia.

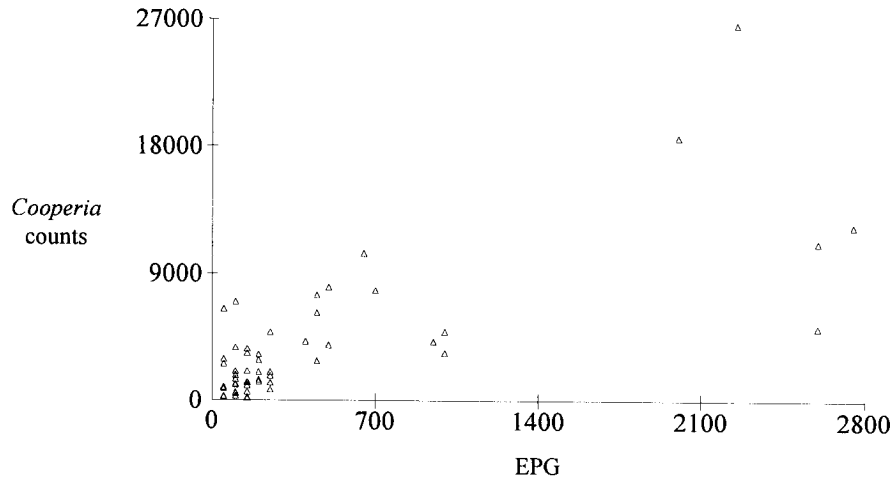


**Figure 12:** Scatter graph for *Cooperia* worm counts compared with faecal egg counts in Traditional cattle of Southern Province of Zambia

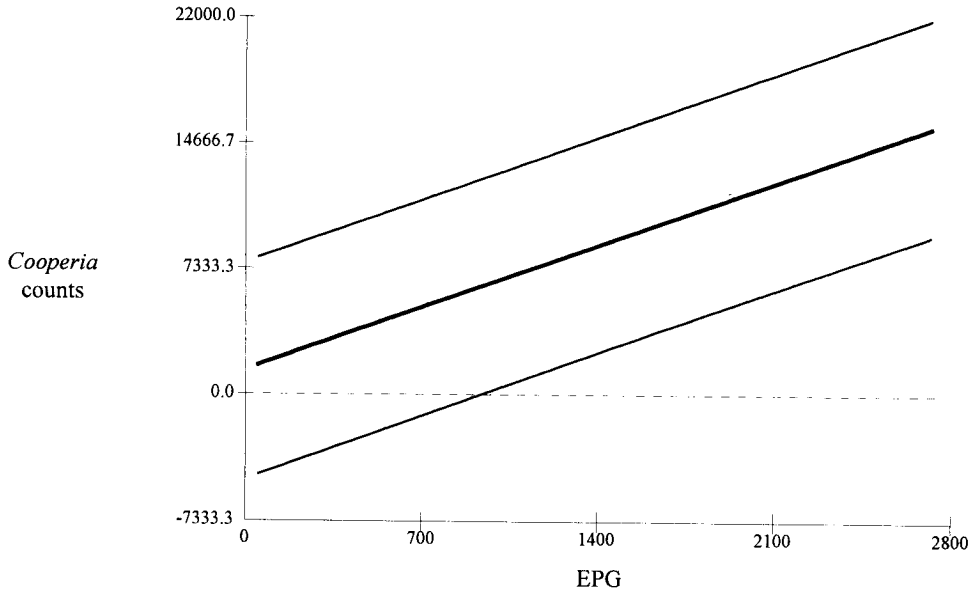


**Figure 13:** Regression line and 95% CI of *Cooperia* compared with faecal egg counts in Traditional cattle of Southern Province of Zambia.

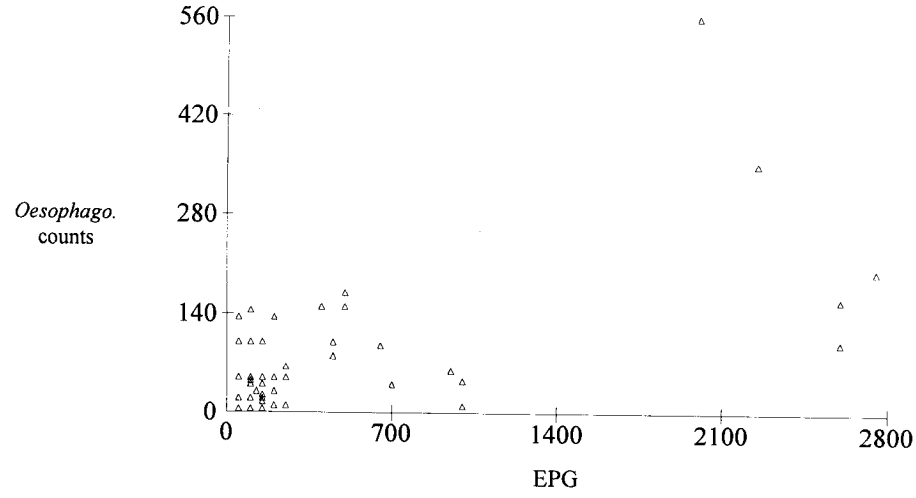


**Figure 14:** Scatter graph for *Oesophagostomum* (*Oesophago.*) worm count compared with faecal egg counts in Traditional cattle of Southern Province of Zambia

