EPIDEMIOLOGY AND CONTROL OF WORM INFECTIONS IN CATTLE ON TRADITIONAL, SMALL-SCALE AND LARGE-SCALE DAIRY FARMS IN IRINGA DISTRICT, TANZANIA

BY

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ABSTRACT

This study aimed to determine worm control practices, anthelmintic usage and the epidemiology of gastrointestinal (GI) nematodes and flukes (*Fasciola* and amphistomes) in cattle on traditional, large-scale and small-scale dairy farms in Iringa District, Tanzania. A combination of qualitative data collection methods was used to obtain information on worm control practices and anthelmintic usage on the cattle farms. Strategic treatments, efficacy trials, an abattoir survey based on examination of GI tracts, cross sectional and longitudinal studies based on monthly coprological examination of cohorts for nematode and fluke eggs, worm counts in tracer calves and pasture larval counts were used to obtain quantitative data on helminth infections. Results indicated that worm control was based entirely on routine use of anthelmintics in 87.7%, 100% and 97.8% of traditional, large-scale and small scale-dairy farms respectively. Dairy farmers (55.5%) treated at least four times a year while traditional farmers (45.8%) treated twice a year. The treatment period on most farms depended on availability of money and not on the epidemiology of parasites. Absence of policy on worm control, lack of quality assurance of anthelmintics, inadequate extension workers and low education among farmers contributed significantly to irrational worm control and anthelmintic usage. The type of management especially the grazing practice had significant influence on the prevalence and intensity of GI nematodes and flukes. The prevalence of GI nematodes in traditional, large-scale and small-scale dairy cattle was 67%, 44.4% and 37% respectively. Worm burdens in tracers were mainly composed of *Cooperia* spp (51.6%), *Oesophagostomum radiatum* (35.7%) and *Haemonchus placei* (10.2%). Faecal egg counts (FEC) and tracer worm counts were generally low and peaked only in calves and weaners. Pasture larval counts, FEC and tracer worm counts peaked toward the end of the rainy season (May). The overall prevalence of *Fasciola gigantica* in traditional, large-scale and small-scale dairy cattle was 63.8%, 46.2% and 28.4% respectively. The prevalence of amphistomes was 81.9%, 55.5% and 41.1% in traditional, large-scale and small-scale dairy cattle respectively. Adult animals had the highest prevalence of both *Fasciola* and amphistomes. The prevalence of flukes was high in all age groups on traditional cattle, only in adults and yearlings in large-scale dairy cattle and low in all age groups in small-scale dairy cattle. The proportion of animals excreting fluke eggs in faeces was highest at the end of the dry season (November) and the early part of the rainy season. Triclabendazole (Fasinex®), nitroxynil (Trodax®) and ivermectin-clorsulon (Ivomec-Super®) were highly effective against *Fasciola*; a reduced efficacy of levamisole-oxyclozanide formulations was suspected. Strategic treatment with triclabendazole at the end of the rainy/early dry season significantly reduced the proportion of animals excreting *Fasciola* eggs while albendazole treatment maintained low nematode FEC until the next rainy season. It was concluded that helminths infection in the district has a seasonal pattern and farmers could save substantial amounts of money through strategic treatment programmes. Strategic treatments at the end of the rainy/early dry season (May/June) and at the end of the dry/early rainy season (November/December) are recommended for sustainable and cost-effective helminth control in the district. An additional treatment against GI nematodes in the middle of the rainy season (February/March) is recommended especially in calves and weaners. Treatment against GI nematodes and flukes in all age groups in small-scale dairy cattle might not be necessary if animals will be given pasture from clean areas.

DECLARATION

I, **JULIUS DOTTO KEYYU**, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work and has not been submitted for a degree award in any other University.

Signature: ....

Date: ......22.07.2004......

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ABBREVIATIONS

DNA	- Deoxyribonucleic acid
EPG	- eggs per gram of faeces
FEC	- Faecal egg counts
HPI	- Heifer Project International
IPM	- Integrated parasite management
kg	- Kilogram
L_1, L_2...L_5	- First, second ...fifth stage larvae
log	- logarithm
LSDF	- Large scale dairy farms
m	- metres
PCR	- Polymerase Chain Reaction
PCV	- Packed cell volume
PGE	- Parasitic gastroenteritis
RFLP	- Restriction fragment length polymorphism
S.e.	- Standard error of the mean
SHDDP	- Southern Highland Dairy Development Project
spp	- species
SSDF	- Small-scale dairy farms
SUA	- Sokoine University of Agriculture
TF	-Traditional farms
VIC	-Veterinary Investigation Centre

CHAPTER 1

INTRODUCTION

Helminth infections are an important cause of disease and loss of productivity in livestock worldwide and their control is *an absolute necessity* (Vercruysse and Claerebout, 2001). The greatest losses associated with parasites are those resulting from sub-clinical infections (Dimander *et al.*, 2000). Economic assessments have repeatedly demonstrated that the losses can be enormous (Morris and Meek, 1980; Morris and Marsh, 1994). Helminths cause significant economic losses due to reduced live weight, reduced fertility, reduced milk production, reduced feed intake and its utilisation, increased mortality and control costs in infected animals (Fox *et al.*, 1989; Mostofa *et al.*, 1997). Parasites may indirectly affect pregnancy, perhaps by their influence on appetite and forage utilisation (Stromberg *et al.*, 1997). Indeed, helminthosis is arguably the most important disease complex of livestock in economic terms on global scale (Keyyu *et al.*, 1998; Perry *et al.*, 2002); and on global basis, worm infection especially gastrointestinal (GI) parasitism emerges with the highest global index as an important animal health constraint to the poor (Perry *et al.*, 2002). Due to losses and costs caused by helminths, there is a great need for control measures. Even using the most conservative estimates, returns from control exceed costs (Vassilev and Jooste, 1991).

Iringa District, in the Southern highlands of Tanzania, is among areas with a high population of indigenous cattle and a steady increase in the dairy industry. The district is known to be a trematode endemic area (Mahlau, 1970; Makundi *et al.*, 1998) due to favourable climate and abundant snail habitats. Slaughter house and cross sectional farm surveys have shown that *Fasciola gigantica*, *Dicrocoelium hospes* and *Schistosoma bovis* are very common in cattle (Mahlau, 1970; Kassuku *et al.*, 1986; Makundi *et al.*, 1998). Moreover, post-weaning stress and helminth infections have been reported to be major causes of mortality in calves on dairy farms (Kifaro and Temba, 1990). Under natural field conditions, concurrent infections with both trematodes and gastrointestinal (GI) nematodes are very common in animals. Little is known on the prevalence and epidemiological patterns of GI nematodes in Iringa and the Southern highlands of Tanzania in general.

The prevalence and intensity of helminth infections in livestock generally vary from one locality to another depending on climate especially rainfall, season, management system and livestock density (Hansen and Perry, 1994). Therefore, for rational and sustainable control of helminth parasites in grazing animals, a comprehensive knowledge of the epidemiology of parasites as it interacts with the host in a specific climate and management system is a pre-requisite (Barger, 1999). Sustainable parasite control programmes require knowledge of seasonal larval availability, origin of larvae contributing to any peak and climatic requirements for worm egg hatching, larval development, survival and translocation (Barger, 1999).

Control of helminths has been shown to be a promising and profitable way to improve livestock productivity (Itty *et al.*, 1997). For many decades, the control of helminths has relied entirely on the use of anthelmintics. In the past, anthelmintics have been used in treating only animals suffering from clinical parasitism. However, the knowledge that sub-clinical parasitism adversely affects productivity has resulted in an increased use of anthelmintics by farmers. Helminths control has dramatically changed to recent programmes whereby animals with sub-clinical parasitism are given routine anthelmintic treatments (Stromberg *et al.*, 1997). Currently, a huge amount of money and effort is being spent annually for deworming livestock and pet animals all over the world; the greater part of these expenses is wasted, largely due to inappropriate usage, even in regions with high standards of management and husbandry mainly due to inappropriate drug choice, timing and unnecessary dosing (Kassai, 1999). Routine, frequent and indiscriminate use of anthelmintics is currently getting unpopular because of the development of resistant strains of helminths to commonly used anthelmintics. Recommendations have therefore been made for rational use of anthelmintics in various countries based on local conditions (Maingi *et al.*, 1996).

In order to improve health and productivity of cattle among management systems in Iringa District, farmers have adapted a variety of helminths control measures including anthelmintic usage. However, the basis of those treatments and their efficiency is doubtful because little data exist on helminth infections in the district. Anthelmintic treatments not based on the epidemiology of parasites can be ineffective, costly, wasteful and can lead to development of anthelmintic resistance (Waller *et al.*, 1995). Moreover, little is known on farmers' knowledge on helminth infections and there is no documentation on farmers' helminth control practices and anthelmintic usage among cattle management systems in Tanzania. Generally, information about anthelmintics used by farmers, the period of the year they are given and the frequency of treatment are important factors to consider in evaluating the efficiency of the control measures used at the farm (Charles and Furlong, 1996).

Due to lack of data on the prevalence and epidemiological pattern of GI nematodes and trematodes among management systems and agro-ecological zones, helminth control practices and anthelmintic usage in the district might be erratic and probably a waste of anthelmintics due to improper timing of treatments. Therefore, the general objective of this study was to determine the prevalence, epidemiology and control practices of helminth infections among cattle management systems in Iringa district. The specific objectives were to explore farmers' knowledge and attitude on helminth infections and to describe worm control practices and anthelmintic usage; to determine the prevalence, intensity and epidemiology of helminth infections; to asses the efficacy of drugs commonly used against flukes (*Fasciola* and amphistomes) in cattle and lastly to evaluate the effect of a single treatment against flukes and GI nematodes at the end of the rainy/early dry season.

Results from this study aimed to generate information on farmers' knowledge on helminths infection, helminth control practices and anthelmintic usage. The study also aimed to obtain data on the prevalence, distribution and epidemiology of helminths infection among management systems in the district in order to formulate a model for rational, cost effective and sustainable helminth control through strategic treatment programmes.

CHAPTER 2

LITERATURE REVIEW

2.1 Gastrointestinal (GI) nematodes of cattle

2.1.1 Species spectrum

The common gastrointestinal nematodes of cattle in tropical countries can be divided into nematodes of the abomasum, small intestines and large intestines. Nematodes found in the abomasum of cattle are *Haemonchus* spp, *Trichostrongylus axei* and *Ostertagia* spp (Soulsby, 1982; Kassai, 1999). Nematodes found in the small intestines are *Cooperia* spp, *Bunostomum* spp, *Trichostrongylus colubriformis*, *Nematodirus* spp and *Strongyloides papillosus* (Reinecke, 1989; Kassai, 1999). Nematodes found in the large intestines are *Oesophagostomum* spp and *Trichuris* spp (Reinecke, 1989). *Toxocara vitulorum* is also found in cattle mainly in young animals (Soulsby, 1982).

Under grazing management systems, animals usually harbour more than one nematode species in the gastrointestinal (GI) tract, a phenomenon called polyparasitism or multiparasitism.

2.1.2 Life cycle

Most GI nematodes of cattle have a direct life cycle. The life cycle is mainly divided into parasitic phase and a pre-parasitic (free-living) phase. The adult nematodes, fourth and fifth stage larvae in the definitive host constitute the parasitic phase and they are found in different sections of the GI tract. The pre-parasitic stages are found freely in the environment on pasture. Adult male and female nematodes in the respective region of the GI tract mate and thereafter females lay eggs that are passed out in faeces. Some female nematodes produce embryonated eggs (e.g. *Strongyloides* spp) while most others lay unembryonated eggs. Under favourable conditions of moisture and temperature, eggs embyonate and hatch into first stage larvae (L_1). The L_1 feed on microbes and then moult into second stage larvae (L_2), shedding the protective cuticle in the process (Hansen and Perry, 1994). The L_2 then moult into third stage larvae (L_3) but the cuticle is not casted. The double cuticle L_3 is the infective stage of GI nematodes on pasture. When conditions of moisture, oxygen and temperature (22-26°C) are favourable, the infective larva is produced in four to six days (Soulsby, 1982).

Infective larvae are transmitted from the cowpat to surrounding pasture by rainfall splash and water currents (Williams and Bilkovick, 1973; Gronvold, 1989); also by vertical and horizontal larvae migration (Silangwa and Todd, 1964) and to a lesser extent by arthropods, invertebrates and birds (Gronvold, 1989). Animals are infected by ingestion of L_3 with contaminated pasture. The acquisition of L_3 by grazing animals depends on the distribution of larvae on pasture and the animals grazing behaviour such that grazers acquire more infective larvae than browsers (Nansen *et al.*, 1988; Boag and Thomas, 1989). Ingested L_3 pass down to the abomasum or intestines and exsheath the cuticle through the host stimuli and enzymes (Soulsby, 1982). The larvae then migrate to respective regions of the GI tract; moult into fourth stage larvae (L_4) and then to pre-adult. The L_3-L_4 of *Haemonchus* and *Ostertagia* migrate into the gastric glands while those of *Trichostrongylus*, *Cooperia* and *Nematodirus* enter the Lieberkühn-crypts of the small intestinal mucosa. Later, the L_4 return to the lumen to become immature adults and then adults. Mature male and female nematodes pair and females start laying eggs. The prepatent period is 2-3 weeks, but under certain circumstances, development in the mucosa is arrested at the early L_4 stage, and the hypobiotic larvae resume maturation 4-6 months later (Kassai, 1999).

2.1.3 Distribution and epidemiology

Gastrointestinal nematode infections in cattle have a worldwide distribution and nematodes are considered to be the most common worms of grazing ruminants (Kassai, 1999). Environmental conditions generally have a significant influence on the global distribution of helminths. The epidemiology of helminths is affected by a number of factors especially rainfall, temperature, management or production system and stocking density (Stear *et al.*, 1997; Barger, 1999). Other factors that may affect the epidemiology and intensity of GI nematodes are sex, breed, age, nutrition and physiological status of the host (Stear *et al.*, 1997). Climatic conditions especially rainfall and temperature have a profound effect on the prevalence and intensity of helminth infections as they determine development and survival of pre-parasitic stages. In tropical and sub-tropical countries, temperature is almost favourable throughout the year for free living stages to hatch and develop (Chiejina and Fakae, 1984; Waller, 1997). Rainfall is the main limiting factor for development and survival in tropical countries and there is a direct correlation between severity of helminth infections and rainfall (Waller, 1997). In the tropics, parasitic gastroenteritis (PGE) is mainly a 'wet season disease' and outbreaks of nematodosis are common during long rains. The dry season is usually unfavourable for development and survival of pre-parasitic stages (Chiejina and Fakae, 1984) and the number of infective larvae on pastures decreases dramatically during the dry season and arrested larvae accumulate in the host (Ogunsussi and Eysker, 1979). However, peak infections at the end of the rainy season may move towards the beginning of the dry season and that infections at the end of the dry season may move into the beginning of the rainy season.

Age or lack of previous exposure of the host has a profound effect on the prevalence and intensity of helminth infections (Gallie, 1973; Coop and Holmes, 1996). First year grazing calves are more susceptible to the establishment of pathogenic worm burdens and that PGE is rarely seen in adults. Yearlings and adult cattle may act as worm carriers and source of spring pasture contamination in temperate climatic conditions (Kassai, 1999). Management systems and farm practices also have a significant influence on the epidemiology of helminths. High stocking rates and mixing of animals (treated and non treated herds) in communal grazing areas and congregation of animals around watering points usually lead to heavy outbreaks of PGE. Stable infections are scarce and insufficient nutrition and other parasitic infections may promote outbreaks of PGE (Kassai, 1999).

2.1.4 Pathophysiology and clinical signs

Gastrointestinal nematodes vary greatly in their pathophysiology mainly depending on the nematodes' pathogenic index and location in the host. The course of infection depends on the number of worms, generic composition of the burden, previous exposure and sex, age and nutritional status of the host (Kassai, 1999).

Pathogenicity is attributable to both developing larvae and adult GI nematodes. The immature stages (L_3-L_4) of GI nematodes in the abomasum or small intestines cause damage to the glands and mucous membranes and the essential pathology is hyperplastic gastritis and catarrhal enteritis (Hansen and Perry, 1994; Kassai, 1999). Blood and proteins leak into the lumen of the abomasum and small intestines. The overall effect is diversion of blood proteins and energy from vital productive processes such as meat and milk production into repair of the damaged gut, synthesis of plasma proteins, mucoproteins and maintenance of circulating blood proteins (Coop and Holmes, 1996).

The effects of adult GI nematodes may be indirect (sub-clinical) or direct (clinical). The indirect effects include reduced feed intake, reduced feed utilization, reduced water, nitrogen and mineral intake and retention (Sykes, 1982; Holmes, 1987; Coop and Holmes, 1996; Knox and Steel, 1997). Gastrointestinal nematodes also cause reduced appetite through reduction in the rate of passage of digesta and reduced abomasal motility (Fox *et al.*, 1989; Mostofa *et al.*, 1997). Parasites may cause reduced pregnancy rates (Stromberg *et al.*, 1997; Stevenson *et al.*, 2002; Maingi *et al.*, 2002) perhaps by their influence on appetite and forage utilisation (Stromberg *et al.*, 1997). The direct effects of parasitism are morbidity and mortality especially in young animals, reduced carcass quality and wool quality. Parasites especially blood sucking parasites (*Haemonchus* spp, *Bunostomum* spp) may also cause mild to severe anaemia. Clinical disease occurs mainly in young animals and rarely in adults. The common clinical signs include diarrhoea, unthriftness, inappetence, rough hair coat, anaemia, oedema, weakness and progressive weight loss.

2.1.5 Immunity to gastrointestinal nematodes

Immunity to GI nematodes and helminths in general is due to an acquired immune response, meaning that resistance is acquired after primary exposure to infection. Animals that have prior exposure to GI nematodes are more resistant to infection than those without previous exposure (Gallie, 1973). Animals kept under worm free conditions or very low exposure to infection might fail to develop acquired resistance. Acquisition of resistance to challenge infections is affected by the size of the primary infection and age of the host. Resistance to challenge infection is usually enhanced with low initial infection, heavy initial primary infection decreases resistance to subsequent infections (Doenhoff *et al.*, 1978). Continued daily initial exposure at low doses (trickle infections) elicits marked immunity than a single primary infection and results in rejection of most worms and partial protection to challenge infections (Kassai, 1999). Older cattle have strong immunity capable of limiting the size of worm burdens to sub-pathological level.

Immunity to helminths is also affected by sex, nutrition and physiological status of the host. Pregnant animals tend to loose the acquired immunity during parturition and lactation period (Barger, 1989; Keyyu *et al.*, 2001). Moreover, immunity to helminths is more enhanced in animals on a good plane of nutrition. Resistance to subsequent infections is also influenced by age of the host and that adults are more resistant than young animals (Gallie, 1973).

The effects of host immune responses against worms are reduction of invasive potential, retarded growth and development, reduced fecundity and expulsion of adult worms (Urquhart *et al.*, 1962; Dobson, 1972). Immune competent animals can therefore prevent establishment of worms as well as get rid of established worms, hypersensitivity to larval intake may occur in resistant hosts (Urquhart *et al.*, 1962; Gallie, 1973). Immunity to helminths is both cell and antibody mediated; factors involved in worm expulsion are sub-epithelia mast cells, intraepithelial globule leucocytes, eosinophils and antibodies (Dobson, 1972).

2.2 Bovine fasciolosis (hepatic distomatosis/liverfluke disease)

2.2.1 Aetiology

The main species that cause bovine fasciolosis are *Fasciola gigantica* and *Fasciola hepatica*. *Fasciola gigantica* is prevalent in all tropical and sub-tropical regions and covers the whole of Africa, Middle East, southern states of USA and Central, East and South East Asia. *Fasciola hepatica* is prevalent in temperate regions and in high altitude areas in tropical and sub-tropical countries (Kassai, 1999). In Tanzania, fasciolosis is mainly due to *F. gigantica*; however, in a study by Makundi *et al.* (2004) at Kitulo farm in southern Tanzania, results obtained strongly suggest that *Fasciola hepatica* may be present on the farm.