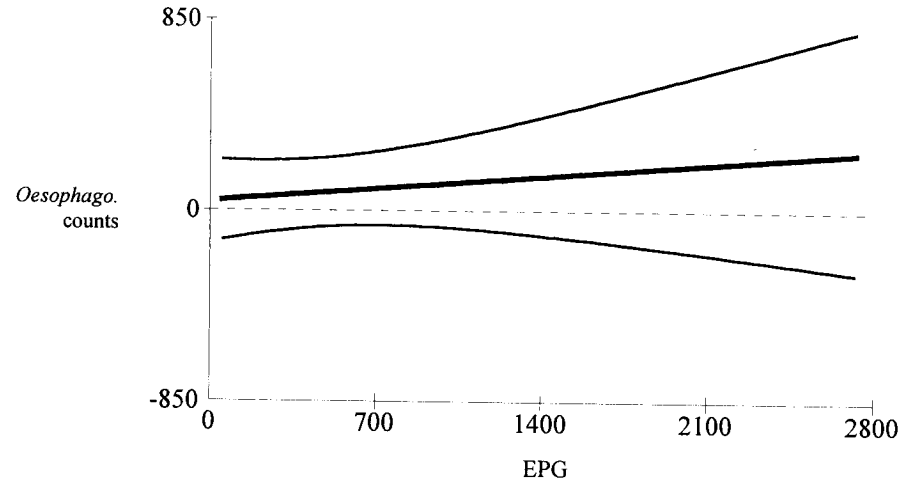


**Figure 15:** Regression line and 95% CI for *Oesophagostomum* (*Oesophago.*) worm counts compared with faecal egg counts in Traditional cattle of Southern Province of Zambia

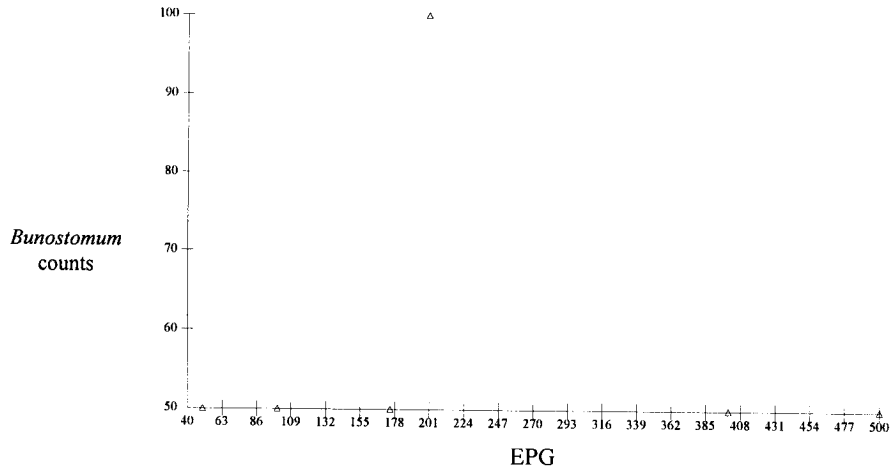


**Figure 16:** Scatter graph for *Bunostomum* worm counts compared with faecal egg counts in Traditional cattle of Southern Province of Zambia.

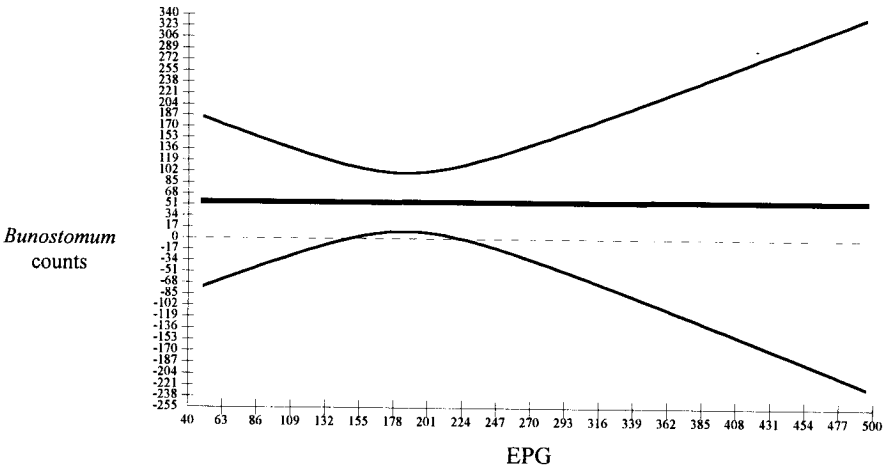


**Figure 17:** Regression line and 95% CI for *Bunostomum* worm counts compared with faecal egg counts in Traditional cattle of Southern Province of Zambia

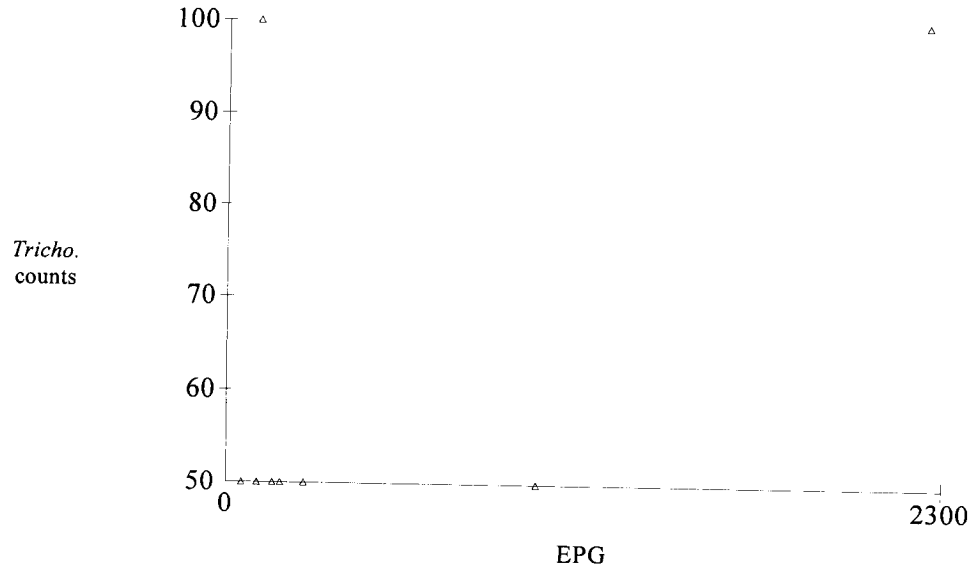


**Figure 18:** Scatter graph for *Trichostrongylus* (*Tricho.*) worm counts compared with faecal egg counts in Traditional cattle of Southern Province of Zambia