2.2.2 Life cycle

Fasciola species have an indirect life cycle that involves an intermediate invertebrate snail host. Adult flukes inhabit the bile ducts in the liver of the definitive host. The adult fluke in the bile duct releases eggs that are passed out in faeces to the environment. Under suitable temperature for development (26°C) the eggs hatch into miracidia in about 17 days for *F. gigantica* and 10-12 days for *F. hepatica* (Soulsby, 1982; Kassai, 1999). The miracidia then seek and penetrate the intermediate host snail and loose the ciliated coat in the process. The intermediate host snails are amphibious fresh water snails of the genus *Lymnaea. Lymnaea natalensis* is the common intermediate host for *F. gigantica* in Africa. Each miracidium develops into a sporocyst in the snail, and each sporocyst develops into 5-8 first generation rediae (mother rediae). Under unfavourable conditions, the first-generation rediae develop into second-generation/daughter rediae (Soulsby, 1982). The daughter rediae then develop into a number of cercariae through asexual reproduction (up to few hundreds). Infected snails may start to release cercariae about 36 days post penetration. Temperature has a profound effect on the development of larval stages in the snail and may take two to several months.

Cercariae released from the snails swim in water until they are in contact with grass or other objects where they are attached, cast off the tail and encyst to become metacercariae. The metacercaria is the infective stage and there is little information on survival on natural vegetation but can survive up to 6 months in stored rice straw (Chiejina, 1994), and have been shown to survive for one year under laboratory conditions (Soulsby, 1982). Experiments by Kendall (1965) showed that herbage remained infected by metacercaria for between 270 and 340 days. Metacercaria may survive on moist hay for eight months and survival below freezing temperatures has been reported (Soulsby, 1982). Failure of metacercaria to survive in silage for 35 days or 37 days has been demonstrated in the Gulf Coast regions of the United States (Soulsby, 1982).

Animals are infected by ingestion of grass or hay containing encysted metacercariae. In the abomasum and duodenum, excystment occurs and young flukes penetrate the abomasal and intestinal wall, migrate through the peritoneum and peritoneal cavity and penetrate the liver capsule by day 4-6 post-excystment (Soulsby, 1982; Kassai, 1999). Immature flukes then migrate through the liver parenchyma for 4-7 weeks before they enter the bile ducts and become adults (Kassai, 1999). The prepatent period is 12 weeks for *F. gigantica* and 6-12 weeks for *F. hepatica*. Adult flukes can survive in the bile ducts of the definitive host for several years (11-20 years).

2.2.3 Epidemiology and distribution

Fasciolosis has worldwide distribution in grazing animals and is endemic in many areas in tropical and subtropical regions (Sousby, 1982). The distribution of *F. gigantica* in East and Central Africa depends exclusively on availability of *L. natalensis* that is the primary intermediate host (Mzembe and Chaudhry, 1979). *Fasciola gigantica* is highly prevalent in tropical Africa. Coprological examinations and abattoir surveys have indicated prevalences between 44.3-98.4% in cattle in Zambia (Silangwa, 1973), 7-50% in cattle in Mali (Tembely *et al.*, 1988), 47.6-54.5% in traditional Zebu cattle in Tanzania (Hammond, 1965; Mahlau, 1970; Ecimovic and Mahlau, 1973), 53.7% in cattle in Uganda (Ogambo-Ongoma, 1972), 16.3-48% in cattle in Kenya (Bitakamire, 1973), 26.3-46.3% in cattle in Zimbabwe (Vassilev and Jooste, 1991) and 60% in sheep in Ethiopia (Heinonen *et al.*, 1995). Presence of suitable intermediate host snail species, infected animals and adequate suitable habitats for the intermediate host snails has a significant influence on the epidemiology and distribution of fasciolosis.

The transmission biology of *Fasciola* has been well reviewed by Chiejina (1994). In tropical regions, snails are abundant during the rainy season but the proportion of snails with cercariae increases at the end of the rainy season and into the dry season (Kassuku *et al.*, 1986; Makundi, 2001). The rainy season favours breeding and spreading of snails over a vast area. During the dry season, snails are found concentrated in water pockets e.g. marshy areas, lagoons, pools and flood plains and there is an enormous number of metacercariae on the surrounding herbage (Silangwa, 1973, 1974). High intake of metacercariae occurs when animals graze on wet areas during drought (Kassai, 1999). Studies have shown that there is a seasonal variation in the prevalence of fasciolosis, and that prevalence is highest at the end of the dry season and the early part of the rainy season (Mahlau, 1970; Asanji, 1989). The prevalence and intensity of infection with *Fasciola* is also affected by the age of the animal. The longevity of infection in definitive hosts, chronic nature of egg production, ability of the snails to aestivate and maintain infectivity and the longevity of metacercariae play important roles in the epidemiology of fasciolosis. Under natural tropical conditions, both intermediate hosts for *Fasciola* and amphistomes are available and concurrent infections of *Fasciola* and amphistomes are common with a positive correlation (Szmidt-Adjidé *et al.*, 2000).

2.2.4 Pathophysiology and clinical signs

Adverse effects of flukes can be grouped into mechanical, toxic and loss of blood (Kassai, 1999). Mechanical effects are due to destruction of the parenchyma and blood vessels of the liver by immature flukes. Toxic effects are due to secretory and excretory products of the flukes. Loss of blood is due to haemorrhages in the liver and the blood sucking habit of flukes (Kassai, 1999). Overall, inflammation of the liver parenchyma (hepatitis) and the bile ducts (cholangitis) are characteristic features of fasciolosis. The severity of lesions usually depends on the host species, number of metacercariae ingested and the duration of the parasites in the host. Fasciolosis can be grouped into three clinical forms namely acute, sub-acute and chronic fasciolosis.

2.2.4.1 Acute fasciolosis

Outbreaks of acute fasciolosis occur when seasonal and climatic conditions result in a massive ingestion of metacercariae by animals over a short period (FAO, 1994). Acute fasciolosis is a result of acute, traumatic, haemorrhagic hepatitis due to extensive damage of the liver parenchyma and capsule by migrating immature flukes. In severe cases especially in sheep, death may occur without obvious signs, later clinical signs include abdominal pain, jaundice and death. The duration of the disease varies between 2-5 weeks. Due to destruction of hepatocytes, the plasma levels of mitochondrial enzymes namely glutamate dehydrogenase (GLDH), aspartate aminotransferase (AST) and sorbital dehydrogenase (SDH) increase (Kassai, 1999). The liver capsule may rapture resulting in sudden death. Post-mortem and pathological findings include blood-tinged fluid in the abdominal cavity, enlarged, haemorrhagic and friable liver, fibrinous peritonitis and migratory tracts in the liver. Immature flukes emerge upon squeezing a cut section of the liver and the size of flukes vary depending on the age of infection.

2.2.4.2 Sub-acute fasciolosis

This form is common in animals that survive the acute form, treated animals or those exposed to massive, longer period intake of metacercariae. The major clinical signs are loss of weight, loss of appetite, anaemia and oedema while the main pathological lesions are traumatic hemorrhagic hepatitis, peritonitis, enlarged liver, cholangitis, liver fibrosis and jaundice. The duration of the disease varies from 4-8 weeks. Post-mortem and pathological findings include mild biliary fibrosis, uneven/thickened liver capsule and migratory tracts and young flukes in bile ducts.

2.2.4.3 Chronic fasciolosis

Chronic fasciolosis is the most common form in ruminants and results from moderate, prolonged intake of metacercariae and the presence of adult flukes in the bile ducts (FAO, 1994; Kassai, 1999). Major clinical signs include anaemia, chronic wasting, reduced appetite, rough hair coat, unthriftness and oedema under the jaw (bottle jaw). Chronic cholangitis, hepatic fibrosis and loss of protein lead to appearance of gamma glutamyl transferase or transpeptidase (GGT) in plasma (Kassai, 1999). Post-mortem and pathological features include remarkable biliary cirrhosis and distorted and reduced liver lobes. On incision, there is extensive liver fibrosis, thickened intrahepatic bile ducts and mature flukes in the bile ducts. Clinical disease due to large numbers of flukes is mainly seen in young animals (FAO, 1994).

2.2.5 Immunity to *Fasciola*

There is a remarkable variation in the ability of mammalian hosts to acquire resistance against challenge infections following primary sensitisation with *F. hepatica* and *F. gigantica* (Haroun and Hillyer, 1986). There is no solid evidence of acquired resistance in sheep and cattle, but infected cattle can partially develop resistance to subsequent infections following primary low dose or continuous doses (Ross, 1967; Chiejina, 1994; Kassai, 1999). The resistance is manifested by decrease in the size and number of flukes recovered from challenge (Haroun and Hillyer, 1986). The resistance acquired by primary infection may persist for a long period after removal of the sensitising infection by anthelmintic treatment (Kendall *et al.*, 1978). Cross-resistance exists between *Fasciola* and *Schistosoma* species in many hosts (Haroun and Hillyer, 1986) and heterologous resistance has been demonstrated in sheep (Monrad *et al.*, 1981), cattle (Sirag *et al.*, 1981; Yagi *et al.*, 1986). Unlike cattle, sheep are unable to resist secondary and subsequent infections after primary infection or prior sensitisation (Boray, 1967; Haroun and Hillyer, 1986) and acute fasciolosis occurs at any age (FAO, 1994). Hepatic fibrosis due to primary infection is probably an important contributory factor to the partial resistance in cattle, the relatively large amount of fibrous tissue hinder migration of young flukes to the bile ducts and eliminates the majority of super infections (Ross and Armour, 1960; Boray, 1967; FAO, 1994; Kassai, 1999).

The mechanism of resistance has not been clearly elucidated though there is evidence that resistance is immunologically mediated through humoral and/or cellular reactions (Haroun and Hillyer, 1986). Immunity to *Fasciola* is expressed both during the immature migrating parenchymal stage and adult bile duct stage of infection. Natural or innate immunity plays a major role in resistance towards the bile duct stage in cattle and can result in elimination of adult flukes about ten months after infection. Immature flukes provoke immunological responses with peak antibody titres six weeks after infection. There is a remarkable decline in antibody titres when flukes enter the bile ducts (Hanna and Jura, 1977). Antibodies (IgA, IgG-1, IgG-2) have little protective role in the host especially during primary infections and flukes do survive peak antibody titres in the parenchyma and for extended periods for adults in bile ducts. Immunity to fasciolosis is also influenced by age, nutrition status of the host and concurrent infections. Calves are usually more susceptible than adult cattle (Howell and Boray, 1994). Immunity to *Fasciola* is complicated by a number of mechanisms used by flukes to evade the host immune mechanism such as absorption of host antigens and shedding of heptolaminate surface (Smithers *et al.*, 1969). Moreover, adult flukes are shielded from immune attack when they enter the bile ducts (Lang, 1967). Attempts to actively stimulate or passively transfer resistance to *Fasciola* through sensitisation by primary homologous or heterologous normal or irradiated infections *per os*, subcutaneous, intramuscular or intraperitoneal implantation with the various fluke stages, or sensitisation by somatic extracts or metabolic products of mature or immature flukes and passive transfer of resistance by immune serum or sensitised lymphocytes have been reviewed by Haroun and Hillyer (1986) and no strong protective immunity has been demonstrated against *Fasciola*.

2.3 Bovine amphistomosis

2.3.1 Aetiology and distribution

Bovine amphistomosis is caused by many species of trematodes in the family Paramphistomidae and other related families. The maggot-shaped flukes are commonly known as amphistomes, stomach/rumen flukes or conical flukes. Adult flukes are found in the rumen and reticulum of ruminants while immature flukes are found in the mucosa of the duodenum and abomasum. A number of genera have been recorded but *Paramphistomum*, *Calicophoron* and *Cotylophoron* are the most common in Africa (Dinnik, 1964). *Calicophoron microbothrium* (Synonym: *Paramphistomum microbothrium*) is the most common specie in Africa responsible for outbreaks of acute amphistomosis in sheep and cattle (Dinnik, 1964; Asanji, 1989).

The recorded species in Tanzania are *Calicophoron microbothrium* and *Cotylophoron jacksoni* in the southern highlands and *P. sukumum*, *P. phillerouxi*, *Calicophoron raja* and *Cotylophoron cotylophorum* in Sukumaland in the lower warmer areas around lake Victoria (Dinnik, 1964). The geographical distribution of amphistomes especially *C. microbothrium* and their intermediate hosts have been reviewed by Dinnik (1964) and Over (1982). The distribution generally overlaps that of the intermediate host snail. Amphistomes have a worldwide distribution and are highly prevalent in tropical and subtropical regions. Amphistomosis is primarily a disease of cattle, sheep, goats and buffaloes and is common in the whole of Africa, East and southern Europe, the Indian subcontinent, South and East Asia, Russia, Indonesia and some of the Mediterranean countries (Horak, 1971; FAO, 1994).

2.3.2 Life cycle and epidemiology of amphistomes

The life cycle of amphistomes is similar to that of liver flukes. The exception is that the intermediate host snails are *Bulinus* spp, *Biomphalaria* spp or *Lymnaea* spp, and young flukes that emerge from the ingested metacercariae invade the mucosa of the duodenum and begin to grow (FAO, 1994). Each amphistome specie requires a specific intermediate host snail; *Bulinus tropicus* is the commonest intermediate host in Africa. From the duodenum, immature flukes later migrate to the rumen and develop into egg laying adult flukes. The prepatent period in cattle is about 3-4 months.

The epidemiology of amphistomosis is similar to that of fasciolosis and depends on the presence of suitable intermediate host snails, high rainfall and temperatures ranging between 10-30°C (FAO, 1994). Factors that influence the epidemiology of amphistomosis include the type of management and grazing habits of cattle (Horak, 1971), the biological potential of the intermediate host snail (Dinnik, 1964; Horak, 1971) and the potential of the fluke stages to infect intermediate and definitive host (Dinnik, 1964; Horak, 1971).

In Africa, many cattle and sheep are infected with amphistomes (Horak, 1971). Some of the factors that maintain amphistomosis are the ability of amphistomes to survive in hosts for some years (Dinnik, 1964), intermediate host snails are prolific breeders and mature rapidly, live for many years in definitive hosts and released metacercariae can live longer at lower temperatures. Moreover, the intermediate host snail (*B. tropicus*) is highly adaptable and may be found in streams, pools, water troughs, dams, marshes, irrigation canals and fountains at any altitude up to 6800 ft (Dinnik, 1964). A number of studies have shown that the prevalence and burdens have a seasonal pattern and are influenced by the number of infected intermediate host snails

(Rolfe *et al.*, 1991). Peak burdens have been recorded to be highest during the dry season and the early part of the rainy season in Sierra Leone (Asanji, 1989), or during prolonged inundation of grazing areas and after inundated areas have dried out in Australia (Rolfe *et al.*, 1991).

High prevalence of amphistomes has been recorded in slaughtered beef cattle (80%) in Kenyan highlands (Dinnik, 1964) and 81.9% in cattle in Sierra Leone (Asanji, 1989). Co-infection with amphistomes and nematodes, cestodes and *Fasciola* has been found to be common in grazing animals (Szmidt-Adjidé *et al.*, 2000). Some years back, and to a large extent in the present production systems in the tropics, amphistomes are usually neglected and considered to be of no effect at all in animals. However, a study conducted by Al-khshali and Altaif (1979) has indicated that amphistomes are a limitation to livestock productivity in the tropics and account for high economic losses especially in weaned animals. Indeed, the economic significance of amphistomosis is grossly under-estimated (FAO, 1994).

2.3.3 Pathophysiology and clinical signs

Clinically, amphistomosis can be divided into acute (intestinal) and chronic (ruminal) forms. The chronic form is due to the presence of adult flukes in the rumen and is the most common form of infection. Adult flukes in the rumen are well tolerated and rarely cause clinical disease apart from indigestion. The acute form is due to infection with heavy migrating immature flukes and causes dramatic outbreaks of acute gastroenteritis or enteritis only with mortality in young animals (FAO, 1994). Moderate infection with immature flukes cause indigestion and retarded growth after weaning. Sub-clinical intestinal amphistomosis in weaners is probably far more important than occasional outbreaks (FAO, 1994).

Outbreaks of intestinal amphistomosis have been recorded in sheep in South Africa with 30-50% mortality (Le Roux, 1930; cited by Dinnik, 1964) and mortality of 4.6% in cows and 96% in calves in the southern highlands of Tanzania (Butler and Yeoman, 1962; cited by Dinnik, 1964). Clinically infected animals had profuse fluid, projectile and foetid-greenish diarrhoea without blood or mucous, were anorexic, thirsty, anaemic, emaciated and the majority died (Dinnik, 1964). The mechanical mucosal damage by immature flukes causes enteritis, erosions, necrosis, bleeding and terminal loss of appetite and death (Horak, 1971). On post-mortem, lesions are mainly in the upper duodenum and the mucosa is thickened, hardened, corrugated and inflamed (verrucose mucosal appearance) and numerous immature flukes can be recovered (Dinnik, 1964; Horak, 1971).

2.3.4 Immunity to amphistomes

Field studies have shown that previous infections particularly in adult cattle provide a degree of resistance enabling animals to withstand subsequent infections and/or eliminate established flukes (Horak, 1971). However, immunity is partial and adult flukes continue to produce eggs (Kassai, 1999). Multiple infections in sheep induce a partial immunity to reinfection (Horak, 1971). The age of an animal also has a significant effect on the outcome of infection, adult animals usually evacuate large amount of amphistome eggs with no clinical signs of infection or disease.

2.4 Diagnosis of helminth infections

Helminth infections can tentatively be diagnosed through clinical signs and history. The common clinical signs like diarrhoea, loss of condition, anorexia, oedema, anaemia, rough hair coat and unthriftness are suggestive of helminth infection in a live animal. The history of the presence of helminth infections in the area, the grazing practice (e.g. in flood plains, swampy areas) and information on whether animals have been dewormed recently or not may assist in approaching a definitive diagnosis. Diagnostic methods for helminth infections can be grouped broadly into parasitological, haematological/serological and molecular biology methods.

2.4.1 Parasitological diagnostic methods

The common parasitological methods used for diagnosis of GI helminth infections are based on direct examination of helminth eggs in faeces. For GI nematodes, the modified McMaster method (MAFF, 1986) is the commonest method used. For *Fasciola* and amphistomes, demonstration of fluke eggs in faeces by the sedimentation technique is the commonest method. Usually, the number of helminth eggs per gram of faeces (EPG) is used as an indirect measure of the worm burden in animals. However, worm eggs in faeces are not uniformly distributed and the number of eggs in faeces is affected by a number of factors e.g. consistency of faeces, duration and the degree of infection, fecundity and age of worms and the host resistance (Hansen and Perry, 1994). Therefore, the correlation between the number of eggs in faeces and worm burden is strong only in sheep, goats and young animals and weak in cattle and adult animals (McKenna, 1981).

Post-mortem worm counts (necropsy) offer direct and clear information on the kind and level of helminth infections as well as the pathological consequences (Kassai, 1999) and allows processing of abomasal and intestinal mucosa for recovery of immature stages of GI nematodes. Necropsy also offers an opportunity for the liver, bile ducts and gall bladder to be opened and checked for flukes or fluke eggs and examination of immature flukes in liver sections, and their subsequent quantification.

2.4.2 Haematological and serological diagnostic methods

The common haematological methods used in assessing sub-clinical effects of blood sucking parasites are packed cell volume (PCV) and haemoglobin (Hb). Packed cell volume and Hb are usually lowered during infections with blood sucking parasites. The use of FAMACHA® chart in the assessment of anaemia caused by blood sucking parasites is becoming popular in southern African countries. The method is based on assessing the pinkish colouration of the mucous membrane of the eye especially in small ruminants. Plasma or serum enzymes and proteins are also useful in the diagnosis of sub-clinical infections, or in the assessment sub-clinical levels of helminth infections, this is due to the fact that the damage of the intestine and liver cells caused by worms results in leakage of proteins and enzymes. Therefore, plasma or serum proteins (albumin, globulin) increase or decrease during infection with GI nematodes; however, it is important to rule out other causes of increased serum or plasma proteins. Total protein and albumin usually decrease due to leakage of plasma into the gut lumen (Ross and Armour, 1960). The inability of coprological methods to detect prepatent helminthoses and their lack of sensitivity with regard to low-level infections has made a need for serological diagnostic techniques (Fagbemi and Guobadia, 1995; Anderson *et al.*, 1999) in order to enable early diagnosis of helminth infections. Plasma or serum enzymes increase or decrease during parasitic infections due to hepatopathies and gastroenteropathies (Simesen and Nansen, 1974). Increased activity is due to damaged cell or decreased clearance while decreased activity may be due to tissue destruction or tissue atrophy (Boyd, 1988). Serum gamma glutamyl transpeptidase (GGT) and aspartate amino-transferase (AST) are useful enzymes in assessing liver damage caused by fasciolosis. Measurement of serum pepsinogen has been recommended as a specific tool in the diagnosis of ostertagiosis (Berghen *et al.*, 1993) and a number of assays are available. Also, serum gastrin levels provide good estimation of adult *Ostertagia* burdens (Hilderson *et al.*, 1989; Berghen *et al.*, 1993).

Measurement of parasite specific serum antibody levels has also been used in the diagnosis of helminth infections. Several immunological assays have been developed for detection of *Fasciola* antigens (e.g. El-Bahi *et al.*, 1992; Dumenigo *et al.*, 1996; Abdel Rahman *et al.*, 1998) or for detection of *Fasciola*-specific antibodies (e.g. Chauvin *et al.*, 1997; Cornellisen *et al.*, 1999) in serum, usually performed by Enzyme Linked Immuno-Sorbent Assay (ELISA); and have been shown to be highly sensitive and specific (Ibarra *et al.*, 1998; Anderson *et al.*, 1999). A monoclonal antibody for direct detection of *Fasciola* antigens in serum or faeces has been developed (Anderson *et al.*, 1999). The main drawbacks of some serological tests include being labour intensive and expensive and having little standardisation and low specificity (Berghen *et al.*, 1993; Scott *et al.*, 1995; Vercruysse and Claerebout, 2001), making comparison from different laboratories difficult. The use of recombinant antigens will probably reduce most of these problems (Vercruysse and Claerebout, 2001).

2.4.3 Molecular biological diagnostic methods

Molecular biology has had a major impact in many areas of parasitology including the identification and systematics of parasites, diagnosis of infections, epidemiology of parasites and in the study of drug resistance and vaccine development (Gasser, 1999). Molecular biological diagnostic methods are highly sensitive and specific nucleic acid based assays that rely on detection and demonstration of the parasite nucleic acid sequence (Kaufmann, 1996; Kassai, 1999). Molecular diagnostic methods can be grouped into Deoxyribonucleic acid (DNA) based and non-DNA based approaches. DNA based approaches include DNA hybridisation, Restriction Fragment Length Polymorphism (RFLP) and PCR based approaches (Kassai, 1999). Non-DNA based approaches make use of other materials in the diagnosis of infection (e.g. use of hormones).

The advent of PCR has revolutionised parasitological research and has found broad applicability mainly because its sensitivity permits the amplification of gene or gene fragments from minute amounts of parasite material (Gasser, 1999). Generally, conventional microscopic parasitological methods for detection of helminth eggs and larvae will continue to be the 'gold standard' for the diagnosis of parasitic diseases. If an unequivocal identification of the parasite cannot be made, the specimens can then be analysed using molecular techniques such as PCR. The PCR amplified fragments can be analysed by RFLP or DNA sequencing if further characterisation is needed. However, molecular methods may have important applications in future anthelmintic control programmes (Kassai, 1999) and in disease eradication programmes.

2.5 Control of helminth infections

The objective of helminth control is to ensure that parasite burdens in animals do not exceed levels that will compromise health and/or production because complete eradication of helminths is not feasible (Okon, 1988; Vercruysse and Claerebout, 2001). Effective helminth control is a major component in ensuring the sustainability of animal production (Waller, 1997). Helminth control measures can be broadly divided into management strategies, use of anthelmintics (chemotherapy) and alternative (novel) control measures. However, the best control option requires integration of the three strategies, termed as integrated parasite management (IPM).

2.5.1 Control by management

The strategy involves the use of a number of simple management practices based on the local epidemiological patterns of parasites and the type of animal management system in a given climatic region. Pastures provide a link between the infective stages of parasites and the parasitic phase of helminths in animals. Therefore, competent pasture management can aid in effective helminth control, and is based on provision of clean pastures on which stock may safely graze (Barger, 1999). The overall aim of grazing management is to reduce pasture contamination and is often referred to as integrated or clean grazing. Options for control under this strategy can be sub-divided into rotational grazing, mixed grazing and alternation of host species, pasture spelling/resting, improved nutrition, pasture sterilisation, adjustment of grazing density and avoidance of valleys and flood plains.

2.5.1.1 Rotational grazing, pasture spelling and sterilisation

Rotational grazing in its original form comprises the withdrawal of susceptible hosts from the pasture until the free-living stages of parasites are not available before animals are replaced (Kassai, 1999). The strategy involves subdivision of a pastureland to small paddocks so that animals graze each constituent paddock for a short period in rotation (Barger, 1999). Basically, rotational grazing utilises the survival time of infective larvae on pasture and aims at breaking the life cycle of parasites. The shorter survival time (3-13 weeks) of infective larvae in the tropics than in temperate regions (Banks *et al.*, 1990; Waller, 1997) makes the option more feasible in tropical regions. Sub-division of pasture land into small plots where sheep grazed in each plot for 4 days before movement to a new plot, returning to the first plot after 30 days indicated a reduction in the number of treatments and that anthelmintics can even be eliminated (Barger *et al.*, 1994). However, there are relatively few such schemes in tropical and sub-tropical regions probably due to the unavailability of grazing land and other resources and the communal grazing practice of traditional herds. Also, the scheme is not practiced because grasses grow too high in the resting period, fencing cost might be too high compared to the benefits and lastly because animals benefit more from being able to select their feed on a large non-divided paddock than when being forced to eat all feed on small plots.

Pasture sterilisation involves rendering contaminated pastures sterile in a short time by physical or chemical means e.g. ploughing, resowing or burning. Pasture spelling involves withdrawing animals from a given area for a considerable long time such that animals will be re-introduced when infective helminth stages have died off. Reduction of stocking density also aids in helminth control through prevention of heavy pasture contamination and overgrazing that forces animals to graze near faecal pats. Regulation of stocking density is the single most important factor influencing levels of herbage infectivity (Kassai, 1999).

2.5.1.2 Alternate and mixed grazing

The strategy involves grazing animals of the same or different species. When animals of the same species are used in this grazing regime, young animals are introduced into pasture to graze first; later adults are introduced in the same paddock when young ones are moved to the next clean paddock. Alternation of animals of different species is employed when animals do not share common parasite species. Alternate grazing of small ruminants and cattle, small ruminants and horses or horses and cattle would appear to be the best candidates as long as *Trichostrongylus axei* is not of major concern (Barger, 1999). Usually, animals that are not definitive hosts (dead end hosts) are introduced first followed by introduction of definitive hosts in the same paddock when dead end hosts are be effective in reducing the extent of peri-parturient rise (PPR) in FEC and post-partum FEC in sheep (Kahn *et al.*, 2003). The control of helminth infections through high protein supplementation might be very expensive for most smallholder farmers and its application is limited especially in developing countries. However, there are options for using low cost nitrogen and energy supplements that are locally available e.g. Urea Molasses Blocks (UMB), bioactive forages and browse plants in tropical areas can be used as a supplement to low quality forage. Protein supplementation is one of the promising and commercially feasible alternative control measures and can make a major contribution to integrated parasite management (IPM) programmes (Kahn *et al.*, 2003).

2.5.1.4 Other herd management control strategies

A number of other management practices might aid in the control of helminth infections e.g. tethering, zero grazing (stall feeding) and avoidance of fluke high transmission areas. Avoidance of grazing animals in flood plains and swampy areas is one of the best control options for flukes. Animals in total confinement are not exposed to trichostrongylid infection (Kassai, 1999). However, this is only true if animals will be provided safe pastures, otherwise there is a great potential for infecting zero grazed animals through pastures obtained from contaminated areas.

2.5.2 Control by use of anthelmintics

Anthelmintic usage is the commonest and most popular worm control method worldwide mainly due to its direct and powerful efficacy, easy and wide scale application and availability of several drugs and formulations. However, anthelmintic usage is associated with high cost, drug resistance and is labour intensive. Currently, consumers are exerting pressure on minimal use of chemicals in animal production (Keyyu *et al.*, 1998; Kassai, 1999). Generally, anthelmintic treatments should have a clearly defined purpose and should be associated with positive cost-benefit ratios. Moreover, for anthelmintic treatments to be effective, the right drug should be used in the right category of animals at the right time using the right dosage.

Anthelmintic treatments are indicated either for therapeutic (curative) or prophylactic (preventive) purposes. Therapeutic treatments aim to save life or cure a sick animal while prophylactic treatments aim to prevent pasture contamination with worm eggs, thereby limiting infection to an acceptable level (Kassai, 1999). Prophylactic (preventive) treatments may be divided into three classes namely strategic, tactical and continuous treatments (Blood and Radostits, 1989).

2.5.2.1 Strategic treatments

These are treatments carried out at pre-determined intervals (same time each year) based on the local epidemiological patterns of parasites and the type of management (Blood and Radostits, 1989; Kassai, 1999). Strategic treatments may also include treatments carried at the same stage in the management programme e.g. treatments at mating, lambing/calving, weaning, drying etc. The overall objective of strategic treatments is to reduce pasture contamination and infection of animals. There are very little precise strategic treatment programmes in developing countries due to lack of data on the pattern of parasites in various countries and management systems. Moreover, in extreme situations of subsistence farming in developing countries, technological advancements or recommendations are either unaffordable or inappropriate mainly due to high price of reputable anthelmintics or inferior quality of local drugs (Waller, 2002).

2.5.2.2 Tactical treatments

Tactical treatments are given on *ad hoc* basis, mainly during periods when rainfall is above average and temperatures favourable for transmission of helminths (Blood and Radostits, 1989). Most often, tactical treatments support strategic treatments for those periods when the set treatments do not seem to be sufficient for the un-expected worm challenge (Kassai, 1999). Occasionally, tactical treatments are given when nutrition is unusually poor such as during the spells or draught or when animals from a worm free environment are moved to a helminth endemic (danger) area (Blood and Radostits, 1989).

2.5.2.3 Continuous (intermittent) treatments

Continuous treatments are appropriate in helminth endemic areas where animals are always at a high risk of infection or re-infection. The strategy involves anthelmintic dosing at low levels calculated to inhibit egg production. The commonest formulations are supplementary feed blocks and intraruminal boluses. The common practice is the use of sustained release devices that are administered intraruminaly to create a depot of anthelmintic from which it is released slowly. The net effect is reduction in establishment of worms and reduction in pasture contamination. The formulations persist for a long period in the body of animals and kill for extended periods, and have been found to be a good option compared to frequent dosing (Blood and Radostits, 1989).

2.5.2.4 Thresholds for treatment against non-treatment

The overall objective of defining a treatment vs. non-treatment threshold is to promote 'better use of anthelmintics' or 'smart use of anthelmintics'. Treatment vs. non-treatment thresholds can also be regarded as parasite levels for decision 'to deworm or not deworm' (Corwin and Stromberg, 1995). At the moment, large amount of anthelmintics are used indiscriminately because the parasite levels are too low to justify (therapeutic) treatment or because the (preventive) treatments are not correctly programmed, resulting into underprotection or overprotection (Vercruysse and Claerebout, 2001). Some possible thresholds for anthelmintic treatments for GI nematodes, lungworms and liver fluke have been well discussed in temperate countries and have been categorised into therapeutic, production-based and preventive thresholds (Vercruysse and Claerebout, 2001). In temperate countries, a therapeutic threshold in calves has been suggested to be based on clinical signs of PGE, and occasionally FEC ≥200 EPG and mean pepsinogen levels ≥5 units tyrosine; production-based threshold has been suggested to be mean pepsinogen levels ≥3-3.5 units tyrosine while the preventive threshold for first year grazing and second year grazing calves has been suggested to be mean FEC ≥200 EPG at turnout and mean pepsinogen levels ≥3.5 units tyrosine at housing (Vercruysse and Claerebout, 2001).

For bovine fasciolosis, a therapeutic threshold appears to be of little relevance because relatively high burdens are rarely seen under natural conditions, and the chronic form is the most common. Defining economic thresholds for fasciolosis has been difficult due to lack of adequate studies. However, in temperate countries, FEC ≥5 EPG, individual gamma glutamyl transferase (GGT) levels above 150 units/l, a herd prevalence of ≥25% or worm burden of 30 flukes per animal can be considered to be an acceptable threshold estimate (Vercruysse and Claerebout, 2001). The preventive threshold for fasciolosis is mainly based on local climatic forecast systems. Climatic forecast systems for predicting acute fasciolosis and appropriate control have been developed and validated for use in Europe, Australia and United States of America (USA). In developing countries, there has been limited work on accurate assessment of economic losses of parasitic diseases in various climatic and management systems. Therefore, it is difficult to have realistic thresholds and there is a great need to have realistic thresholds for treatment vs. non-treatment based on local management systems and parasite species in order to promote appropriate use of anthelmintics.

2.5.3 Alternative (novel) control measures

Problems of resistance to anthelmintics, high cost of anthelmintics, an increasing pressure from consumers on presence of minimal or no chemicals in animal products and some environmental consequences of anthelmintics have generated serious concerns on alternative (novel) control measures. Alternative control measures can be divided into biological, breeding for worm resistant hosts and vaccination.

2.5.3.1 Biological control

Biological control is one of the alternative methods for control of parasitic nematodes that has achieved a steadily increasing interest within the past 5-10 years (Larsen, 2002). The strategy is based on the use of living organisms that can utilise nematodes as source of food. The nematode trapping/destroying fungi have proved most promising as potential biological control agents (Kassai, 1999). The fungi belong to a taxonomically diverse group with both endoparasitic and predacious nematode-trapping fungi (Larsen, 2002). Among the predacious fungi, *Duddingtonia flagrans* has displayed superior ability with regard to GI tract survival as well as subsequent destruction of developing parasitic larvae (Gronvold *et al.*, 1993; Waller *et al.*, 1994). The fungi act on free-living stages of nematodes, i.e. L_1, L_2 and L_3 on pasture that represent the greatest proportion of the parasites biomass. The fungi produce trapping organs such as constricting (active) or non-constricting (passive) rings, sticky hyphae, sticky knobs, sticky branches or sticky networks along the vegetative hyphal system (Gronvold *et al.*, 1993).

Fungal material can be incorporated into fresh dung, added to feed supplement, intraruminal sustained device or applied as a drench. Laboratory, plot and field trials with spores of *D. flagrans* have shown a good reduction of free-living larval stages of parasitic nematodes of cattle (Fernandez *et al.*, 1999; Faedo *et al.*, 2002), sheep (Pena *et al.*, 2002), pigs (Nansen *et al.*, 1996; Petkevicius *et al.*, 1998) and horses (Baudena *et al.*, 2000). *Duddingtonia flagrans* has also been shown to be highly effective in reducing larval development of *Teladosargia circumcincta* in goat faeces (Paraud and Chartier, 2003). Biological control using predacious fungi has not yet reached practical commercial application in the field. Some of the challenges to be met before commercial exploitation are on means of delivery of fungal spores, environmental impact of spores and the cost-effective means of production and deployment of fungal materials (Kassai, 1999). Preliminary results are promising and the fungi offer some prospects in future control or in integrated parasite control programmes.

2.5.3.2 Breeding for worm resistant animals (Genetic selection)

Genetic control is based on selection of animals that appear to have the ability to resist or tolerate worm infection. This may be the ability of the host to suppress established and/or subsequent infections (resistance) or the ability of the host to thrive well in spite of the established worm infections, termed as resilience (Kassai, 1999). Usually animals of superior genetic trait (high responder) are selected and utilised in selective breeding programmes. The aim is genetic manipulation of host resistance through selective breeding. Removing low responders (susceptible) from the breeding programme improves the general level of worm resistance of the host population. Differences in the ability of livestock to resist parasitic infections are well documented (Owen and Axford, 1991), variations have been observed both between and within breeds and strains of sheep, goats and cattle (Gray, 1991; Baker *et al.*, 1994; Eady *et al.*, 1996; Keyyu *et al.*, 1998). Variation in resistance is due to differences in immune responsiveness of the host to infection and partly due to genetic factors (Stear and Murray, 1994).

Resistance to helminths is genetically determined and is heritable in sheep, goats and cattle. Resistant animals carry few worms and require few anthelmintic treatments for adequate worm control (Barger, 1989). The risk of worms to be resistant to anthelmintics is reduced if the timing and frequency of treatment are properly managed. Worms in resistant animals are less fecund and contamination of pastures with worm eggs is greatly reduced. Most studies on variation in resistance to helminths have been conducted in sheep, limited data is available for goats and cattle (Keyyu *et al.*, 1998; Kassai, 1999). Moreover, most studies have concentrated on GI nematodes with few studies on trematode and cestode infections. Breeding for animals with high resistance to GI nematodes has reached some level of success (Larsen, 2002). Commercial breeding programmes for sheep are now operating in Australia and New Zealand and some experimental flocks exist in Hungary and Poland. Though genetic selection is far from implementation in many countries since it is time consuming, costly and the fact that such improvement sometimes has some trade off with respect to productivity (Woolaston and Baker, 1996; Gray, 1997; Kassai, 1999); it is still one of the promising and feasible alternative control method and may make useful contributions to IPM programmes (Kahn *et al.*, 2003).

2.5.3.3 Vaccination

Immunisation of hosts with antigens derived from appropriate helminth stages might be one of the best ways of controlling helminth infections in livestock. Three strategies for vaccine production against helminths have been tried, i.e. immunization with dead parasite antigens (dead vaccine), immunization with live attenuated parasites and immunization with excretory-secretory parasite antigens (Nansen, 1985). The most successful of these strategies has been the live attenuated vaccines that involve exposing parasite stages to X-ray, gamma rays or ultraviolet (UV) radiation. Since successful production of the first irradiated larvae vaccine against *Dictyocaulus viviparus* in cattle in the 1960s (Jarrett *et al.*, 1960; Urquhart, 1985) and the canine hookworm (*Ancylostoma caninum*), there was great optimism that similar procedures could be used for other helminths. This has not been the case (Gray, 1995). No other vaccines followed and the same attenuation procedures have failed to produce commercial helminth vaccines (Waller, 1997; Kassai, 1999).

Efforts to produce vaccines against helminths is complicated by the poor immune responsiveness of animals against helminths. Immunity elicited by helminths occurs later and is more labile compared to those induced by microbial infections, hence the production of powerful and cost-effective helminth vaccines is a difficult task (Kassai, 1999). Moreover, parasites have evolved several mechanisms to escape (evade) the hosts' immune response which include rapid shedding of surface molecules after antibody binding, mimicry and absorption of host antigens, secretory of toxic immunomodulatory molecules and the active movement to different tissue sites (Soulsby, 1982; Weir, 1988; Tizard, 1994; Meeusen, 1999). The lumen and mucosal surface of the GI tract seem to be good places for nematodes to escape the full force of immune response than other organs (Gray, 1995).