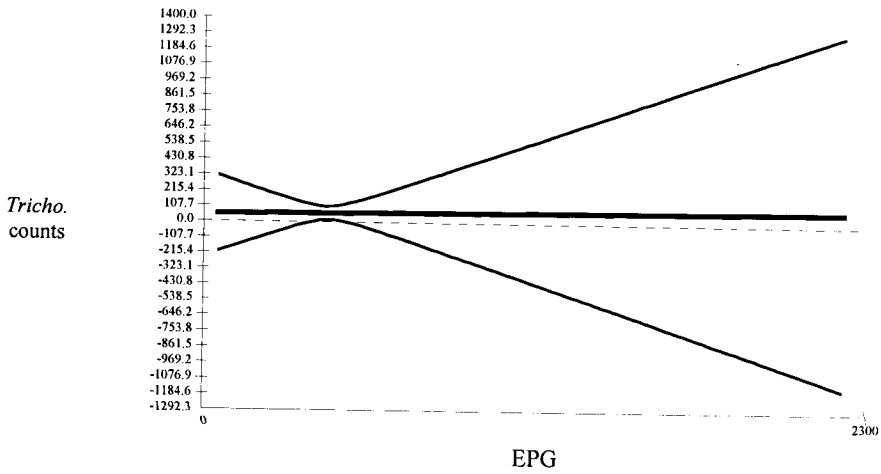


**Figure 19:** Regression line and 95% CI for *Trichostrongylus* (*Tricho.*) worm counts compared with faecal egg counts in Traditional cattle of Southern Province of Zambia

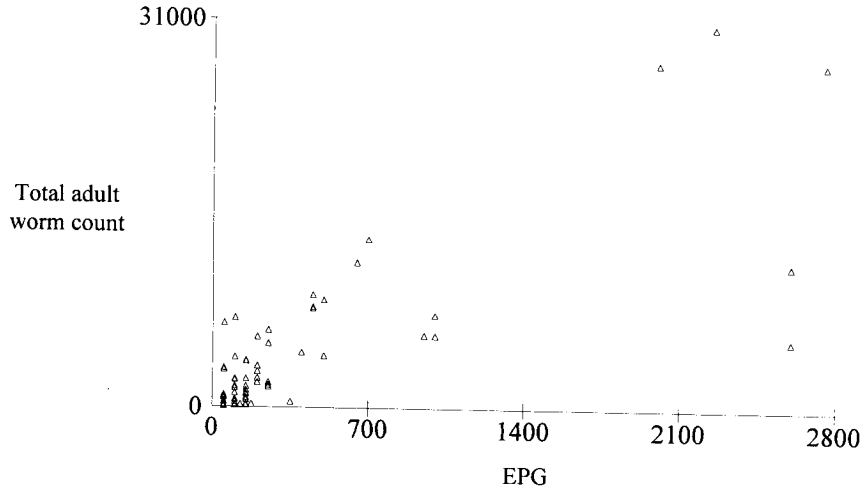


**Figure 20:** Scatter graph for total worm counts compared with faecal egg counts in Traditional cattle of Southern Province of Zambia

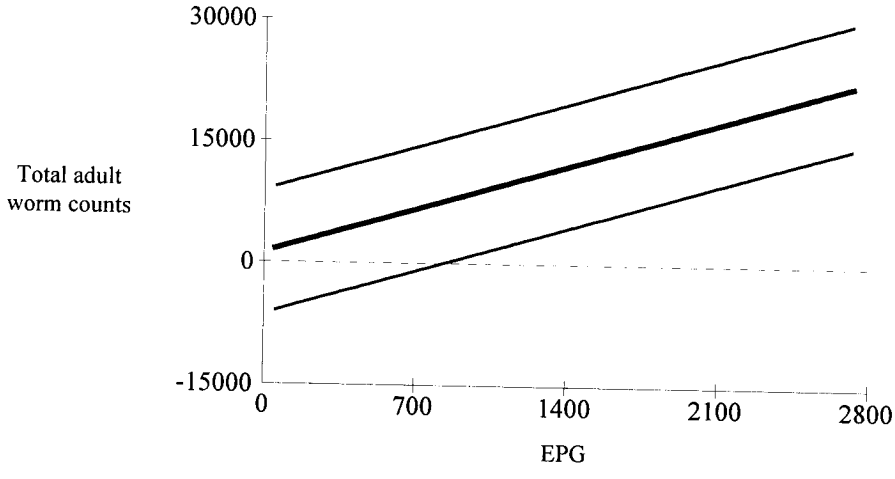


**Figure 21:** Regression line and 95% CI for total worm counts compared with faecal egg counts in Traditional cattle of Southern Province of Zambia

### 3.5. Hypobiosis

All the animals in the study showed no evidence of L4 (inhibited larvae) after pepsin digestion. This means that throughout the study period, no hypobiosis was observed in all the sampled animals.

# CHAPTER FOUR

## DISCUSSION AND CONCLUSION

### 4.1. Discussion

All the genera of worms that were identified in this particular study have been documented in Zambia (Tada *et al.,* 1995). The only genus that was not found in this study but has been reported in Zambia is *Trichuris.* This may be because it is not found in the particular area (Southern Province) or its prevalence is too low such that it was not detected in the present study. Additional worms that have been reported in Southern Africa but not found in the present study are *Ostertargia ostertagi* and *Toxocara* which have been reported in South Africa and Zimbabwe respectively (Pandey *et al.*, 1993; Swan *et al.,* 1985; Schroder, 1979).

The most predominant worms in cattle in Zambia are *Cooperia* and *Haemonchus* (Tada *et al.*, (1995) and similar observations have been made in the present study. It can also be concluded that most cattle in the traditional sector are infected with *Haemonchus* and *Cooperia* species throughout the year. The high levels of these two species may be because the two are more resistant to adverse climatic conditions (Schroder, 1979) and are more predominant in older animals than in the younger age groups (Fritsche *et al.,* 1993). Worm infestations in cattle are common as shown by the prevalence of at least one genus of gastrointestinal nematodes in all the animals examined. A few of these infestations were not detected by egg counts.