Most of the conventional vaccines produced have been crude homogenates and extracts, and therefore not worthwhile in terms of immunogenicity, safety and effectiveness in inducing protection (Waller, 1997). Vaccines based on naturally exposed or hidden antigens have been thoroughly investigated (Smith, 1999; Dalton and Mulcahy, 2001) over the last 10-15 years, but no commercial product has been released on the market based upon these results (Larsen, 2002). The contribution of molecular biology to the development of vaccines against helminths has been well reviewed by Knox *et al.* (2001). Molecular biology has provided means to identify antigens and define their functions and pattern of expression and means to produce parasite antigens in substantial quantity. Moreover, molecular biology has provided new means for antigen delivery (e.g. DNA vaccination) and antigen targeting (e.g. microarray and proteomic programmes) which offer the prospect of large scale, rapid antigen screening and identification (Knox *et al.*, 2001). Recent developments in immunology and molecular biology are showing promises, but production of the long awaited commercial vaccines for practical use in the field is 'some years' away (Gray, 1995; Waller, 1997).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study area

3.1.1 Location and climatic zones

The study was conducted in Iringa District, Iringa region, in the southern highlands of Tanzania. Currently the district has been divided into Iringa and Kilolo districts. The district lies between latitudes 6°-10°S and longitudes 33°-37°E and has an area of about 28 000 km^2 (50% of Iringa region). The district has three agro-climatic zones namely the highland zone (temperate plateau), mid zone (central plain) and the lowland zone (lower plain). The highland zone is at an altitude of between 1600-2700 m above sea level and the mean annual rainfall is between 1000-1600 mm. The mid zone lies at an altitude of between 1200-1600 m above sea level and the mean annual rainfall is 600-1000 mm while the lowland zone lies at an altitude of between 900-1200 m above sea level and the mean annual rainfall is below 600 mm.

Vegetation cover in the highland zone is comprised of rainfall forests while savannah grasslands predominate in the mid zone. The lowland zone is arid and semi-arid in nature and is mainly comprised of thickets and scattered bushes. The district has one rainy season that is from December to May; the other months form the dry season. Temperature varies greatly, among the three zones, between 6-35°C. There is a typical cool climate especially in the highland zone from June to August. This study was conducted in four livestock markets in the lowland zone, three villages in the highland zone and two villages in the central zone (Fig 1).

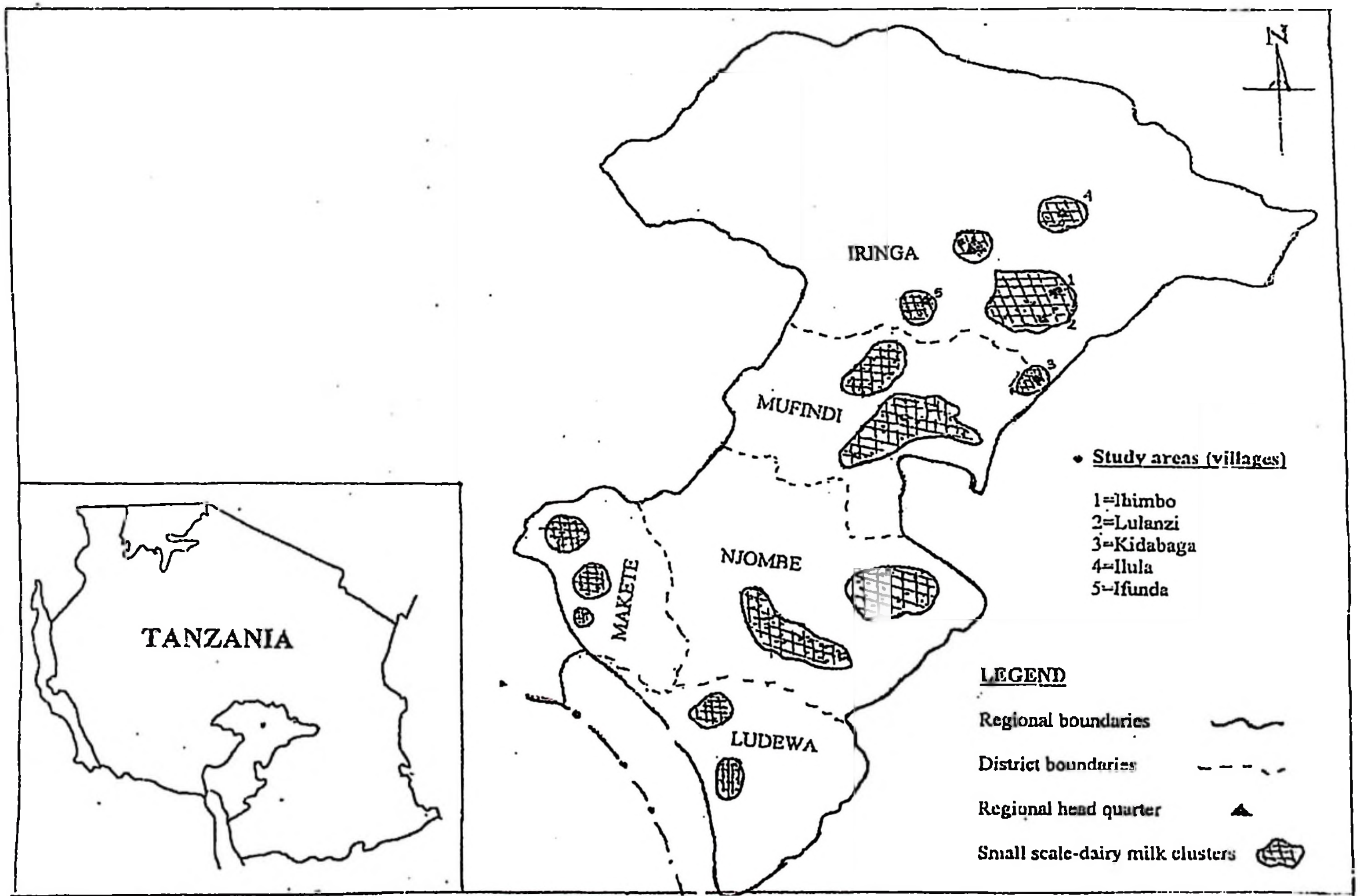


Fig 1. Map of Iringa District in Iringa region showing the study areas

3.1.2 Topography and agricultural activities

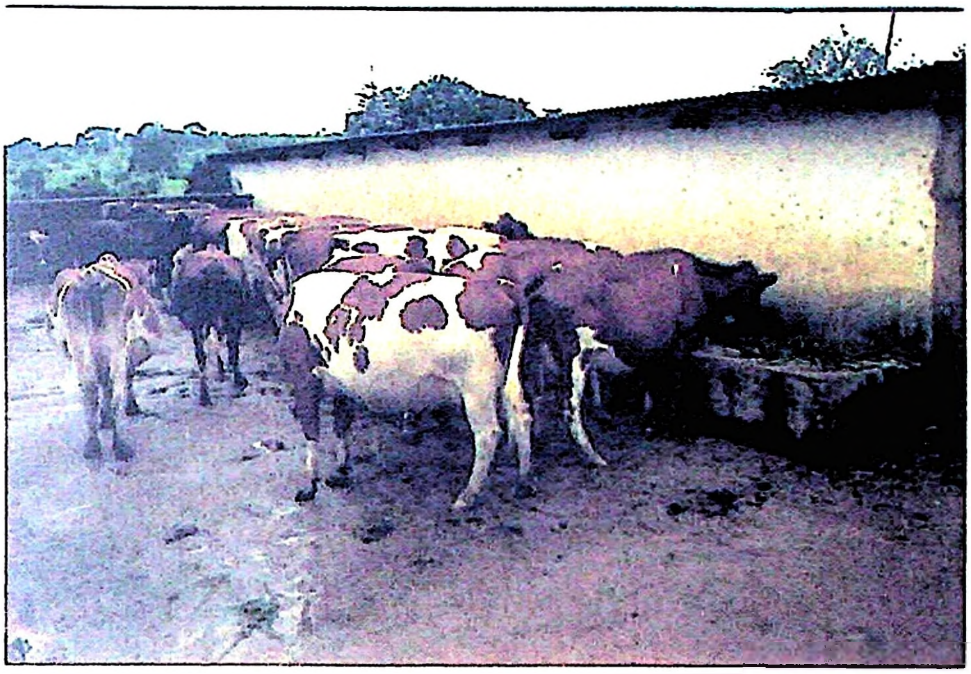
Iringa District and the southern highlands comprise of many hills, valleys and a number of rivers, streams, tributaries and flood plains. In the plateau and the lowland areas there are a number of springs, swamps and permanent or temporary ponds that serve as major watering points for cattle. These water bodies are also potential snail habitats. The moisture and temperatures in the district are favourable for development and survival of free-living stages of parasitic nematodes during most times of the year. The district is traversed by the great East African Rift Valley and is drained mainly by the little and great Ruaha rivers. The rift valley and flood plains are important grazing areas during the dry season.

The main agricultural crop in all zones is maize, however, vegetable production and forestry play important roles in the economy of households in the highland and mid zones.

3.1.3 Cattle management systems

The investigated cattle management systems were large-scale, small-scale dairy and traditional system. Large-scale farms practiced semi-improved management systems. They had their own grazing land that was fenced or not fenced and animals were given concentrates, chopped grass and mineral supplementation. Animals were dipped in acaricides twice a week during the rainy season and once a week during the dry season. Ayrshire and Friesian were the common breeds found on the large-scale dairy farms (Fig. 2). The median herd size on the large-scale dairy farms was 127 animals (range 50-370) and a median farm area of 455 acres (range 100-1900).

(a)



(b)

Fig 2. Ayrshire breed of cattle (a) and an animal house (b) on one large-scale dairy farm in Iringa District

The small-scale dairy system was subdivided into zero grazing, semi-zero grazing and tethering systems based on the feeding/grazing practice. Animals in the zero grazing system were housed/confined throughout while those in the semi-zero system were housed during the rainy season and taken out for grazing during the dry season. The commonest system was the zero-grazing system whereby pasture was 'cut and carried' for feeding permanently housed animals. Tick control was through hand spraying twice a week during the rainy season and once a week during the dry season. Supplementation was very low, irregular and confined to milking cows. Ayrshire and Friesian crosses with Boran or Zebu were the dominant animals on small-scale dairy farms (Fig. 3). The median herd size was 3 animals (range 1-15) and a median farm area of 1 acre (range 0.25-7).

Traditional farms were subdivided into sedentary and migratory herds. Traditional sedentary herds predominated in the highland and mid zones where farmers also practiced agricultural activities. Animals grazed in communal areas during the day and were housed around households in open kraals/'bomas' at night. The median herd size in sedentary herds was 10 animals (range 1-80) and a median farm area of 7.5 acres (range 0.5-500). Traditional migratory herds predominated in the lowland zone and involved migration over space and time, and extensive use of grazing areas. Migratory herds were further divided into semi-nomadism and total nomadism depending on the extent of migration. The indigenous breed, the Tanzania Short Horn Zebu (TSHZ, Iringa red ecotype) dominated on all traditional farms (Fig. 4). Traditional cattle were denied of any form of modern animal husbandry, and tick control was seasonal (only during the rainy season) and only in sedentary herds.

(a)

(b)

Fig 3. Friesian-Ayrshire cross-bred cattle and an animal house in urban (a) and rural (b) areas on small-scale dairy farms in Iringa District

(a)

(b)

Fig 4. Tanganyika Short Horn Zebu cattle at Lulanzi communal grazing area (a) and an animal kraal or night boma (b) on one traditional farm in Iringa District

3.2 Study on worm control practices and anthelmintic usage, farmers' knowledge on anthelmintics and livestock extension system

3.2.1 Study area and farms

A total of 177 farms in the highland and mid zones were randomly selected and included in the survey. These farms comprised of all 9 large-scale dairy farms in the two zones, 92 small-scale dairy farms and 76 traditional farms. A total of eleven villages with both traditional and dairy cattle were randomly selected and covered in the interview. Eight-village extension officers (1-2 in each village) were also interviewed by the same interviewer and using the same questionnaire. The villages were from four divisions (Mazombe, Kilolo, Mlolo and Kiponzelo) out of the nine divisions in the district. Twenty-one small-scale dairy farms in the municipality were also surveyed. Overall, twenty-one farms within the municipality (urban) and 156 farms in peri-urban and rural areas were randomly selected using the village and farmers' list in the district. All traditional and large-scale dairy farms were in rural areas. Most small-scale dairy holders obtained their dairy cattle through "Credit in Kind" from livestock development projects mainly the Southern Highlands Dairy Development Project (SHDDP) and the Heifer Project International (HPI). For this study, a farm was defined as any animal establishment with a minimum of one cow/animal.

3.2.2 Data collection methods

A combination of qualitative data collection methods was used. Information on worm control practices and anthelmintic usage was collected through key informant interviews using a semi-structured questionnaire (Appendix 1).

Respondents were farm owners/managers or animal attendants on each farm. The same interviewer administered all questionnaires right on the farm. The questionnaire had two sections; the first section was on farm particulars and some aspects of management while the second section dealt with specific questions on worm control and anthelmintic usage on the farm. Both closed and open-ended questions were used.

The main information sought from farmers was worm control methods practiced, the type of anthelmintic used by the farmer during the period (year) of the study, the type of anthelmintic used by the farmer the previous year, milk and slaughter clearance in treated animals, sources of anthelmintics, frequency of treatment and interruption of pre-planned treatments. There were also questions on alternation/change of anthelmintic classes, source of information on anthelmintic usage, use of ethno-veterinary medicine, seminars on helminths and anthelmintics attended and farmers' knowledge on sources of worm infections in cattle. Disease ranking was used to obtain information on the most important diseases hindering productivity of cattle. Moreover, there were focus group discussions on worm control and anthelmintic usage at Ng'ang'ange and Ihimbo villages. The enumerator also observed and recorded the type and shelf life of anthelmintic the farmer had (if any) on the day of the visit at the farm and also made observations on body condition of animals, animal house, animal feed and grazing areas right on the farm.

3.3 Abattoir survey on GI nematodes in indigenous cattle under semi-nomadic and nomadic management systems in the lowland zone

3.3.1 Study area

This study was conducted in the lowland zone that forms part of the extensive plains in the southern highlands of Tanzania. The lowland zone is traversed by the great East African Rift Valley and is drained by the Little Ruaha, Great Ruaha and Kizigo rivers. The Rift Valley and the flood plains of the rivers are important grazing areas during the dry season. The zone is semi-arid and arid in nature and agricultural activities are very limited. Pastoral, semi-nomadic and nomadic communities dominate the lowland zone. The study was conducted in three divisions in the lowland zone namely Pawaga, Idodi and Izazi. The cattle are owned by different ethnic groups and involve constant migration over space and time and extensive use of grazing areas. During the dry season, cattle in the lowland zone wonder throughout the plains and into neighbouring regions in search of pasture and water. There is no supplementary feeding and anthelmintic treatments are rare, mainly, therapeutic and in young animals.

3.3.2 Animals

The animals examined in the survey were indigenous Zebu cattle bought by butchermen from four livestock markets in the lowland zone namely, Izazi, Ismani, Nyang'oro and Pawaga. Animals purchased by butcher men were brought for slaughter at the regional abattoir in Iringa (namely Mlandege) located at Mlandege in Iringa Municipality; some of the animals were brought for slaughter at Ipogolo and Kihesa slaughter slabs in the Municipality. Prior to slaughter, the live animals' grade scores were recorded and was coded as 1=very good, 2=good, 3=satisfactory and 4=poor. The sex, age and livestock market of origin for each animal were also recorded at the abattoir prior to slaughter. Slaughtered cattle were categorised into immature (< 3 years) or mature (≥3 years) based on visual appraisal and with the assistance of butchermen. Only animals from the lowland zone of Iringa District were included in the study.

Forty animals were examined during the mid-rainy season (February/March), 40 during the late rainy/early dry season (May/June), 44 during the mid-dry season (August/September), while 20 animals were examined during the late dry/early rainy season (November/December). The gastrointestinal tracts of the slaughtered animals were randomly selected and purchased from the butchermen depending on their willingness to sell them. Additionally, the grazing areas in the lowland zone were visited and in-depth interviews with herdsmen were made on the grazing pattern/cycle of animals in 12 months of the year.

3.3.3 Samples, sample processing and examination

After slaughter, the GI tracts of randomly selected animals were double ligated into three sections to prevent mixing of the abomasal, small intestinal and large intestinal contents. Three or four GI tracts were purchased on every visit to the abattoir. The selected GI tract was placed in a bucket, labelled and transported to the Veterinary Investigation Centre (VIC) in Iringa for processing. In the laboratory, the whole abomasum of each GI tract was isolated and opened along the greater curvature, washed and the contents collected into a bucket. The abomasal contents were then passed through a 140 μm wire mesh sieve and washed with tap water under pressure to clean the contents and retain the worms. The contents retained by the sieve were resuspended in 4 litres of water and an aliquot of 400 ml was taken in small amounts over time while mixing. The small intestines were separated from the large intestines with the caecum, and both were freed of the mesentery, opened along their length and processed separately. The entire length of the small intestine and the whole length of the large intestine were processed. The intestinal contents and washings were processed in the same way as the abomasum, except that contents of the large intestines were passed through a 200 μm wire mesh sieve.

The aliquot of each section was then examined in black trays, a few millilitres at a time, with the aid of a magnifying lens with an illuminated light. The worms were isolated, counted and placed in a plastic jar containing 70% ethyl alcohol for later identification to species level based on identification keys described in the MAFF (1986) manual and by Hansen and Perry (1994).

3.4 Cross sectional prevalence of helminth infections among cattle management systems

3.4.1 Study design, selection of farms and sampling

The study was a cross sectional observational study conducted in the highland and mid zones. A multi stage stratified random sampling method was used to select animals from zones, villages, management systems, farms and age groups. Farms in each zone/village were categorised into large-scale dairy, small-scale dairy and traditional farms based on the management system especially the grazing/feeding practice. Furthermore, animals on each farm were stratified into three age groups of calves (<8 months), weaners/yearlings (9-24 months) and adults (>24 months). The study was carried out on four large-scale dairy farms namely Amani, Tommy, Muhesa and Mshughulika and five villages, three in the highland zone (Lulanzi, Ihimbo and Nga'ng'ange) and two in the central zone (Ilula and Ifunda). The selected villages were those with both small-scale dairy and traditional farms and within a one-day return field trip from the Iringa municipality (63 km radius). At least two traditional and five small-scale dairy farms were selected in each village.

A total of 236 animals on large-scale dairy farms, 127 on small-scale dairy farms and 150 on traditional farms were sampled during the study (total of 513 animals). At least thirty animals were randomly sampled on each of the large-scale dairy farms, a minimum of ten animals on each traditional farm and a maximum of five animals on small-scale dairy farms. For this study a farm was defined as any establishment with a minimum of one animal.

3.4.2 Samples and sample processing

Samples taken were faecal samples for determination of the presence of *Fasciola* and amphistome eggs and for quantitative determination of nematode eggs. The number of nematode eggs per gram of faeces (EPG) was used as an indirect measure of worm burdens in animals. Faecal samples were collected per rectum using gloved fingers. The collected samples were labelled and placed in cool boxes and transported to the Veterinary Investigation Centre (VIC) in Iringa for examination. The EPG for GI nematodes was determined by the modified McMaster method (MAFF, 1986; Appendix 2). The presence of fluke eggs was determined by the sedimentation technique (Hansen and Perry, 1994; Appendix 3). When the amount of faecal sample was small, it was processed either for nematode eggs or fluke eggs only. All samples collected were processed and examined within 36 hours. Faecal samples that remained were pooled by management system and cultured as described by Hansen and Perry (1994; Appendix 4) for recovery of third stage larvae (L_3) of nematodes. The L_3 recovered were identified to genus level based on length and morphology using identification keys (MAFF, 1986). In addition, 16 infected cattle slaughtered at the regional abattoir were randomly selected and flukes collected for identification of species infecting cattle.

3.5 Epidemiology of helminth infections among cattle management systems

3.5.1 Study design, selection of farms and animals

This was an observational descriptive longitudinal study conducted in the highland zone and the mid zone. A multi stage stratified random sampling method was used to select animals from zones, villages, management systems, farms and age groups. Farms in each zone/village were categorised into large-scale, small-scale and traditional, respectively. The study was carried out on five large-scale dairy farms; three in the highland zone (Tommy, Muhesa and Turlia) and two in the mid zone (Mshughulika and Igumbiro); and in five villages, three in the highland zone (Lulanzi, Ihimbo and Kidabaga) and two in the mid zone (Ilula and Ifunda). The selected villages were those with both small-scale dairy and traditional farms and within a day return trip from the Iringa municipality (63 km radius). At least two traditional and five small-scale dairy farms were selected in each village. Animals on each farm were categorised into three age groups i.e. calves (<8 months), weaners/yearlings (9-24 months) and adults (>24 months). A total of 138 animals on 5 large-scale dairy farms, 69 animals on 24 small-scale dairy farms and 94 animals on 11 traditional farms (total of 301 animals) were selected and included in the study.

The common breeds in large scale-dairy farms were Ayrshire, Friesian and their crosses, small-scale dairy farms had crosses between Ayrshire or Friesian with Boran or Zebu cattle while the indigenous Zebu cattle dominated in all traditional farms. The selected animals on each farm were ear-tagged during the first sampling day in January 2002 to ease their identification in subsequent monthly samplings. The ear-tagged animals were sampled every month for a period of one year (January-December 2002).

Only Ilula village had a communal operating dip tank for traditional cattle. Farmers in the other villages hand-sprayed animals or trekked them over long distances to dip tanks in other villages. All large-scale dairy farms had their own dip tanks on the farm. For this study, a farm was defined as any livestock establishment with a minimum of one cow/animal. Each farm continued with its normal management practices including routine anthelmintic treatments (usually 2-4 times per year) during the study.

3.5.2 Samples and sample processing

3.5.2.1 Faecal and blood samples

Samples taken were faeces for determination of the number of nematode EPG and qualitative determination of the presence of *Fasciola* and amphistome eggs. Whole blood was collected for determination of PCV as a measure of anaemia due to worm infection. Faecal samples were taken per rectum in gloves while blood was taken from the jugular vein into evacuated blood collecting tubes (Vacutainers®). Collected samples were labeled, placed in a cool box and transported to the VIC in Iringa for examination. Samples were collected on the last week of every month for one calendar year (January-December 2002). Salvage treatments were carried out for animals that showed clinical signs of helminthosis (e.g. diarrhoea, weight loss, oedema, anaemia etc) and FEC > 500 EPG. Monthly rainfall data was obtained from Kilolo meteorological centre at the end of each month. The number of worm eggs in faeces was used as an indirect measure of worm burden. Faecal egg counts (FEC) for nematode eggs was determined by the modified McMaster method (MAFF, 1986; Appendix 2). The presence of *Fasciola* and amphistome eggs was determined by the sedimentation technique as described by Hansen and Perry (1994; Appendix 3). Packed cell volume (haematocrit) was determined by the micro-haematocrit centrifuge technique (Anonymous, 1983; Appendix 5).

3.5.2.2 Pasture samples and larval counts

Pasture samples were collected in order to determine the monthly amount of infective nematode larvae available for grazing animals. Two grazing paddocks at Tommy dairy farm and one communal grazing area of indigenous cattle at Lulanzi village were selected. The grazing area/paddock was traversed in a 'W'-shape route stopping every 5-10 metres and small wisps of pasture picked by hands/scissors and placed in polythene bags. The collected pastures were transported to the VIC in Iringa for processing, recovery, counting and identification of infective larvae to genus level. Pasture samples were collected from each grazing area on the last week of every month for one year (January-December 2002). The number of infective nematode larvae per kilogram of pasture in grazing areas was determined by the modified Baermann technique (MAFF,1986). Infective larvae were identified to genus level based on tail length and cuticle morphology (Hansen and Perry, 1994).

3.5.2.3 Tracers and worm counts

To monitor monthly pasture infectivity, weaned castrated male calves of the same breed (Ayrshire) of about 6-7 months were used as tracers. The tracers used had prior exposure to helminth infections because it was not possible to get worm free tracers. Prior to introduction into their respective farms/areas, the tracers were treated with levamisole (Alfamisole®, Alfasan Company, Holland) and triclabendazole (Fasinex®, Novartis, Basel, Switzerland) in order to clear pre-existing nematode and *Fasciola* infections. After anthelmintic treatment at Sokoine University of Agriculture (SUA), tracers were placed in a pen with concrete floor, fed on hay free from infective larvae or metacercariae for 30 days before being introduced onto the farm. Tracers were then transported to Iringa for introduction into respective farms on the first two days of every month to graze with resident cattle for 30 days. Two tracer calves were used every month; one was introduced at Tommy large-scale dairy farm while the second was introduced at Lulanzi traditional communal grazing land. Tracers on the large-scale dairy farm grazed with dry cows and heifers herd.

After 30 days, tracers were removed from those farms and transported to SUA where they were kept in a clean pen with concrete floor and fed on safe hay for another 30 days. The animals were then slaughtered for recovery, counting and identification of GI nematodes and flukes. After opening the carcass, the main bile duct was ligated to prevent worms from escaping. The bile ducts were then opened using scissors to examine for mature liver flukes. The liver was then sliced into small pieces of less than 5 mm that were placed in warm saline for 30 minutes; the slices were then squeezed to expel immature flukes. The fore-stomachs were opened for examination and enumeration of amphistomes. The procedures for processing GI tracts, recovery and identification of nematodes were similar to those described under the abattoir study (3.3.3).

3.6 Study on the efficacy of drugs commonly used against *Fasciola* and amphistomes in naturally infected cattle

3.6.1 Study area

The study was conducted at Amani dairy farm in the highland zone of Iringa District. The farm is located on a high ground and to the west it is bordered by the Little Ruaha River. The farm has an area of about 510 acres that include a small area of improved pastures. Animals are usually grazed in three groups namely the milking herd, heifers herd and weaner-calves. In addition to dairying, the farm also had sheep and camels. The farm is among *Fasciola* endemic farms in Iringa District and in August 2001 there was an outbreak of acute fasciolosis in sheep on this farm, which resulted in a mortality of 33%. Animals grazed on pastures on the farm during the day and were housed at night.

3.6.2 Study design and animals

The study was a clinical field trial that involved 46 (29 adults and 17 weaned) Ayrshire cows of both sexes, naturally infected with both *Fasciola* and amphistomes. The adult cattle used in the study were >2 years old while weaned animals were 9-12 months old. The animals were selected after screening the dry herd, weaner calves and heifers in the morning (Day 0) for *Fasciola* and amphistome eggs in faeces. The selected animals excreted moderate to heavy fluke eggs in faeces. Selected animals were later in the evening of the same day (day 0) randomly allocated to seven treatment groups; whereby five groups were treated with anthelmintics commonly used in Iringa and two groups were treated with anthelmintics not commonly used in Iringa (Ivomec®-Super and Fasinex®). The weight of each animal was determined using a tape measure and the anthelmintic's dosage calculated based on the manufacturers recommendations.

Seven animals (Grp1) were treated with Milsan® (1.5% levamisole and 3% oxyclozanide, Interchem Pharma Ltd, Moshi, Tanzania) at a dose of 7.5mg/kg levamisole and 15mg/kg oxyclozanide orally. Seven animals (Grp 2) were treated with Levoxy® (1.5% levamisole and 3% oxyclozanide, Unga feeds Ltd, Nairobi, Kenya) at the same dose orally. Seven animals (Grp 3) were treated orally with Albenda® (Albendazole bolus, Interchem Pharma Ltd, Veterinary Division, Moshi, Tanzania) at 1½ times the recommended dose of 7.5mg/kg orally while 7 animals in-group 4 were treated with Farbenda® (10% Albendazole suspension, Farvet Bladel, Holland) at the same dose (e.g. 11.25mg/kg). Six animals (Grp 5) were treated with Ivomec®-Super (1% ivermectin m/v and 10% clorsulon m/v, South Africa Ltd, Halfway House, South Africa) at 1 ml/50kg subcutaneously. Six animals (Grp 6) were treated with Trodax® 34% (Nitroxynil, Merial, Lyson, France) at 1.5ml/50kg subcutaneously. Seven animals (Grp 7) were treated with Fasinex® (10% w/v triclabendazole, Novartis Animal Health, Basel, Switzerland) at 12 mg/kg orally. Treated animals continued to graze with other untreated animals on the farm for a period of 10 weeks, when the farmer terminated the trial by treating all animals with Farbenda® (10% Albendazole oral suspension, Farvet Bladel, Holland) at 7.5 mg/kg.

3.6.3 Sampling and sample processing

Faecal samples from all treated animals were taken on day 0 (before treatment), and then at 2, 6 and 10 weeks after treatment (e.g. at 14, 42 and 70 days after treatment). Thereafter, the farmer treated all animals in the farm on routine basis for fear of clinical helminthosis and therefore ending the study. The faecal samples were collected per rectum in plastic gloves and stored in a cool box and transported to the VIC in Iringa for examination. Samples from all animals were collected and processed on the same day. Animals without faeces in the rectum were sampled again later during the day (evening) to reduce missing values. Faecal samples were processed for examination of the presence of *Fasciola* and amphistome eggs by the sedimentation technique (Hansen and Perry, 1994; Appendix 3).

3.7 Study on strategic treatment against flukes and GI nematodes at the end of the rainy/early dry season

3.7.1 Study area and animals

The study was conducted on one traditional farm (Chelestino) in Ihimbo village in the highland zone, and at one beef farm (Mshughulika) in Ifunda village in the mid zone. The traditional farm had the indigenous Zebu cattle while the beef farm had crosses of Ayrshire with Zebu and Boran. At the traditional farm, animals were grazed and watered on communal areas while the beef farm had its own grazing land that was occasionally grazed by traditional cattle from other farms due to lack of perimeter fencing.

3.7.2 Study design and anthelmintic treatments

This was an intervention study in the form of a field trial conducted on naturally infected animals. Prior to commencement of the trial (early May 2001), faecal samples from all animals on both farms were taken for screening for the presence of *Fasciola* and amphistome eggs in faeces. Thereafter, animals with both *Fasciola* and amphistome eggs in faeces on each farm were selected. Fourteen infected animals (10 adults and 4 weaned) on the traditional farm and 19 animals (13 adults and 6 weaned) on the beef farm were selected. The selected animals on both farms were ear tagged to ease identification during subsequent monthly sample collection.

On the first week of May 2001, the selected animals at the traditional farm were treated with Fasinex® (10% w/v triclabendazole, Novartis Animal Health, Basel, Switzerland) orally at 12mg/kg. At the beef farm, the selected animals were treated with oral Farbenda® (10% albendazole suspension, Farvet Bladel, Holland) at 1½ times the recommended dose of 7.5mg/kg (e.g. 11.25mg/kg). Thereafter, the treated animals on both farms continued to graze with other animals under the same farm management practices except for routine anthelmintic treatments for one year.

3.7.3 Samples and sample processing

Faecal samples were collected from each animal for examination of *Fasciola* and/or amphistome eggs in both farms, and also for determination of nematode egg counts at the beef farm. Whole blood from the jugular vein was also collected at the beef farm for determination of PCV. The first sampling was 21 days (three weeks) after treatment (end of May); thereafter samples were taken at monthly intervals for one year (May 2001-April 2002). Samples were collected within the last 3 days of every month and transported to the laboratory at VIC in Iringa for examination. The presence of *Fasciola* and amphistome eggs was determined by the sedimentation technique (Hansen and Perry, 1994; Appendix 3) while EPG for each animal at the beef farm was determined by the modified McMaster method (MAFF, 1986; Appendix 2).

3.8 Data analysis

Data was entered, validated and cleaned in excel, then imported into either Statistix® for Windows (Statistix®, 1994) or Statistical Analysis System (SAS®, 1990) for analysis. Anthelmintics were grouped into four classes: Benzimidazoles and pro-benzimidazoles (Class I), combinations of levamisole and oxyclozanide (Class II), levamisole alone (Class III) and nitroxynil (Class IV)). Alternation of anthelmintic classes was grouped into regular alternation (after every 1 year), moderate alternation (2 years), slow (3 years) and no alternation (>3 years). For qualitative data, descriptive statistics and frequency of responses, data were analysed by Statistix® for Windows (Statistix®, 1994). Data from group discussions and in-depth interviews were analysed manually for content and recurrent themes in the text.

Faecal egg counts were analysed by mixed model linear procedure using village as a random effect. The statistical model fitted included fixed effects of management systems, age, month (season) and their interactions. Due to skewed distribution of FEC and worm counts, a logarithmic transformation was employed for total worm counts ([log (TWC + 1)] and faecal egg counts ([log (FEC + 25)] to normalize data before analysis. Adjustment for multiple comparisons was made using the least significant difference (LSD). Worm burden between sex and zones were analysed by two-sample t-test on logarithm-transformed data.

The proportion of animals excreting *Fasciola* or amphistome eggs by management system, sex, age, location, month (season) and treatment group were analysed by Chi square (χ^2) test. The risk of an animal being infected by *Fasciola*/amphistome was analysed by SAS® logistic regression (SAS®, 1990). Based on presence of *Fasciola*/amphistome eggs in faeces, efficacy of an anthelmintic was determined as the proportion of animals negative for eggs in faeces over the total number of animals treated in a group. The cost of treatment of an animal was calculated on the basis of an albendazole bolus that is sold at 500/= TShs and that an average cow uses 3 boluses while calves are usually given half of the bolus. Pasture contamination was determined as the number of infective larvae per kilogram of pasture/herbage. In all statistical analysis, the level of significance (p-value) was taken to be $p<0.05$.

CHAPTER 4

RESULTS

4.1. Worm control practices and anthelmintic usage

4.1.1 Worm control methods and type of anthelmintics

Results from the questionnaire survey on worm control methods among management systems are shown in Table 1. Out of 177 interviewed farmers, 172 (97.2%) responded to the question on worm control methods at the farm. Out of all 172 interviewed farmers, 161 (93.6%) used anthelmintics to control worm infection in cattle. A small proportion of traditional farmers were even not aware of the presence of commercial anthelmintics.

Results from the questionnaire survey on the types of anthelmintics used on farms among management systems are shown in Table 2. Out of 177 interviewed farmers, 149 responded to the question on types of anthelmintics used at the farm. Out of 149 (84.2%) interviewed farmers, 68 (45.6%) used anthelmintics with a combination of levamisole and oxyclozanide, followed by benzimidazoles (28.2%). The use of levamisole and oxyclozanide was highest among all three management systems. At least nine brands of anthelmintics (range 4-13) were simultaneously being used in each village (i.e. Albendazole bolus, Farbenda®, Tramazole®, Milsan®, Levoxy®, Milverm®, Polystrongyle, Wormicid® and Trodax®). Most farmers selected the cheapest anthelmintics and used them for several years. Nitroxynil was mainly used on large-scale dairy farms (33.3%) and few small-scale dairy farms (8.6%) in urban areas. Small-scale dairy and traditional farmers in peri-urban and rural areas used the majority of cheap anthelmintics such as albendazole boluses. Almost all farmers and some extension officers thought that one type of anthelmintic could eliminate all helminths in an animal, and they were not aware of the different classes of helminths (i.e. trematodes, cestodes and nematodes). Moreover, farmers and extension workers were not aware of the need to increase the dose of some drugs in order to achieve activity against mature or immature flukes.

Table 1. Proportion of interviewed farmers (%) in Iringa District practicing various worm-control methods for cattle under different management systems.

	Management system			Total
Worm control method	LSDF (n=9)	SSDF (n=90)	TF (n=73)	(n=172)
Anthelmintics	100.0	97.8	87.7	93.6
Traditional medicines	0.0	0.0	2.7	1.2
Management	0.0	2.2	0.0	0.0
None	0.0	0.0	9.6	5.2

n= number of responding farmers, LSDF =Large-scale dairy farms, SSDF =Small-scale dairy farms, TF =Traditional farms

The questionnaire survey indicated that out of 159 interviewed farmers, 62 (39%) had at one time used traditional medicines as an alternative to commercial anthelmintics due to lack of money. The 'reported' efficacy of traditional medicines varied from low to highly effective. The survey also indicated that out of 161 interviewed farmers, 112 (69.6%) used anthelmintics prophylactically with few treatments mainly on traditional farms based on clinical helminthosis (28.6%). Preventive treatments on traditional farms were mainly given to calves, weaned and working animals (oxen). Almost all farmers assessed the efficacy of any anthelmintic by improvement of an animal body condition and health after treatment.

Table 2. Proportion of interviewed farmers (%) in Iringa District using various types of anthelmintics in cattle under different management systems.

Type of anthelmintic	Management system			Total
	LSDF (n=9)	SSDF (n=81)	TF (n=59)	(n=149)
Benzimidazoles	0.0	32.1	25.4	28.2
Levamisole & Oxyclozanide	66.7	35.8	57.6	45.6
Levamisole alone	0.0	22.2	13.6	17.4
Nitroxynil (Trodax®)	33.3	8.6	1.7	7.4
Other	0.0	1.2	1.7	1.3

n= number of responding farmers, LSDF =Large-scale dairy farms, SSDF =Small-scale dairy farms, TF= Traditional farms

4.1.2 Source of anthelmintics and frequency of treatments

The questionnaire survey indicated a significant difference on the source of anthelmintics among management systems (P=0.004). Out of 157 interviewed farmers, 98 (62.4%) obtained anthelmintics and information on anthelmintics from private drug shops locally known as '*duka la dawa*' in urban areas and '*malimbichi*' in rural areas. The survey indicated that village extension officers were a source of anthelmintics in 29 (33.3%) of the 87 small-scale dairy farms and 25 (41%) of the 61 traditional farms. There was a multitude of anthelmintic brands, drug vendors and service providers especially in urban and peri-urban areas. The survey also indicated that out of 158 interviewed farmers, 58 (36.7%) administered anthelmintics themselves, and when farmers were asked to estimate weight of an animal and the amount of anthelmintic to be administered, the study indicated that there were instances of under-dosing due to inaccurate weight estimates. The main reason for self-administration was unavailability or unreliability of village extension officers because they were also involved in other development projects. Farmers said that village extension officers and fellow farmers had shown them how to estimate dosage and administer anthelmintics. Farmers preferred to have anthelmintics in their homes because it reduced the cost of treatment and that sometimes village extension officers had no anthelmintics, thus it was so risky to depend on them. Expired anthelmintics were encountered in few small-scale dairy and traditional farms.

Results from the questionnaire survey on the frequency of treatments in different management systems are shown in Table 3. Out of 177 interviewed farmers, 140 (79%) responded to the question on frequency of anthelmintic treatment at the farm. Out of 140 interviewed farmers, 57 (40.7%) treated their cattle three or four times in a year, while 14 (10%) treated more than four times per year (mean 6.5). The number of treatments per year was significantly low on traditional than on dairy farms (P=0.0001). Anthelmintic treatments on many farms were not based on the epidemiology of parasites; months to treat cattle depended mainly on availability of money and anthelmintics. The survey indicated a remarkable variation in the time of the year to treat cattle among farms within the same village and the three months interval of treatment depended on the time one started keeping cattle.

The questionnaire survey also indicated that villages with farmers' associations that practised group treatments (e.g. Lulanzi and Ilula-Itunda) had more co-ordinated treatment programmes despite the fact that they were also not based on the epidemiology of parasites. Out of 134 interviewed farmers who responded to the question on interrupted treatments, 62 (46.3%) failed to follow their pre-planned drenching intervals mainly due to lack of money (86%) and unavailability of drugs (6.6%). A significant high rate of failure to follow pre-planned treatment intervals was among farmers in rural areas (P=0.007).

Table 3. Proportion of interviewed farmers (%) in Iringa District who were treating 1,2,3-4 and >4 times a year in different management systems.

Number of treatments	Management system			Total
per year	LSDF (n=9)	SSDF (n=83)	TF (n=48)	(n=140)
Once	11.1	12.1	35.4	20.0
Twice	22.2	20.5	45.8	29.3
3-4	55.6	55.4	12.5	40.7
>4	11.1	12.0	6.3	10.0

n = number of responding farmers, LSDF =Large-scale dairy farms, SSDF =Small-scale dairy farms, TF= Traditional farms

4.1.3 Alternation of anthelmintics

The questionnaire survey indicated a significant difference on alternation of anthelmintic classes among management systems (P=0.02). Large-scale dairy farms had a moderate alternation between anthelmintic classes while traditional and small-scale dairy farms had slow and irregular change of anthelmintics brands, mostly within the same anthelmintic class (which is not alternation). Alternation or change of anthelmintics was mainly influenced by availability of anthelmintics. Out of 121 interviewed farmers, 33 (27.3%) have used the same anthelmintic for more that four years (maximum of 11 years). A high proportion (32.3%) of farmers in the small-scale management system used the same anthelmintic for a longer period than in large-scale (20%) and traditional (16%) farms. Out of 95 interviewed farmers who had used a certain anthelmintic for more than four years, 81 (85.3%) would like to continue with the same anthelmintic. Out of 41 farmers who responded to have changed the type of anthelmintic, 15 (36.6%) changed the type of anthelmintic due to lack of improvement after treatment and 11 (26.8%) due to unavailability of the previous anthelmintic. Respondents at Ihimbo village 'reported' a reduced efficacy of Milsan® (1.5% levamisole and 3% oxyclozanide) in cattle after it had been used continuously for more than ten years.