All the worms, except *Cooperia,* were found in organs of the gastrointestinal tract where they are expected to be found (compare Table 1 with Table 5). *Cooperia* is normally found in the small intestines (Urquhart *et al.,* 1992; Soulsby, 1986) but in the present study, 65 percent of the animals with *Cooperia* had some of the *Cooperia* in the abomasum. One possible explanation for this occurrence is that the worm could have migrated from the small intestines to the abomasum after the animals were slaughtered. This however is doubtful as the percentage of the occurrence is quite high and one could have expected the worm to have migrated to the large intestines as well. It may therefore be suggested that the abomasum is their normal habitat.

*Cooperia pectinata* and *C. punctata* are the more pathogenic of the *Cooperia* group in cattle as they are known to penetrate the epithelial surface of the small intestines and cause an atrophy of villi and reduction in nutrient absorption (Urquhart *et al.,* 1992; Soulsby, 1986). These worms were found in very high numbers in the animals and may be a cause of concern even in the old animals more so because these animals are usually undernourished.

The 22 percent prevalence rate of *Haemonchus,* though high, would be of little concern as the worm is rarely likely to cause problems in animals older than two years (all the animals sampled were at least more than two years of age) due to the development of immunity (Urquhart *et al* 1992). The same applies to the rest of the worms isolated. Despite the fact that they are likely to cause less harm to the old animals, these animals

continuously contaminate the pastures posing a danger to the young and more naïve animals.

The animals were found to have very high worm burdens and persistently infected during the whole study period. The observed high prevalence and intensity of worm infestations could be related to management conditions of communal grazing areas where most animals came from (Pandey *et al.,* 1993). The pastures in these areas are generally poor and hence the animals are exposed to very low nutritional pastures. Additionally in the traditional sector anthelmintic treatment is either rare or non existent (Kadohira *et al.*, 1996). If at all it is practised , it is usually given to a few sick animals only. It can hence be assumed that the pasture are constantly contaminated with worm eggs and infective larvae, and due to the poor nutrition, the worm burden is thus maintained at high levels even in adult animals.

The differential larval counts may not necessarily predict the worm burden in the animal but may estimate the proportions of the different worm species in the animal and predict which species are in higher numbers. This then gives the investigator a picture of the types of helminths that are in the host and which species are predominant and can thus help in deciding what sort of treatment regime is required if the need arises. The differential larval counts are supplementary to as faecal egg counts which are widely used but cannot differentiate the different species in the animal in a routine laboratory diagnosis (Hansen and Perry 1994). The value of differential larval counts in predicting the relative abundance of worm species in cattle is low because of the differences in the egg laying capacities of the various worm species (Bryan and Kerr, 1989).

Faecal egg counts are generally high in the warm wet season when conditions for infestation are also more favourable (Nalubamba, 1996; Fritsche *et al.,* 1993; Rahman, 1992; Schroder, 1979). In the present study it was observed that faecal egg counts correlated with the availability of moisture and changes in ambient temperature. They were generally high during the warm-wet months of February , March and April and dropped in the cold-dry months of May, June and July remaining low through the hot and dry months of August and September. These findings are consistent with the findings of Nalubamba (1996) in a study he carried out on goats in the traditional grasslands of Zambia. The same trend was also seen in a similar study done by Rahman (1992) in Malaysia.

The drop in faecal egg counts in the dry season is thought to coincide with the hypermetabolic state of most worms in the host. This explains the low levels of faecal egg counts during the dry season. An increase in the activity of these worms coincides with environmental conditions being conducive to their free living development (Urquhart *et al.,* 1992; Soulsby, 1986), and consequently, an increase in the faecal egg counts.

It was found in this study that statistically, a strong positive correlation ($r = 0.7564$, $p<0.05$) was observed between faecal egg counts and total worm burden. The study also reveals that there was a positive correlation between *Haemonchus, Cooperia, Oesophagostomum* and faecal egg counts. This was not so for *Trichostrongylus* and *Bunostomum* and this maybe because these worms were found in very small numbers compared to the rest. The use of faecal egg counts to estimate the total worm burden has

been questioned by many authors (Ndao *et al.,* 1995; Duwel, 1990; Barth *et al.,* 1981). The main reason advanced for this is that the worms differ in their egg laying capacities (Wamae and Ihigi, 1990) and also due to the external influences of the outside environment (Nalubamba, 1996; Urquhart *et al.,* 1992; Soulsby, 1986).

Previous studies have shown that faecal egg counts can be used as an estimator of total worm burden in first grazing season and in young animals (Aumont et al., 1991; Bisset et al., 1996; Ploeger et al., 1994). This means that as the animals grow older, the usefulness of faecal egg counts diminishes. However in the present study, older animals of at least two years old were used and a significant strong positive correlation ($r = 0.7564$, $p<0.05$) was observed between faecal egg counts and total worm burden. The reason for this trend can not be easily understood, but one reason may be because the data for all animals was pooled together before the analysis was done. Roberts and Swan (1981), caution against the use of faecal egg counts to predict individual animal's parasite burden, but state that the technique provides information with a satisfactory degree of precision, and aid rational decision-making in the diagnosis and control of worms in flocks and herds. Faecal egg counts could be used to estimate worm burden in large groups of animals. The *results in the present confirms the aforesaid.*

Since the majority of the animals had mixed infections of worms and since it is known that the different species of worms have different egg laying capacities, the correlation between the faecal egg counts and worm burden of individual species should be treated with caution. For example, *Haemonchus* may produce between 5000 - 15000 eggs per female daily whilst *Trichostrongylus* will only produce 100 - 200 eggs per female daily

(Hansen and Perry, 1994). The results will therefore have to be analysed with the results of a differential larval count to see which species of worms are likely to have contributed the majority of the eggs. In the present study, for example, most animals had a very high infection rate of *Cooperia* which explains the very strong positive correlation that was observed ($r = 0.7469$, $p<0.05$) between faecal egg counts and total *Cooperia* worm counts.

Hypobiosis was not observed in all the animals that were examined. Despite its absence in this study the phenomena has however been reported in goats in Zambia (Nalubamba, 1996) and hence the fact that it was not noticed in this study does not necessarily mean it is absent in Zambia. Hypobiosis is a worm's survival strategy in adverse conditions and is thus expected in Zambia in the cold and hot dry months when temperatures are either very low or very high and the moisture levels are low. One reason that hypobiosis was not observed may be that the conditions were not so adverse as to illicit a survival response (i.e. arrested growth). Gibbs (1982) suggests that temperatures should be higher than 30°C to adversely affect the survival of larvae and since the temperatures in southern Province during the study period did not even exceed 27°C the environment might not have been too adverse to illicit hypobiosis.