4.1.4 Constraints of productivity in cattle

Results from the questionnaire on the most important constraints of productivity in cattle in the district are shown in Table 4. Out of 177 interviewed farmers, 173 (97.7%) responded to the question on important constraints of livestock productivity. Out of 173 farmers that responded, 109 (63%) mentioned East Coast Fever (ECF), locally known as '*Makatu*' as the most important constrain while 29 (16.8%) mentioned worm infestation (helminthosis), locally called '*Minyoo*' as the second important constraint of productivity in cattle.

Out of 173 interviewed farmers, 69 (40%) ranked the worm problem to be highest on their farms. The maximum number of eggs per gram of faeces (EPG) recorded in animals randomly sampled during the survey was 6300 for large-scale dairy, 1200 for small-scale dairy and 1400 for traditional cattle. The survey also indicated that of all chemical compounds that were used at the farm level, anthelmintics appeared to be the mostly used, followed by antibiotics.

Table 4. Important constraints (%) hindering productivity of cattle among management systems in Iringa District

	Management system			Total
Constrain	LSDF (n=9)	SSDF (n=88)	TF (n=76)	(n=173)
East Coast Fever (ECF)	55.6	58.0	69.7	63.0
Helminths (Worms)	11.1	15.9	19.7	16.8
Mastitis	11.1	14.8	1.3	8.7
Pasture shortage (Feed)	22.2	5.7	2.6	5.2
Other	0.0	5.7	6.6	6.4

n = number of responding farmers, LSDF =Large-scale dairy farms, SSDF =Small-scale dairy farms, TF= Traditional farms

4.1.5 Farmers' awareness and knowledge on helminths in cattle

The questionnaire survey indicated that almost all farmers were aware of worm infestation in cattle and the need for preventive or therapeutic treatments mainly in the dairy sector. Despite the awareness on worm infestation and the associated signs; out of 160 interviewed farmers, 68 (42.5%) had limited knowledge about the life cycle, uptake and epidemiology of worms. The knowledge on worms and anthelmintics mainly depended on the period one had owned cattle. Farmers and extension officers diagnosed worm infestation purely from theoretical estimation (visual appraisal) like poor growth, poor body condition, rough hair coat, reduced appetite and reduced milk. Farmers were not able to differentiate worm infestation from other causes like poor nutrition and trypanosomosis.

Out of 167 interviewed farmers, 78 (46.7%) had not attended any seminar on animal health or production. Results of the questionnaire study showed that the number of traditional and large-scale dairy farmers that had been involved in seminars on animal health or production was significantly lower than that of small-scale dairy farmers (P=0.001). While 47.1% of small-scale dairy farmers had attended more than 4 seminars, about 78.7% of traditional farmers and 60% of large-scale dairy farmers had not attended any seminar apart from involvement on vaccination campaigns. Helminthosis was not a priority in those seminars but the farmers were advised to deworm their cattle at least four times in a year without mention of the appropriate months.

4.1.6 Information on anthelmintics, extension system and withdraw period

The survey indicated that out of 145 interviewed farmers, 133 (91.7%) depended on village extension officers for information on worm control and anthelmintic usage. The survey also showed serious extension problems in most villages; in terms of number of extension officers, skills and availability. One village had no any extension officer and depended on those in neighbouring villages. Two extension officers had no means of transport and therefore could not serve farmers adequately. In some villages, village extension officers lacked adequate knowledge on helminths and anthelmintics because they had formal training in crop production but were responsible for both crops and animal health at the village level.

The survey indicated that out of 148 interviewed farmers, 73 (49.3%) lacked knowledge on withdrawal period in animals treated with anthelmintics. Most small-scale dairy farmers allowed only a one-day withdrawal period for milk regardless of the type of anthelmintic used and there was almost no milk and slaughter clearance in treated animals among traditional farms.

4.2 Abattoir survey of GI nematodes in Zebu cattle in the lowland zone

The rainfall pattern during the period of the study is shown in Fig 5. The rainy season was from December to May. The abattoir survey indicated that 97.2% of all cattle that were examined (144) were infected with GI nematodes. The worm species recovered, their prevalence and burdens are shown in Table 5. Eight nematode species were identified; the common ones were *Cooperia* spp (67.8%), *Haemonchus* spp (24.7%) and *Oesophagostomum radiatum* (5.1%). Most of the infected animals harboured more than one nematode species. Worm burden in animals was generally low although a few; mainly young animals were heavily infected.

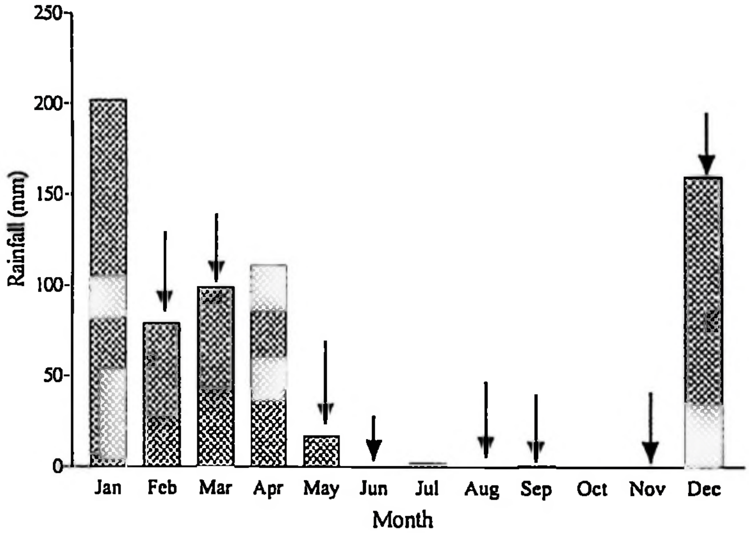


Fig 5. Monthly rainfall at Kilolo centre in Iringa District from January-December 2001

NB: Arrows indicate sampling months

The mean worm burdens in animals slaughtered at different seasons of the year are shown in Fig 6. The worm burden was significantly low at the end of the dry /early rainy season ($P < 0.001$) compared to the late rainy season. The burden increased from the mid-rainy season and peaked at the end of the rainy season/early dry season. Immature animals had significantly higher worm burdens than mature ones ($P < 0.01$).

The species composition of worm burdens in cattle at different seasons of the year is shown in Fig 7. There was no succession of nematode species across the seasons, the ranking being more or less the same throughout the year except that *Bunostomum phlebotomum* was isolated only in the middle of the rainy season.

There was a weak positive correlation between a poor grade for the live animal and worm burden but only in immature animals and mainly in the dry season (r =0.456, P<0.001). There was no significant difference in worm burdens between male and female cattle (P>0.05). The mean worm burden in cattle from different livestock markets is shown in Fig. 8. There was no significant difference in worm burden between animals from different livestock markets (P>0.05).

Table 5. Worm species, prevalence and worm burdens in slaughtered indigenous Zebu cattle from the lowland zone of Iringa District

Location and worm species	Prevalence (%)	Worm burdens		
		%	Mean ± S.e	Range
Abomasum				
Haemonchus placei	84.7	24.60	316±46	20-3420
Haemonchus similis	5.6	0.08	1±0.35	10-20
Small intestines				
Cooperia pectinata	55.6	55.50	713±122	30-6480
Cooperia punctata	44.4	12.30	158±37	20-3450
Bunostomum phlebotomum	5.6	0.39	5±30	10-320
Trichostrongylus colubriformis	1.4	1.90	24±17	60-1730
Large intestines				
Oesophagostomum radiatum	79.2	5.10	66±15	10-1390
Trichuris globulosa	5.6	0.16	2±1.04	10-100
Overall	97.2	-	1284±183	10-12 600

S.e. = Standard error

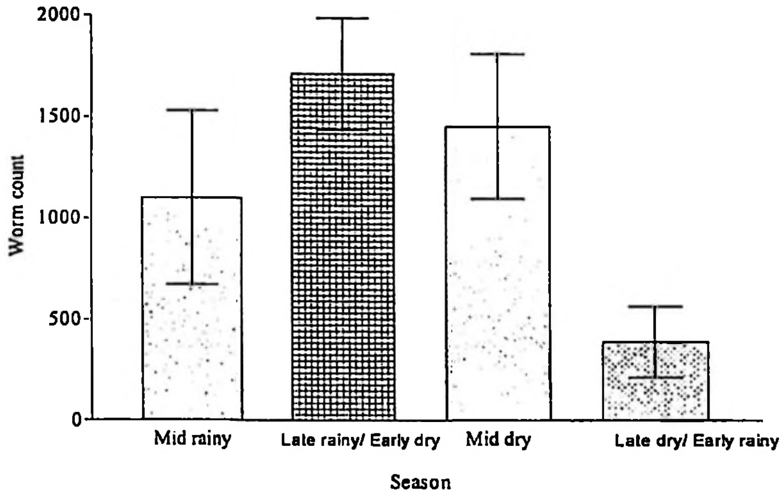


Fig 6. Mean worm burdens of cattle slaughtered in the lowland zone of Iringa District during different seasons of the year

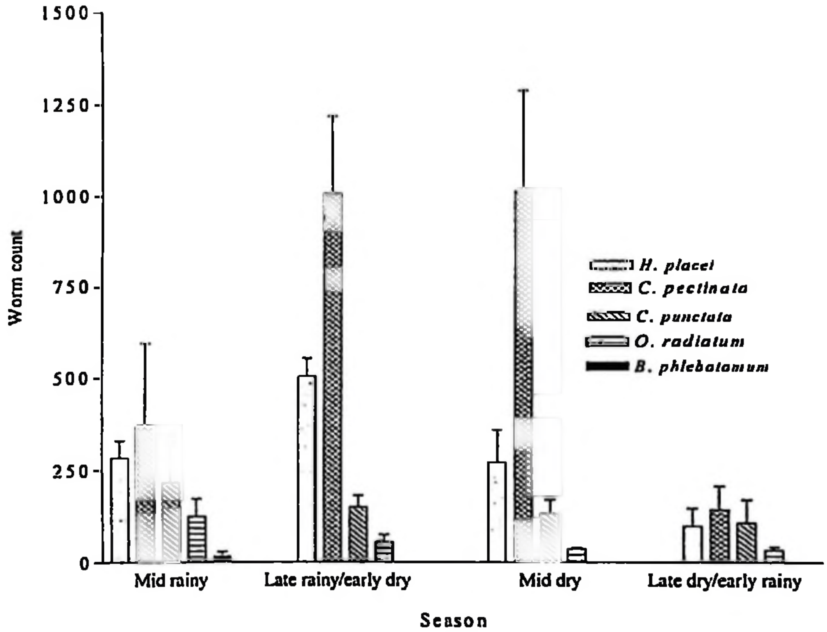


Fig 7. The species composition of worm burdens in cattle slaughtered during different seasons of the year in Iringa District during a period of one year (2001)

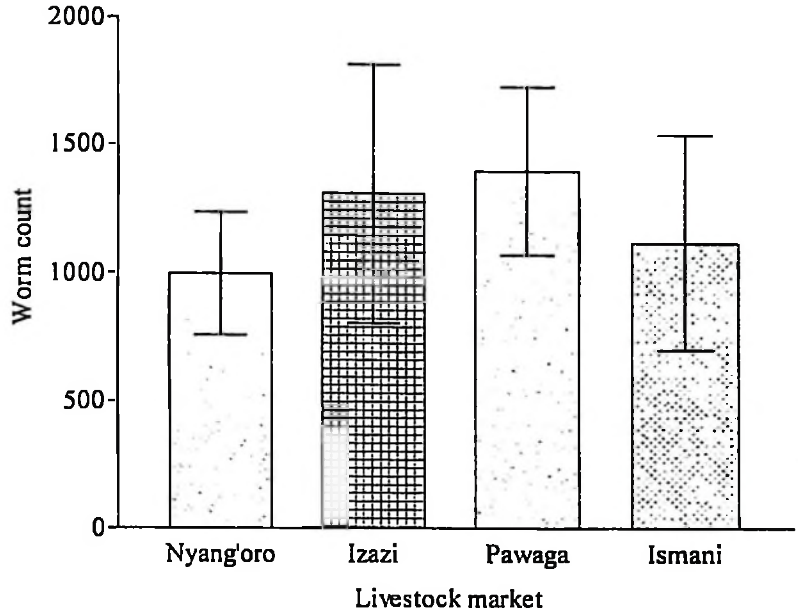


Fig 8. Mean worm burdens in cattle from different livestock markets in the lowland zone of Iringa District

4.3 Cross sectional prevalence of helminth infections

4.3.1 *Fasciola* and amphistomes

Results from the 16 infected animals examined at the abattoir indicated that *Fasciola gigantica* was the only liver fluke specie recovered while *Calicophoron microbothrium* and *Cotylophoron jacksoni* were the only amphistome species recovered. The prevalence of *Fasciola gigantica* among management systems is shown in Table 6. The management system was found to have a significant association with the prevalence of *F. gigantica* (χ^2 =30.52, df=2, P=0.0001) as traditional cattle had significantly higher risk of infection than both small-scale (P=0.02) and large-scale (P=0.008) dairy cattle.

The prevalence of amphistomes among management systems is shown in Table 7. The prevalence of amphistomes was associated to the management system

(χ^2 =46.05, df=2, P=0.0001). The prevalence of amphistomes was highest in cattle under the traditional management system. The prevalence of amphistomes was always higher than that of *Fasciola* in all managements systems, farms and villages. Out of the 482 animals examined, 203 (42.1%) were infected with both *Fasciola* and amphistomes.

Table 6. Prevalence of *Fasciola gigantica* among cattle management systems in Iringa District

Management system	Animals examined	Prevalence (%)		
		Overall	Minimum	Maximum
Large-scale dairy	236	46.2	4.0	92
Small-scale dairy	102	28.4	0.0	54
Traditional	144	63.8	19.0	75
Overall	482	46.1	7.7	74

Both *Fasciola* and amphistomes were prevalent in all villages and farms surveyed and the prevalence varied greatly among villages and farms in the same management system. Ifunda village had the highest prevalence of both *Fasciola* (73.2%) and amphistomes (79%); Ilula village had the lowest prevalence of *Fasciola* (14.8%) while Lulanzi village had the lowest prevalence of amphistomes (59.6%). There was no significant difference (P=0.91) on the prevalence of *Fasciola* between the highland zone (47.5%) and the central zone (48%), the prevalence of amphistomes was also not different between the highland and the central zone (60 Vs 60.9%, P=0.84).

Table 7. Prevalence of amphistomes among cattle management systems in Iringa District

Management system	Animals examined	Prevalence (%)		
		Overall	Minimum	Maximum
Large-scale dairy	236	55.5	4.0	100
Small-scale dairy	102	41.1	0.0	83
Traditional	144	81.9	67.0	92
Overall	482	62.6	23.6	92

The age-specific prevalence of *F. gigantica* among management system is shown in Table 8. There was an association between age and prevalence in animals (χ^2 =34.94, df=2, P=0.001). On large-scale dairy and traditional farms, adults (P=0.05) and weaners/yearlings (P=0.042) had higher risk of infection than calves. The age-specific prevalence of amphistomes among management systems is shown in Table 9.

The prevalence of amphistomes among management systems was associated with the age of animal (χ^2 =38.06, df=2, P=0.0001). The prevalence of amphistomes was highest in adults than in calves in large-scale and small-scale dairy farms. There was no significant association between sex and prevalence of *Fasciola* (χ^2 =0.64, df=1, P=0.42) or amphistomes (χ^2 =0.06, df=1, P=0.81).

Table 8. Age-specific prevalence of *Fasciola gigantica* among cattle management systems and age groups in Iringa District

Management system	Age					
	Adults		Weaners/Yearlings		Calves	
	No.	Prevalence (%)	No.	Prevalence (%)	No.	Prevalence (%)
Large-scale dairy	52	61.5	146	50.0	36	11.0
Small-scale dairy	54	42.6	17	0.0	29	13.7
Traditional	56	71.4	74	59.5	12	50.0
Total	162	58.5	249	36.5	65	24.9

No.=number of examined animals

Table 9. Age-specific prevalence of amphistomes among cattle management systems and age groups in Iringa District

Management system	Age					
	Adults		Weaners/Yearlings		Calves	
	No.	Prevalence (%)	No.	Prevalence (%)	No.	Prevalence (%)
Large-scale dairy	52	76.9	146	55.5	36	27.7
Small-scale dairy	54	59.3	29	23.5	29	13.8
Traditional	56	89.3	74	75.6	12	100.0
Total	162	75.2	249	51.5	65	47.2

No. =number of examined animals

4.3.2 Gastrointestinal nematodes

Clinical helminthosis occurred mainly in calves especially during the rainy season. The individual FEC in calves that showed clinical signs of helminthosis was > 500 EPG and therefore it was considered as a cut off point. The prevalence, intensity and the proportion of animals with FEC less than 500 EPG among management systems are shown in Table 10. There was a significant difference in FEC among management systems (P=0.0001). The traditional system had the highest mean FEC, while the small-scale dairy system had the lowest FEC. Pair wise comparison of FEC indicated that there was not difference between small-scale dairy and large-scale dairy systems. There was a significant difference in mean FEC among age groups (P=0.001). Calves had the highest mean FEC (280±66), weaners/yearlings were intermediate (191±22) while adults had the lowest mean FEC (78±10). Pair wise comparison of means indicated that FEC in calves and weaners/yearlings were not significantly different. There was no difference in FEC between male and female animals (175±24 vs. 165±22, P=0.76). The intensity of infection was not different between the highland and the lowland zone (P=0.81). Faecal egg counts varied significantly among villages (P=0.001), being highest at Ilula village (607±167) and lowest at Ifunda village (99±17). Overall, a high proportion (89.8%) of animals had low FEC (< 500 EPG). A moderate proportion (21.6%) of animals had FEC greater than 500 EPG and were mainly calves/weaners.

4.3.3 Faecal culture

The overall proportion of infective larvae from cultures among management systems indicated that the common nematodes were *Cooperia* spp (39.8%), *Oesophagostomum* spp (35.9%), *Haemonchus* spp (21.6%) and *Trichostrongylus* spp (2.5%). Other GI nematodes identified through egg morphology were *Trichuris* spp, *Capillaria* spp and *Strongyloides* spp. *Haemonchus* spp was isolated frequently in faecal cultures from dairy cattle while *Oesophagostomum* spp was most frequently isolated in pooled faecal cultures from traditional cattle.

Table 10: Prevalence and intensity of GI nematodes and the proportion of animals with FEC less than 500 EPG among cattle management systems

Management system	Animals examined	Prevalence (%)	Mean FEC ±se	Range	FEC <500 EPG (%)
Small-scale dairy	127	37.0	107±19	0-1200	100.0
Large-scale dairy	236	44.4	117±14	0-1300	94.4
Traditional farms	150	67.0	296 ±46	0-2900	80.0
Overall	513	50.0	167±16	0-2900	89.8

4.4 Epidemiology of gastrointestinal nematodes

4.4.1 Faecal egg counts (FEC)

The trend of faecal egg counts among management systems and age groups is shown in Fig 9 (note the different Y-axis scale for FEC in calves and note the representative rainfall pattern during the study on the graph for calves in the small-scale dairy system). There was a clear seasonal pattern and peak in FEC in large-scale and traditional management systems. Faecal egg counts increased during the rainy season (December-April) in calves and weaners. The small-scale dairy cattle generally had low FEC throughout the year, and without any peak in any age group. Adult cattle in all management systems had very low FEC and without any peak throughout the year. Faecal egg count was highest with a remarkable peak in calves and weaners/yearlings on large-scale and traditional farms. The period where peak FEC occurred varied considerably among management systems. Faecal egg counts in traditional farms peaked during the early part of the rainy season (February) while on large-scale dairy farms the peak occurred towards the end of the rainy season (April). There was a significant difference in FEC among management systems (P=0.001) with traditional cattle having the highest FEC. There was a significant difference in FEC among age groups (P=0.0001) and calves had the highest overall FEC.

Faecal egg counts in all management systems were generally low with a high proportion of animals (85.5%) having FEC less than 500 EPG. Animals with FEC greater than 500 EPG were mainly calves (24.2%). Out of 49 calves, clinical helminthosis occurred in 13 (27%) calves mainly during the rainy season and necessitated anthelmintic treatments. The body condition score in adult animals was good during the rainy season and deteriorated in most animals during the dry season, especially on traditional and small-scale dairy farms. Faecal egg counts varied significantly among villages (P=0.003), Ilula village had the highest FEC throughout the year while FEC was lowest at Ifunda village during most times of the year. There were significant interactions between age and management, age and month (season) and also between age, management and month ($P<0.05$). The highland zone had higher FEC than the central zone but the difference was not significant (P=0.07).

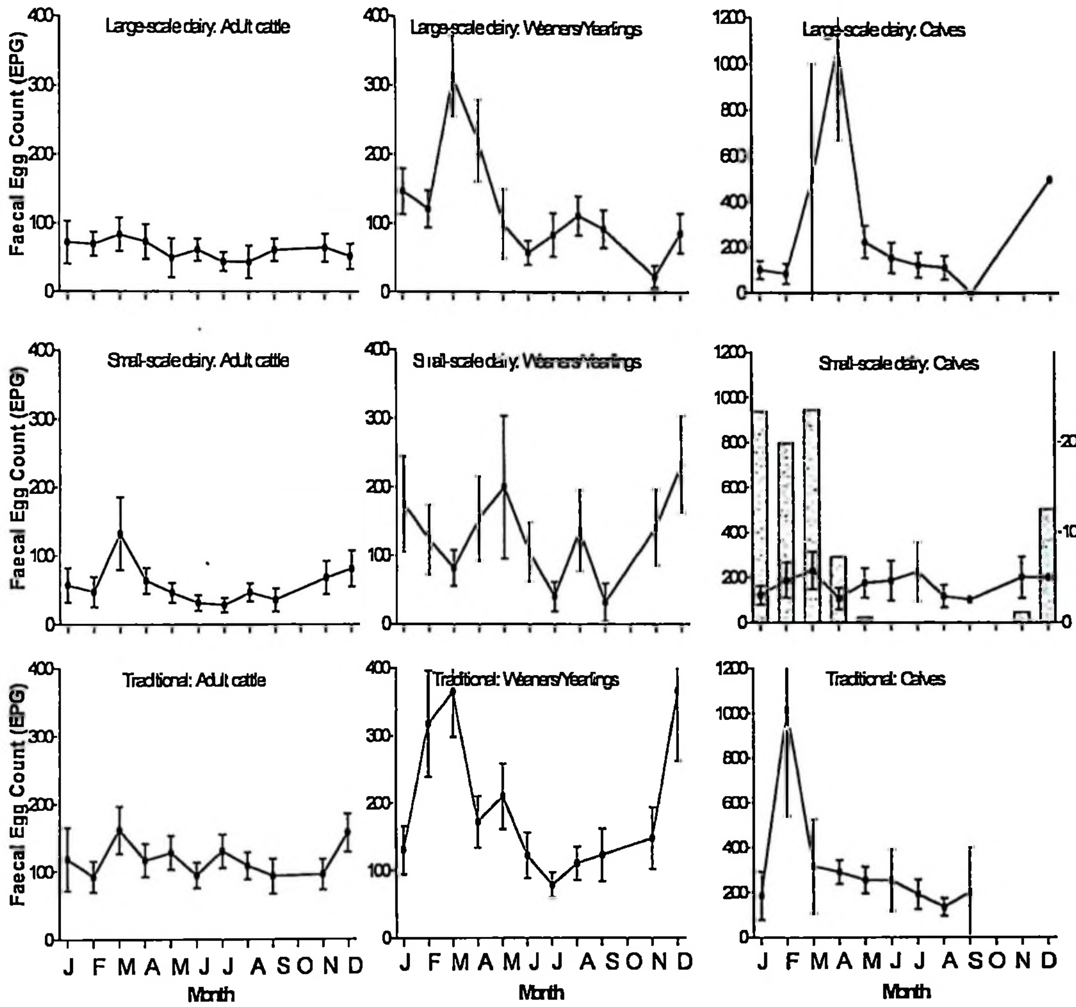


Fig 9. Patterns of faecal egg counts (mean ±se) in cattle within and between age groups of cattle and management systems in Iringa District during a period of 12 months (Jan-Dec 2002)

NB: Note the different scale for the Y-axis on graphs for FEC in calves and also note the representative rainfall pattern during the study on the graph for calves in the small-scale dairy system

4.4.2 Worm counts in tracers

One tracer died in March at Tommy dairy farm due to East Coast Fever (ECF) and another one died at Lulanzi village in September due to progressive loss of body condition. Worm counts in tracers on traditional and large-scale dairy farms during the year are shown in Fig 10. The worm burdens in tracers at the large-scale dairy farm increased gradually through the rainy season and peaked towards the end of the rainy season (April). The worm burden of tracers at the traditional farm was generally low. It was difficult to maintain Ayrshire tracers in communal grazing areas, they had a different grazing behaviour and there were times where they were lost or grazed in different areas. The worm burden of tracers at the large-scale dairy farm generally followed the rainfall pattern, with high worm burdens occurring during the rainy season.

The worm species composition and proportion in tracers between management systems is show in Table 11. Of the total worms recovered in tracers, the predominant parasites were *Cooperia* spp (51.6%), *Oesophagostomum radiatum* (35.7%) and *Haemonchus placei* (10.2%). *Ostertagia ostertagi* was recovered and confirmed for the first time in Tanzania. The total worm burden was dominated by *Cooperia* spp in the large-scale dairy farm (83%) while *Oesophagostomum radiatum* was dominant in traditional cattle (60.8%). Four immature *Calicophoron microbothrium* were isolated in one tracer in July while four immature *Fasciola gigantica* were recovered in one tracer in September. *Trichuris globulosa* was isolated in two tracers in July and each tracer had four worms. Adult worms of *Cooperia* spp; *Haemonchus placei* and *Oesophagostomum* *radiatum* were recovered from tracers throughout the dry season. However, the proportion of adult *Haemonchus placei* decreased in tracers on traditional grazing areas during the dry season.

Gastrointestinal parasites identified through egg morphology but were not recovered in tracers were *Nematodirus* spp, *Strongyloides* spp (up to 300 EPG), *Toxocara vitulorum* (up to 7100 EPG) and *Capillaria* spp (up 500 EPG). Eggs of *Toxocara* spp and *Strongyloides* spp were only present in calves. Eggs of *Moniezia* spp (up to 1500 EPG) were present in faeces of few animals in all age groups.

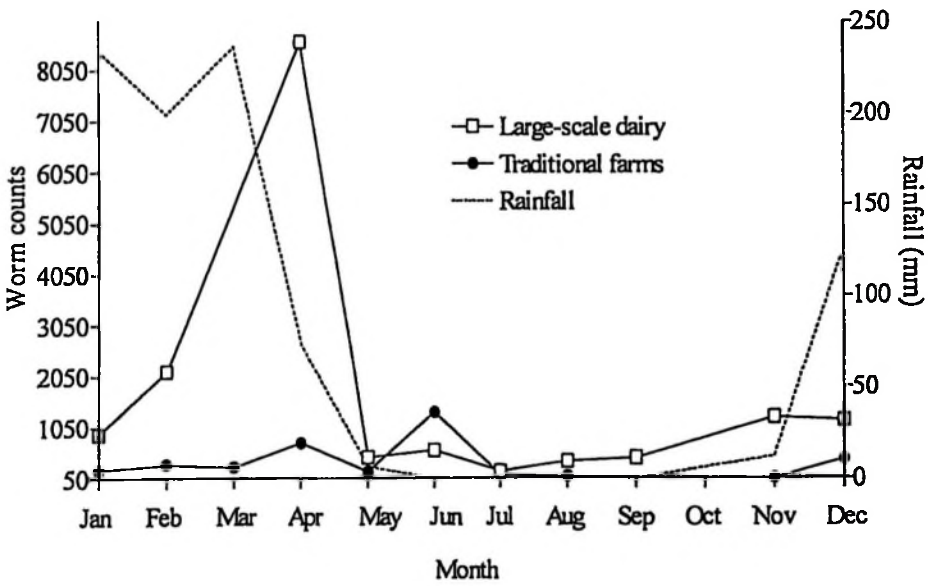


Fig 10. Patterns of worm burdens in tracer calves under different management systems in Iringa District during a period of 12 months (Jan - Dec 2002)

Note: It was difficult to maintain Ayrshire tracers in traditional communal grazing areas; they grazed abnormally, frequently went astray or even got lost

Table 11: Mean nematode species composition (%) for worm burdens in tracer calves on dairy and traditional farms in Iringa District during a period of 12 months

Nematode specie	Management system		Overall
	Large-scale dairy	Traditional	mean
	(%)	(%)	(%)
Haemonchus placei	6.7	13.7	10.2
Cooperia pectinata	33.0	16.8	24.9
Cooperia punctata	39.0	2.7	20.8
Cooperia spatulata	11.0	0.8	5.9
Oesophagostomum radiatum	10.5	60.8	35.7
Trichostrongylus colubriformis		2.4	1.2
Ostertagia ostertagi		1.3	0.7
Trichuris globulosa		1.2	0.6

4.4.3 Pasture larval counts

The number of infective larvae (L_3) on pasture at the large-scale dairy farm and the traditional communal grazing area is shown in Fig 11. The number of infective larvae on pastures followed the rainfall pattern and was similar to the pattern of FEC and tracer worm counts. Pasture infectivity peaked towards the end of the rainy season (April) on the communal grazing land. Infective larvae on pastures on the large-scale dairy farm peaked in the mid rainy and the end of the rainy/early dry season. Infective larvae on pasture were very low to virtually zero on the communal grazing area during the last part of the dry season (September-November). The common infective nematode larvae identified were *Cooperia* spp, *Oesophagostomum* spp and *Haemonchus* spp in the order of decreasing abundance.

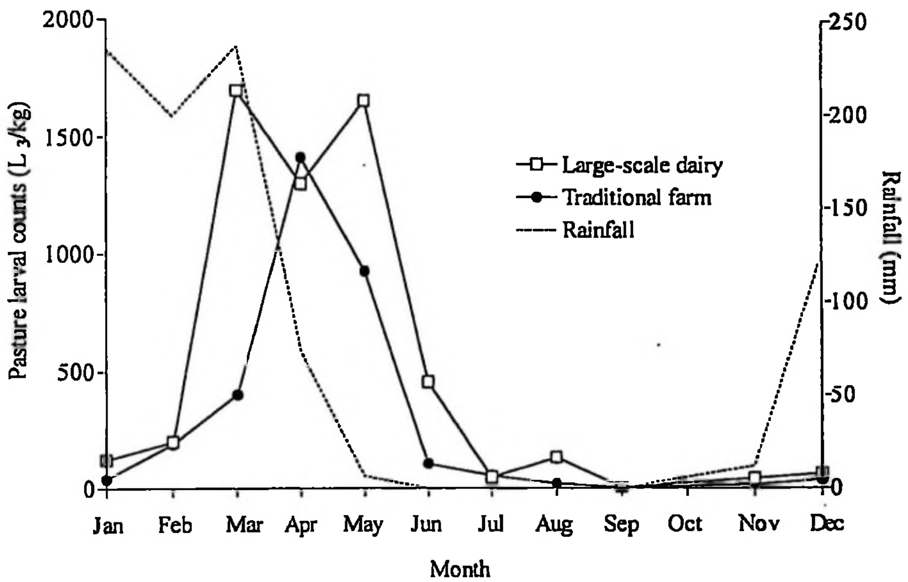


Fig 11. Patterns of larval counts on large-scale dairy and traditional communal grazing area in Iringa district during a period of 12 months (Jan-Dec 2002)

4.4.4 Packed cell volume (Haematocrit)

The pattern of mean packed cell volume (PCV) in cattle in the three management systems is shown in Fig 12. Packed cell volume was high in cattle in all management systems during the rainy season and decreased after the rainy season and in the last three months of the dry season. The indigenous Zebu cattle had higher PCV than exotic or crossbred cattle in dairy farms in most times of the year. There was no significant difference in PCV among age groups (P=0.06) and there was no difference in PCV between male and female animals (P=0.13). Generally, animals in all management systems maintained a good PCV throughout the year. The correlation between PCV and faecal egg counts (FEC) was weakly negative and not significant (r=-0.04, P=0.08).

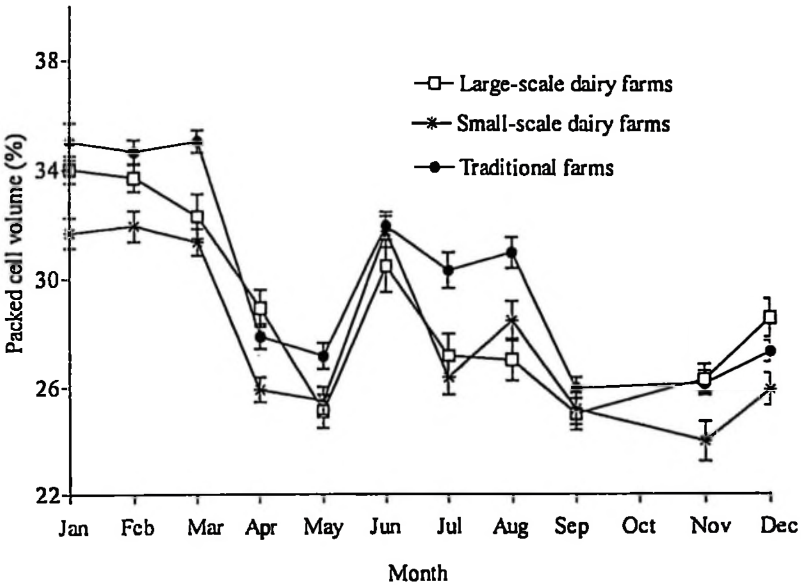


Fig 12. Patterns of packed cell volume in cattle under different management systems in Iringa District during a period of 12 months (Jan-Dec 2002)

4.4.5 Threshold for treatment and cost-effectiveness of anthelmintic treatments

Based on faecal egg counts, clinical signs, body condition score and taking into consideration the low fecundity of the predominant parasite specie (*Cooperia* spp, 200 eggs /day). Salvage anthelmintic treatments were done mainly in calves; out of 49 calves, 17 (35%) were treated when FEC > 500 EPG. When animals were categorised into age groups, FEC showed that pasture contamination attributed to adult cattle was very low. The mean and standard error of pasture contamination due to one calf (247±30 EPG) was equivalent to four adult cattle (60±4 EPG). When albendazole bolus or most other anthelmintics were used, the cost of treatment of one adult cow (1500 Tshs) was equivalent to the cost of treating 6-8 calves (250 Tshs). Analysis of costs of treatment indicated that despite the low contribution of adults to total pasture contamination, adults used about 67% of the money or anthelmintic requirement for the whole herd, mainly due to their high body weights.

4.5 Epidemiology of *Fasciola* and amphistomes

4.5.1 Epidemiology of *Fasciola*

The proportion of animals excreting *Fasciola* eggs in faeces among management systems and age groups is shown in Fig 13 (note the representative rainfall pattern during the study on the graph for calves in the small-scale dairy system). The proportion of egg positive animals was high in the traditional system on all age groups throughout the year. Only adults and weaners/yearlings in large-scale dairy farms had a high prevalence throughout the year. Except for adults that had a moderate proportion, animals passing *Fasciola* eggs in the small-scale dairy system were very low throughout the year. There was a seasonal trend in the proportion of animals passing *Fasciola* eggs. The proportion of egg positive animals decreased toward the end of the rainy season, then increased gradually through the dry season and peaked toward the beginning of the rainy season (December).

The prevalence generally was associated to the type of management especially the grazing practice (χ^2 =124, P=0.001, df=2). The annual prevalence was highest in the traditional system (44.9%), followed by large-scale dairy system

(30.2%) and lowest on small-scale dairy system (17.8%). There was a significant association between the prevalence and the age of animal (χ^2=72, P=0.001, df=2). In all management systems, adults had the highest annual prevalence (40.4%); weaners/yearlings were intermediate (27.8%) while calves had the lowest prevalence (13.6%). The prevalence was higher in the central zone than in the highland zone (44.9 vs. 24%). The prevalence varied greatly among large-scale dairy farms and villages. Mshughulika had the highest annual prevalence (69.1%) among farms while Ifunda had the highest annual prevalence (62.2%) among villages.

4.5.2 Epidemiology of amphistomes

The proportion of animals excreting amphistome eggs over time among management systems and age groups is shown in Fig. 14 (note the representative rainfall pattern during the study on the graph for calves in the small-scale dairy system). The proportion of egg positive animals was high in the traditional system in all age groups throughout the year. Only adults and yearlings on large-scale dairy farms had a high prevalence during most periods of the year. Except for adults that had a moderate portion, the proportion of animals passing amphistome eggs in faeces in small-scale dairy cattle was low throughout the year.

There was a difference in the proportion of egg positive animals among management systems and was associated to the type of management (χ^2=388, df=2, P=0.001). The traditional management system had the highest annual prevalence (82.7%); the large-scale-dairy system was intermediate (47.4%) while the small-scale dairy system had the lowest annual prevalence (35.9%). The prevalence of amphistomes was significantly associated to the age of animal ($\chi^2=80.9$, df=2, P=0.001). In all management systems, adults had the highest annual prevalence (65.4%); weaners/yearlings had a moderate prevalence (52%) while calves had the lowest prevalence (40%). The annual prevalence in the central zone (66.5%) was higher than the highland zone (52%). The prevalence of amphistomes also varied greatly among farms and villages; Mshughulika had the highest annual prevalence (91.3%) among farms while Ifunda had the highest annual prevalence (78.5%) among villages. The proportion of animals excreting amphistome eggs in faeces was always higher than that of animals excreting *Fasciola* eggs in all zones, villages, management systems, farms, sex and age groups.