Therefore the lack of recovery of hypobiotic larvae should not suggest that arrested growth does not occur in Zambia as the study did not cover the whole year but only concentrated on a portion of the year (from February to September) which did not include the normally hotter months of October to December. A more thorough study that will run

for the whole year to include all seasons is required to check for the presence of arrested worms in traditional cattle.

The results presented in this study can at least give indications of what sort of helminth control programmes could be carried out in traditional animals of the Southern Province to reduce or eliminate helminth problems. A control programme should be based on the following:

a) Worm infestations in herds is prevalent at all times, and

b) Continuous contamination of the pasture.

It should also be borne in mind that adult worms do not have a long lifespan in the host and the worms' free living stages on pastures develop at different rates during the various seasons of the year (Van Wyk, 1990). Therefore the use of anthelmintics merely removes worms that could have died soon in any case and hence sometimes the effects of drenching is very little. Furthermore, unless the infested animals are removed from the pastures when they are dewormed, reinfection would take place rapidly making drenching an activity of limited value.

Drenching in highly infected animals may help to animal lives but even then for the infection to have reached very high levels means that the animal has already lost considerable productivity and the pastures have already been highly contaminated.

Based on the above it should be worthwhile to have the animals dewormed just after the rainy season to get rid of the infection acquired during the rainy season and also to help

the animals withstand the nutritional stress of the dry season. An early deworming just before the rains should then be carried out to prevent larval build-up in the pasture in the rainy season. The dewormings should be done in conjunction with the removal of the animals from the contaminated pasture to avoid reinfection. In a traditional set up it is advisable to include everyone in the locality at village or local community level so that all animals sharing communal pastures are drenched at the same time. The nutrition of the animals should also be improved so the animals can be able to withstand challenges of reinfection.

## *4.2. Conclusion*

The study has shown that gastrointestinal nematodes are common in traditional cattle and that mixed infections are also very common. Faecal egg counts were found to give a good estimation of total worm burdens in herds and can therefore be of value in determining prevalence; more especially in helminth control programmes that will involve herds of cattle. The potential of faecal egg counts is even greater in areas where communal grazing is practised.

It can also be concluded that temperature and rainfall are very important attributes to the development and establishment of gastrointestinal nematode infections. These two factors should always be borne in mind every time a gastrointestinal nematode infection control programme is to be implemented.

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# APPENDICES

## APPENDIX 1

**Helminths found in cattle** (Adopted from Soulsby 1986)

**Digestive Tract**

Trematodes
*Ogmocotyle indica*
*Paramphistoma cervi*
*P. Scotiae*
*P. hiberniae*
*P. ichikawi*
*P. gotoi*
*P. liorchis*
*P. microbothrioides*
*Caliocophoron calicophorum*
*C. raja*
*C. cauliorchis*
*Cotylophoron cotylophoron*
*Gastrothylax crumenifer*
*Fischoederius cobboldi*
*F. elongatus*
*Carmyerius spatiousus*
*C. gregarius*
*Ceylonocotyle streptocoelim*

Cestodes
*Moniezia expansa*
*M. benedeni*
*Avitellina spp*
*Thysanosoma actinioides*
*Thysaniezia giardi*

Nematodes
*Toxocara vitulorum*
*Gongylonema pulchrum*
*G verrucosum*
*Mecistocirrus digitatus*
*Haemonchus contortus*
*H. placei*
*H. similis*
*Trichostrongylus axei*
*T. colubriformis*
*T. longipicularis*
*Ostertagia ostertagi*
*O. trufurcata*
*O. lyrata*
*O. leptopicuris*
*O. bisonis*
*O. orloffi*
O. *podjapolskyi*
*Skrabinagia boevi*
*Cooperia puntata*
*C. pectinata*
*C. onchophora*
*C. surnabada*
*C. spatulata*
*Nematodirus spathiger*
*N. filicolis*
*N. hervetianus*
*Agriostomum vryburgi*
*Bunostomum phlebotomum*
*Strongyloides papillosus*
*Trichuris ovis*
*T. globulosa*
*T. discolor*
*Capillaria bovis*
*C. Longipes*
*C bilobata*
*Oesophagostomum radiatum*
*Charbertia ovina*

**Liver**

Trematodes
*Dicrocoerum dendriticum*
*D. hoepes*
*Eurytrema pancreaticum*
*E. coelomaticum*
*Fasiola hepatica*
*F. gigantica*
*Fascioloides magna*
*Gigantocotyle explanatum*

Cestodes
Hydatid cyst
*Taenia hydatigena (Cysticercus tenuicollis)*
*Stilesia hepatica*
*S. globipunctata*

*Thysanosoma actnioides*

**Circulatory system**

Trematodes
*Schistosoma japonicum*
*S. bovis*
*S. mattheei*
*S. spindale*
*S. mansoni*
*S. nasalis*
*S. margrebowiei*
*S. indicum*
*Ornithobilharzia turkestanicum*
*O. bomfordi*

Nematodes
*Elacophora poeli*
*Onchocerca armillata*

**Urogenital system**

Nematodes
*Stephanurus dentatus*
*Dioctopyma renale*

**Respiratory system**

Trematodes
*Fasciola hepatica*
*Schistosoma nasalis*

Cestodes
Hydatid cyst

Nematodes
*Dictyocaulus viviparus*
*Mammomonogamus larngeus*
*M. nasicola*

**Skin and subcutaneous tissue**

Nematodes
*Rhabditis bovis*
*Stephanofilaria dedoesi*
*S. stilesi*
*S. kaeli*
*S. assamensis*
*S. okinawaesis*
*Parafilaria bovicola*
*Onchocerca dukei*
*Dracunculus medinesis*

**Muscles and Tendons**

Cestodes
*Taenia saginata (Cysticercus bovis)*

Nematodes
*Orchocerca gibsoni*
*O. gutturosa*
*O. lienalis*

**Eye**

Nematodes
*Thelazia rhodesii*
*T. gulosa*
*T. alforrtensis*
*T. skrjabini*
*Setaria spp.* (immature)

**Central nervous system**

Cestodes
*Coenurus cerebralis*

**Serous cavities**

Nematodes
*Setaria labiato-papillosa*
*S. digitata*
*S. yehi*

## APPENDIX 2

**Livestock populations in Zambia according to provinces (1995)**

| PROVINCE | Cattle | Sheep | Goat | Pigs |
|---|---|---|---|---|
| Central | 269,307 | 1,355 | 48,798 | 16,426 |
| Copperbelt | 55,534 | 2,386 | 14,444 | 24,183 |
| Eastern | 240,926 | 18,672 | 167,967 | 151,152 |
| Luapula | 2,905 | 0 | 0 | 0 |
| Lusaka | * | * | * | * |
| Northern | 1,909 | 1,713 | 4,371 | 462 |
| N-western | 38,980 | 1,173 | 12,125 | 4,151 |
| Southern | 815,851 | 20,770 | 241,054 | 0 |
| Western | * | * | * | * |

* = Data not available

**Source**: Department of Animal Production and Health

## APPENDIX 3

## RESEARCH PROTOCOL

### *FAECAL EGG COUNTS*

*(Using the modified McMaster's technique)*

**Equipment**

- Plastic beakers
- Measuring Scale
- Sieve or Tea strainer
- Measuring cylinder
- Stirring device
- Pasteur pipette with a rubber teat
- Flotation Fluid (saturated salt solution)
- McMaster counting chamber
- Microscope

**Procedure**

1. Weigh 4g of faeces in a plastic beaker
2. Add 56 ml of saturated floatation solution
3. Mix thoroughly and pour through a tea strainer into a second beaker
4. Crush the large pellets on the tea strainer and pour the filtrate from the second beaker into the first beaker through the tea strainer.
5. Repeat step 4 until only undigested material remain on the tea strainer.
6. Stir the filtrate thoroughly and withdraw sufficient sub-sample with a Pasteur pipette and fill one side of the counting chamber.
7. Repeat step 6 to fill the other side of the counting chamber
8. Mount the counting chamber on the microscope and let it to stand for at least 5 minutes.
9. Examine at 10 X 10 magnification and count all nematode eggs within the engraved area of both chambers.
10. Eggs per gram = Total # in both chambers x 50

**NOTE:**

Volume under one side of chamber = 1 cm x 1 cm x 0.15 cm = 0.15 $cm^3$ = 0.15 ml.
4 grams yielded 60 ml
Then 1 gram is equivalent to 15 ml
Since two sides were examined, total volume examined is 0.15 ml x 2 = 0.3 ml
The factor therefore is 15 ml / 0.3 ml = 50
Therefore Eggs per gram = Total number in both squares x 50.

### *COPROCULTURES*

**Equipment**

- Plastic /glass jar
- Aluminium foil
- Spatula

- Vermiculite or charcoal
- incubator

**Procedure**

1. Place between 5 and 10 grams of faecal sample into the plastic jar
2. Break the faeces into fine particles with a spatula.
3. Mix with vermiculite or charcoal to obtain a moist consistency. If too dry add water and if too wet add Charcoal or vermiculite.
4. Close the lid loosely with aluminium foil
5. Place in the incubator at 27°C for 7 days
6. During the incubation period stir the culture each day to aerate the lower layers of the culture and to inhibit fungi growth. Add water when necessary to maintain moist consistency.
7. After the incubation period harvest the infective larvae using the Baermann apparatus method overnight.

***RECOVERY USING THE BEARMANN APPARATUS***

**Equipment**

- Funnel.
- Funnel stand
- Rubber tubing
- Clump
- Milk filter
- Strainer
- Test tubes
- Pasteur pipettes
- Microscope.

**Procedure**

1. Fit a short tubing which is closed at one end with a clump, to the stem of the funnel of appropriate size
2. Support the funnel with a stand
3. Fill the funnel with tap water to about 1 cm below the brim of the funnel taking extra caution not to trap any air bubbles.
4. Moisten the milk filter and place the cultured faecal sample on it and put it in a tea strainer.
5. Place the tea strainer into the funnel and allow to stand overnight.
6. Draw a few ml of fluid from the funnel stem into a test tube and place the tube in refrigerator at 4°C for at least 30 minutes.
7. Remove the supernatant with a Pasteur pipette to leave about a ml of fluid

8. Transfer a small aliquot of the remaining fluid using a Pasteur pipette to a microslide, add a few drops of iodine and cover with a cover slip.
9. Examine under 10 X 10 magnification for larval identification.
10. Continue to examine until at least 50 larvae have been examined.

## *POST-MORTEM DIFFERENTIAL WORM COUNTS*

**Equipment**

- A large bucket of about 10 to 20*l* (calibrated on the side in litres)
- Plastic bottles of about 500 ml
- 40% formalin.
- An enterotome

**Procedure**

***Processing the abomasum***

1. Open abomasum along the greater curvature.
2. Collect all the contents in the bucket.
3. Wash the empty abomasum thoroughly in the Bucket several times with tap water using a squeeze bottle or running tap water
4. Measure all the collected contents (For cattle it is advisable to make the total volume above 4*l*)
5. Mix thoroughly and collect 200 mls of the well mixed contents into the plastic bottle and add 20 mls of 40% Formalin for preservation and store for future worm counts.(collect paired samples)
6. Scrap off the mucosa of half the abomasum using a metallic scrapper and place the scrapings in a bottle for pepsin digestion. The bottle can be placed in a deep freezer till the digestion

***Processing the small intestines***

1. Free small intestines from the mesentery.
2. Place the intestines in the bucket and open the intestines along its entire length using an enterotomy scissors.
3. Rinse the intestine using a minimum amount of water and note the volume. After thorough mixing, collect 200 mls into specimen bottles and add 20 mls of 40% formalin.(collect paired samples)
4. Collect about 60% of the well mixed contents.

***Processing the large intestines and caecum.***

1. Open the organs lengthwise and then pour contents into a bucket.
2. Wash the mucosa with running tap water thoroughly to remove adherent worms
3. Collect 20% of the original volume and add to it 40% Formalin The formalin should be added in such amount as it is 10% of the sample.

***Procedure for carrying out differential worm counts for abomasum and small intestines.***

1. Pour contents into a conical flask and mix using a magnetic stirrer.
2. Using a pipette collect 11 ml of the well mixed contents and wash them in a endocot sieve and pour the washed contents in a counting chamber.

3. Examine using a stereo microscope and pick all the worms taking note of the number picked.
4. Identify all the picked worms to either genus or species level where possible.
5. Continue the washings until 2% of the original volume has been examined

***Procedure for worm counts of the large intestine.***

Wash all the collected contents using a # endcot sieve and count all the worm collected and identify them

***Procedure for isolating inhibited larvae from the abomasal mucosae by acid pepsin digestion.***

1. Thaw if the mucosa was kept in the freezer by leaving at room temperature for 2 to 3 hours.
2. Add 500 ml per 100g of mucosa acid pepsin (37°C) to each bottle
3. Mix thoroughly and incubate at 37°C for 10 hours
4. Remove all large undigested material with thumb forceps.
5. Sieve the digesta and make the volume of it to 2*l*.
6. Using a dissecting microscope, examine aliquots of 10 mls in a petri dish and count the larvae. Examine at least 5% of the 2*l* (100 mls)
7. To identify the parasite species, transfer sub sample by Pasteur pipette to a microslide and examine under a microscope.
8. Total # of larvae = # in 100 mls x 20.

***Procedure for preparing acid pepsin***

1. Add 40g pepsin to 4,850 mls distilled water.
2. Stir vigorously with a magnetic stirrer until all the pepsin has dissolved.
3. Add 115 mls of saturated sodium chloride solution.
4. Cautiously add 100 mls Fuming Hydrochloric acid to the solution.

NO HEATING REQUIRED.

## APPENDIX. 4

**Key to the infective larvae of some common nematodes of cattle** (After Soulsby 1965)

| KIND | TOTAL LENGTH | OESOPHAGUS LENGTH | ANUS - BODY END | BODYEND - SHEATH END | ANUS - SHEATH END |
|---|---|---|---|---|---|
| *Mecistocirrus digitatus* | 675 - 725 | 140 - 170 | 56 - 72 | - | 40 - 55 |
| *H. placei* | 750 - 850 | 135 - 164 | 42 - 60 | 90 - 110 | 160 - 190 |
| *H. contortus* | 650 - 751 | 122 - 150 | 54 - 68 | 65 - 68 | 119 - 146 |
| *O. ostertagi* | 825 - 925 | 140 - 182 | 55 - 75 | 55 - 70 | 130 - 160 |
| *T. axei* | 620 - 760 | 150 - 180 | 40 - 72 | 25 - 39 | 83 - 107 |
| *T. colubriformis* | 690 | 165 | 60 | - | 94 |
| *C. onchophora* | 850 - 950 | 150 - 180 | 63 - 80 | 80 - 100 | 150 - 180 |
| *C. punctata* | 670 - 870 | 142 - 170 | 52 - 75 | 47 - 71 | 109 - 142 |
| *C. pectinata* | same as C. punctata. Tail of sheath is longer. | | | | |
| *Nematodirus spp* | 920 - 1250 | 170 - 252 | 32 - 96 | - | 211 - 313 |
| *Bunostomum phlebotomum* | 525 - 575 | 130 - 152 | 42 - 63 | 60 - 80 | 140 - 150 |
| *Bunostomum trigonocephalum* | 570 | 160 | 60 | - | 140 |
| *Strongyloides papillosus* | 550 - 650 | 210 - 255 | 90 | sheath is absent | |
| *O. radiatum* | 750 - 850 | 138 - 160 | 55 - 75 | 140 - 170 | 220 - 250 |
| *O. columbianum* | 790 | 160 | 70 | - | 214 |
| *Chabertia ovina* | 730 | 165 | 64 | - | 165 |

## APPENDIX 5

**Key to the infective larvae of some common nematodes of cattle** (After Keith 1953)

| | |
|---|---|
| 1. Sheath absent, oesophagus more than 1/3 the length of the body. | *Strongyloides* |
| Sheath present, oesophagus short | 2 |
| 2. Length, including sheath, less than 600μ | *Bunostomum* |
| Length, including sheath, more than 600μ | 3 |
| 3. Tail of sheath less than 200μ | 4 |
| Tail of sheath more than 200μ | 5 |
| 4. Two conspicuous oval bodies at anterior end of oesophagus | 6 |
| No such structures at anterior end of oesophagus | 7 |
| 5. Length including sheath more than 1000μ; tail of sheath dorsal and ventral lobes with a rod-like process between | *Nematodirus* |
| Length including sheath less than 1000μ, tail of larva ending in a simple point | *Oesophagostomum radium* |
| 6. Length including sheath, usually more than 850μ; tail of sheath usually more than 150μ long, gradually to end bluntly | *Cooperia onchophora* |
| Length, including sheath, usually less than 850μ; tail of sheath tapering rapidly to a point or short fine filament less than 150μ long | *C. punctata*<br>*C. pectinata* |
| 7. Tail of sheath short and conical, less than 110μ long | *Trichostrongylus axei* |
| Tail of sheath at least 126μ long | 8 |
| 8. Tail of sheath ending bluntly | *Ostertagia ostertagi* |
| Tail of sheath ending in a fine whip like filament | *Haemonchus contortus* |

## APPENDIX 6

### The numbers of worms isolated per individual animal

| I.D. # | *Haem.* | Coop. | Oeso. | *Buno.* | *Trich.* | Immature | EPG | Total Adults | Source |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 950 | 1150 | 100 | | 50 | 100 | 100 | 2250 | Mazabuka |
| 2 | 2900 | 26600 | 350 | 600 | 100 | 450 | 2250 | 30550 | Mazabuka |
| 3 | 800 | | | | | | 50 | 800 | Mazabuka |
| 4 | 150 | | | | 50 | | 100 | 200 | Mazabuka |
| 5 | 300 | 200 | 100 | | | | 150 | 600 | Mazabuka |
| 6 | 50 | | 100 | | | | 50 | 150 | Mazabuka |
| 7 | 300 | | 20 | | 100 | 50 | 100 | 420 | Mazabuka |
| 8 | 950 | 750 | 65 | | | 1605 | 250 | 1765 | Gwembe |
| 9 | 100 | | | | | 100 | 50 | 100 | Gwembe |
| 10 | 450 | | | | | | 350 | 450 | Gwembe |
| 11 | 2450 | 4900 | 10 | | 50 | | 1000 | 7410 | Gwembe |
| 12 | 5550 | 7850 | 40 | | | 250 | 700 | 13440 | Siavonga |
| 13 | 1050 | 10450 | 95 | | | 200 | 650 | 11595 | Siavonga |
| 14 | 2650 | 6250 | 80 | | | | 450 | 8980 | Gwembe |
| 15 | 200 | | 30 | | | | 125 | 230 | Gwembe |
| 16 | 2350 | 3400 | 45 | | | 650 | 1000 | 5795 | Gwembe |
| 17 | 50 | 300 | 50 | 50 | | | 50 | 450 | Siavonga |
| 18 | 1550 | 4200 | 60 | | | | 950 | 5810 | Gwembe |
| 19 | 200 | 2050 | 15 | | | | 150 | 2265 | Gwembe |
| 20 | | 3250 | 30 | | | 100 | 200 | 3280 | Siavonga |
| 21 | 50 | 1300 | 5 | | | | 150 | 1355 | Gwembe |
| 22 | 200 | 4850 | 10 | | | | 250 | 5060 | Mazabuka |
| 23 | | 250 | 5 | | | | 50 | 255 | Siavonga |
| 24 | 8450 | 18600 | 560 | | | 715 | 2000 | 27610 | Gwembe |
| 25 | 300 | 300 | 45 | | | 50 | 100 | 645 | Gwembe |
| 26 | 13200 | 12350 | 200 | | | 820 | 2750 | 27550 | Mazabuka |
| 27 | 950 | 550 | | 50 | | 100 | 100 | 1550 | Gwembe |
| 28 | | 3650 | 25 | | | | 150 | 3675 | Mazabuka |
| 29 | 350 | 3350 | 20 | | | | 150 | 3720 | Gwembe |
| 30 | 150 | | | 50 | 50 | | 175 | 250 | Siavonga |
| 31 | | 2850 | | | | 150 | 200 | 2850 | Gwembe |
| 32 | 300 | 1800 | 145 | | | 150 | 100 | 2245 | Gwembe |
| 33 | 100 | 500 | 5 | 50 | | | 100 | 655 | Gwembe |
| 34 | 4100 | 1350 | 50 | 100 | | | 200 | 5600 | Gwembe |
| 35 | | 1050 | 40 | | | | 150 | 1090 | Gwembe |

*continued on next page.*

| I.D. # | *Haem.* | Coop. | Oeso. | *Buno.* | *Trich.* | Immature | EPG | Total Adults | Source |
|---|---|---|---|---|---|---|---|---|---|
| 36 | | 4200 | 150 | 50 | | | 400 | 4400 | Mazabuka |
| 37 | 350 | 8050 | 170 | | | | 500 | 8570 | Mazabuka |
| 38 | 50 | 600 | 100 | | 50 | | 150 | 800 | Gwembe |
| 39 | 400 | 1450 | 135 | | | | 200 | 1985 | Gwembe |
| 40 | 5100 | 2800 | | | | | 450 | 7900 | Siavonga |
| 41 | 400 | 1450 | 135 | | | | 200 | 1985 | Mazabuka |
| 42 | | 2950 | 50 | | | | 50 | 3000 | Gwembe |
| 43 | 200 | 6500 | 5 | | | | 50 | 6705 | Mazabuka |
| 44 | 4100 | 2000 | 50 | | | | 250 | 6150 | Siavonga |
| 45 | | 3950 | 150 | 50 | | | 500 | 4150 | Gwembe |
| 46 | 400 | 1250 | | | | | 150 | 1650 | Mazabuka |
| 47 | 100 | 7000 | 20 | | | | 100 | 7120 | Gwembe |
| 48 | 400 | 2600 | 135 | | | | 50 | 3135 | Siavonga |
| 49 | 400 | 7500 | 100 | | | | 450 | 8000 | Gwembe |
| 50 | 50 | 300 | 100 | | 50 | | 50 | 500 | Siavonga |
| 51 | | 150 | | | 50 | | 150 | 200 | Choma |
| 52 | | 950 | 5 | 50 | | 100 | 50 | 1005 | Gwembe |
| 53 | * | * | * | * | * | * | * | * | |
| 54 | 400 | 1250 | | | | | 250 | 1650 | Choma |
| 55 | 200 | 3750 | 50 | | | | 100 | 4000 | Mazabuka |
| 56 | | 950 | 5 | | | | 50 | 955 | Mazabuka |
| 57 | 150 | 11200 | 160 | | | | 2600 | 11510 | Choma |
| 58 | 50 | 900 | | | | | 50 | 950 | Gwembe |
| 59 | 100 | 2050 | 40 | | | | 100 | 2190 | Gwembe |
| 60 | | 200 | 50 | | | | 150 | 250 | Mazabuka |
| 61 | 300 | 2000 | 10 | | | | 200 | 2310 | Gwembe |
| 62 | 150 | 1750 | 50 | | 50 | | 250 | 2000 | Mazabuka |
| 63 | | 850 | | | | | 50 | 850 | Siavonga |
| 64 | | 100 | | | | | | 100 | Gwembe |
| 65 | 200 | 5200 | 100 | | | | 2600 | 5500 | Mazabuka |
| 66 | | 900 | 20 | | | | 50 | 920 | Gwembe |
| 67 | 200 | 1500 | | | | | 100 | 1700 | Siavonga |
| 68 | 50 | 1050 | 5 | | | | 150 | 1105 | Gwembe |
| 69 | | | 50 | | | | | 50 | Siavonga |
| 70 | 100 | 1100 | | 50 | | | 100 | 1150 | Choma |

* = Destroyed sample