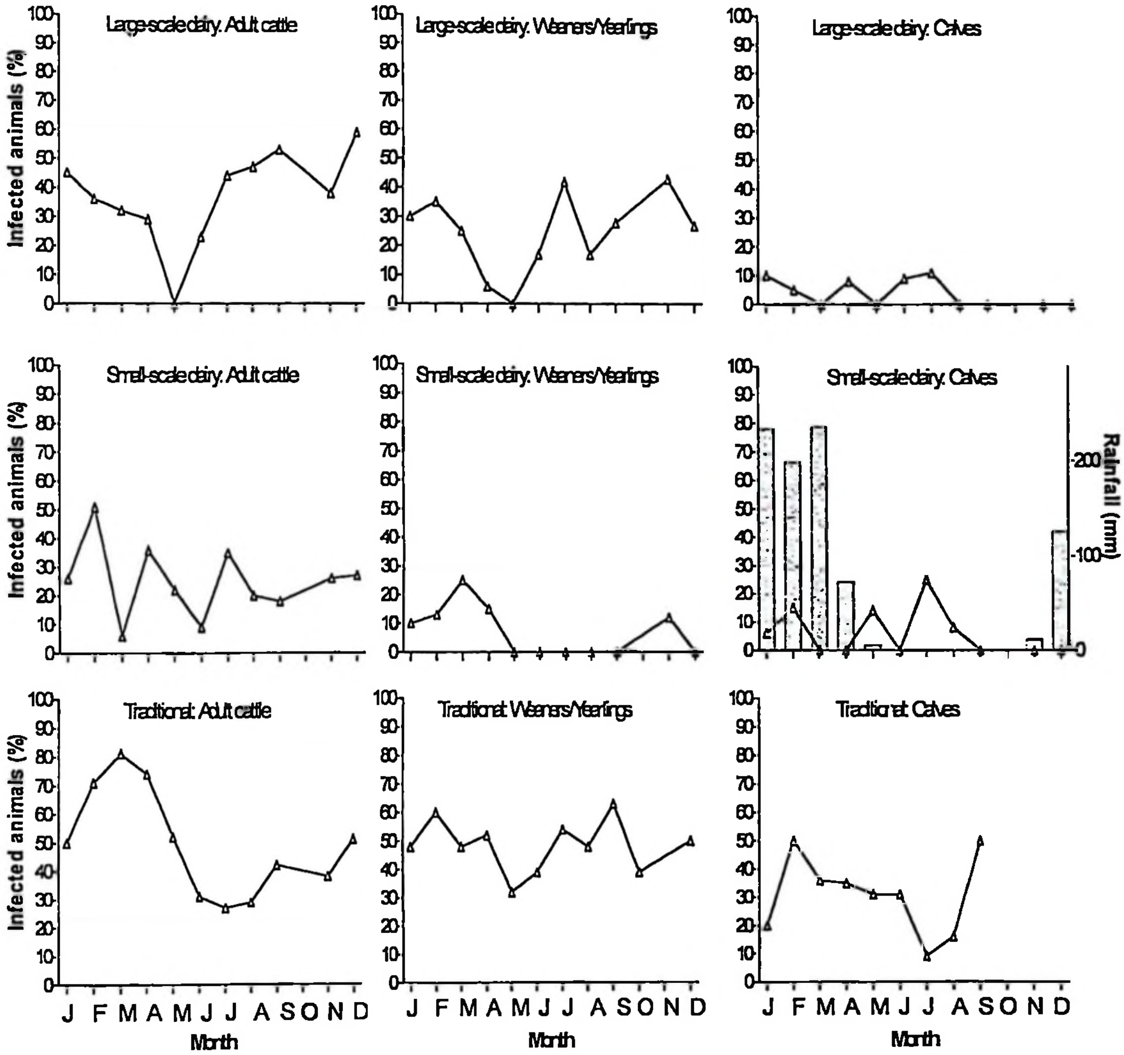


Fig 13. Proportion of cattle (%) excreting *Fasciola* eggs within and between age groups and management systems in Iringa District during a period of 12 months (Jan-Dec 2002)

NB: Note the representative rainfall pattern during the study on the graph for calves in the small-scale dairy system

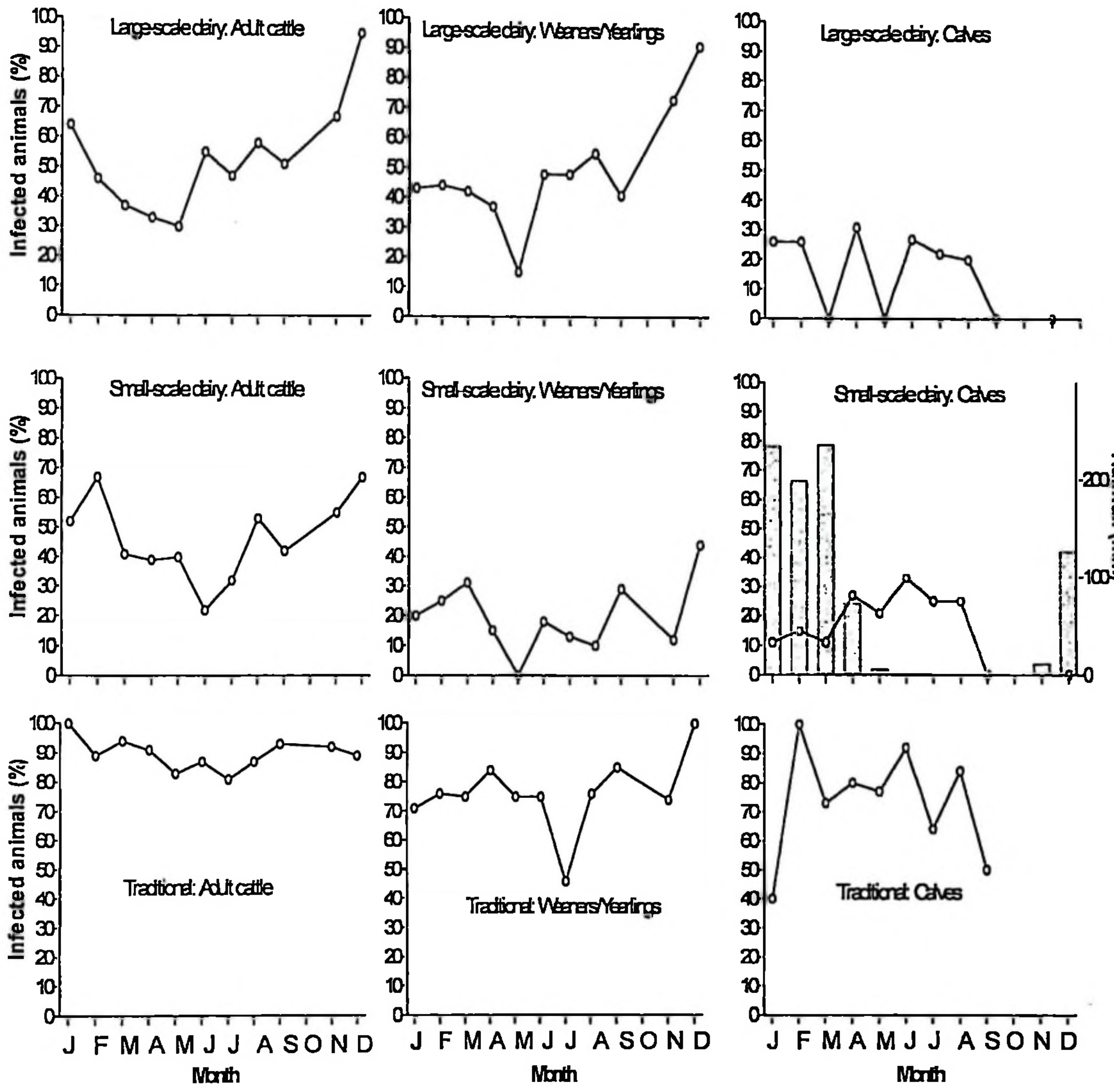


Fig 14. Proportion of cattle (%) excreting amphistome eggs within and between age groups and management systems in Iringa District during a period of 12 months (Jan-Dec 2002)

NB: Note the representative rainfall pattern during the study on the graph for calves in the small-scale dairy system

4.6 Efficacy of drugs against *Fasciola* and amphistomes

The number of animals that were excreting *Fasciola* eggs in faeces over time after treatment with anthelmintics commonly used against *Fasciola* in Iringa District is shown in Table 12. Triclabendazole, nitroxynil and ivermectin-clorsulon were 100% effective based on the presence of *Fasciola* eggs in faeces 2 weeks after treatment. One to two (14-33%) animals in groups treated with levamisole-oxyclozanide or albendazole were egg positive 2 weeks days after treatment. The number of animals passing *Fasciola* eggs increased steadily over time in groups treated with levamisole-oxyclozanide or albendazole, and most animals (86%) were passing *Fasciola* eggs 10 weeks after treatment. A reduced efficacy of albendazole and levamisole-oxyclozanide (Milsan® and Levoxy®) preparations against *Fasciola* was suspected.

The number of animals that were excreting amphistome eggs over time after treatment with anthelmintics commonly used against flukes in Iringa is shown in Table 13. Two weeks after treatment, two to three animals (29-43%) in groups treated with levamisole-oxyclozanide formulations were passing amphistome eggs in faeces. The number of animals excreting eggs then increased steadily over time and most animals (43-71%) were egg positive ten weeks after treatment. Triclabendazole, nitroxynil and ivermectin-clorsulon had no activity at all against amphistomes and treated animals continued to excrete amphistome eggs throughout the study period. A reduced efficacy of levamisole-oxyclozanide preparations (Milsan® or Levoxy®) against amphistomes was suspected at Amani dairy farm.

Table 12: The number of animals positive for *Fasciola* eggs in faeces after treatment with flukicides over time

Anthelmintic	Number of positive animals			
	Day 0	2 weeks	6 weeks	10 weeks
Levamisole-Oxyclozanide (Milsan®)	7	1 **	2	4
Levamisole-oxyclozanide (Levoxy®)	7	1 **	3	6
Albendazole bolus	6	2	3	5
Albendazole (Farbenda®)	7	2 *	3	5
Ivermectin-clorsulon (Ivomec-Super®)	6	0 ***	1*	1*
Nitroxynil (Trodax®)	6	0***	0***	1*
Triclabendazole (Fasinex®)	7	0***	0***	0***

*=P<0.05 **=P<0.01 ***P=<0.001 (egg positive animals compared to day 0)

Table 13: The number of animals excreting amphistome eggs in faeces after treatment with flukicides over time

Type of anthelmintic	Number of positive animals			
	Day 0	2 weeks	6 weeks	10 weeks
Levamisole-Oxyclozanide (Milsan®)	7	2	2	3
Levamisole-oxyclozanide (Levoxy®)	7	3	4	5
Ivermectin-clorsulon (Ivomec-Super®)	6	6	6	6
Nitroxynil (Trodax®)	6	6	6	6
Triclabendazole (Fasinex®)	7	6	6	6

4.7 Strategic treatment of flukes and GI nematodes at the end of the rainy/early dry season

4.7.1 Strategic treatment with Triclabendazole (Fasinex®)

None of the treated animals on both farms showed clinical signs of fasciolosis during the study. The proportion of animals on the traditional farm (Chelestino) that were excreting *Fasciola* or amphistome eggs in faeces over time after treatment with triclabendazole is shown in Fig 15. Triclabendazole was effective against *Fasciola* in all (100%) treated animals and none of them passed eggs in faeces up to four months following treatment (May- September). Two out of 13 (15.4%) treated animals started to excrete *Fasciola* eggs in September and the proportion increased steadily over time such that 7 out of 13 (54%) treated animals were egg positive at the end of the dry /early rainy season (December). Thirteen out of 14 (93%) treated animals were egg positive during the early part of the rainy season (February). The mean monthly infection rate of *Fasciola* was 16% at the beginning of the dry season (June) and 38.4% toward the end of the dry season (November). Triclabendazole was not effective at all against amphistomes. Most of treated animals (78.6–100%) continued to excrete amphistomes eggs throughout the study period.

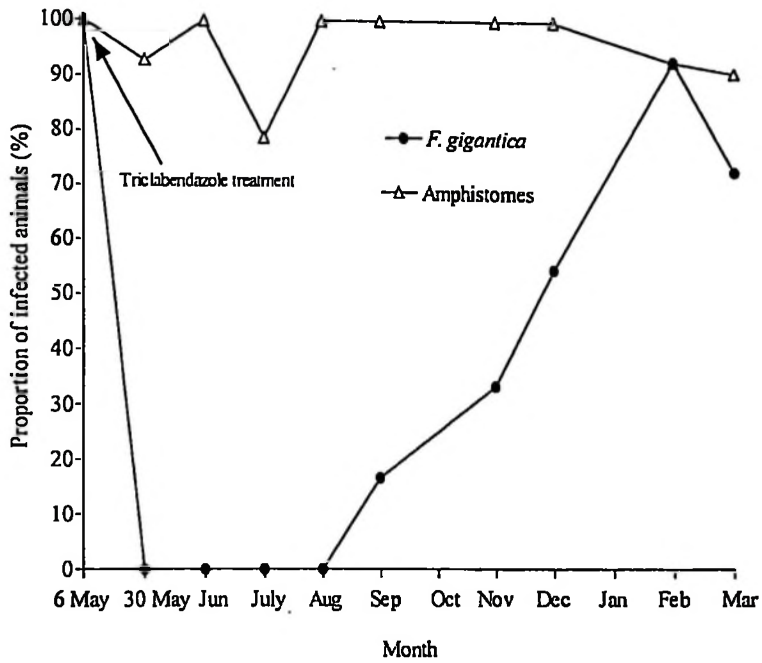


Fig 15. Proportion of cattle (%) excreting *Fasciola* and amphistome eggs after strategic treatment with triclabendazole at the traditional farm in Iringa District

4.7.2 Strategic treatment with albendazole (Tramazole®)

The proportion of animals at the beef farm (Mshughulika) that were excreting *Fasciola* or amphistome eggs in faeces over time after treatment with albendazole (Tramazole®) is shown in Fig 16. Two out of 19 (11%) treated animals were positive for *Fasciola* eggs within three weeks after treatment. The proportion then increased steadily over time such that 12 out of 18 (67%) treated animals were passing *Fasciola* eggs three months after treatment. Most treated animals (85-95%) were egg positive toward the end of the dry/early rainy season (November/December). The monthly infection rate was 11.7% at the beginning of the dry season and 38.6% at the end of the dry season. Albendazole at the given increased dosage was not effective against amphistomes and almost all animals (77.8-100%) passed amphistome eggs in faeces throughout the study.

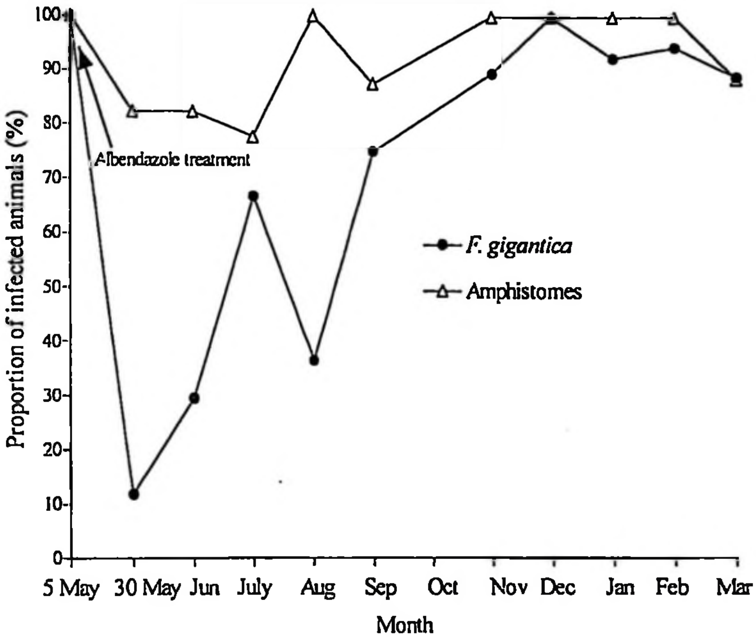


Fig 16. Proportion of cattle (%) excreting *Fasciola* and amphistome eggs after strategic treatment with albendazole at the beef farm in Iringa District

Faecal nematode egg counts and PCV pattern after strategic treatment with albendazole (Tramazole®) at the beef farm are shown in Fig 17. Faecal egg counts dropped sharply to low levels after treatment and remained low (<200 EPG) throughout the dry season. Faecal egg counts started to increase gradually at the beginning of the rainy season but dropped following routine treatment of all animals by the owner in January, and later increased during the rainy season (March). Packed cell volume was normal throughout with a slight decline during the dry season.

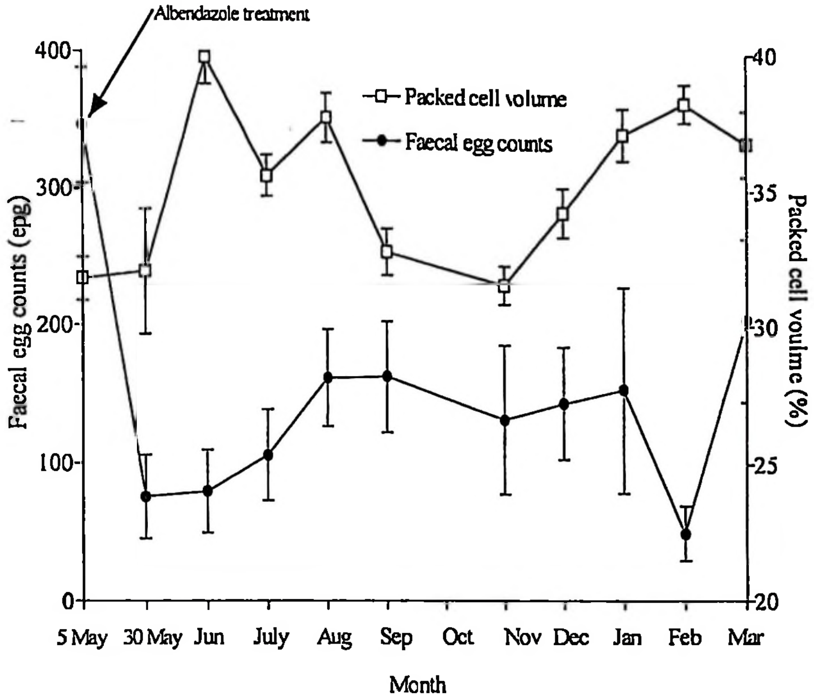


Fig 17. Nematode faecal egg counts and packed cell volume over time in cattle after strategic treatment with albendazole at the beef farm in Iringa District

CHAPTER 5

DISCUSSION

5.1 Worm control practices and anthelmintic usage

The survey has shown that worm control in all management systems was mostly based on the use of anthelmintics. The results are similar to worm control studies in other countries in different classes of livestock (Kinoti *et al.*, 1994; Maingi *et al.*, 1996; Charles and Furlong, 1996; Maingi *et al.*, 1997). Anthelmintic treatments in almost all farms surveyed depended on the availability of money or drugs and not the epidemiology of parasites. Epidemiological studies in most tropical countries have indicated high burden of gastrointestinal nematodes during the rainy season and lowest during the dry season (Kaufmann and Pfister, 1990; Moyo *et al.*, 1996; Waruiru *et al.*, 2001). Peak transmission of trematodes in the district has been reported to occur at the end of the rainy season (Makundi, 2001) with an increase in the prevalence of *Fasciola* at the end of the dry season (Mahlau, 1970). Therefore, there is a great potential for utilisation of the epidemiological knowledge in better targeting of anthelmintic treatments in Iringa District as opposed to the observed situation where most treatments were based on availability of money or drugs.

Similar to results in Brazil by Charles and Furlong (1996), the number of anthelmintic treatments in this survey was surprisingly high for resource poor farmers to buy these drugs especially in the small-scale dairy production system. The high frequency of treatment might be related to advise from extension workers where farmers were advised to deworm cattle at least four times a year regardless of the epidemiological pattern and management system. Results from this survey in animals randomly sampled during the rainy season have indicated that such frequency is not justifiable, and there is a good opportunity for farmers to save money by avoiding unnecessary treatments. The study has also shown that the frequency of treatment was the same in pasture grazed large-scale dairy and zero-grazed small-scale dairy cattle. Though the need to treat zero-grazed animals is debatable, this survey has highlighted a justification only in animals fed pastures obtained from contaminated areas by traditional or untreated cattle. However, the number of treatments per year even in that situation has to be low compared to other management systems.

This study has shown that rural small-scale dairy and traditional holders used the majority of cheap anthelmintics, and used them for several years due to limited access and information on anthelmintics. The multitude of anthelmintic brands, service providers, drug shops and drug vendors revealed in the survey as a result of trade liberalisation has indicated a great need for quality assurance of anthelmintics in urban and rural areas. Despite the fact that the study area was a trematode enzootic area (Mahlau, 1970; Makundi *et al.*, 1998), the survey indicated that 17.4% of farmers used anthelmintics that were only effective against nematodes to control all helminths. The belief that any anthelmintic can kill all types of helminths and all stages might be contributing to maintenance of trematodes in the area. Therefore, there is a great need to educate farmers and extension officers on appropriate anthelmintics to be used in their locality and there should be regular seminars and visits in rural areas by state veterinarians.

The study has indicated that worm control programmes through farmer's organisations can be a 'turning point' towards rational worm control at a community level. This is from the fact that they offer prospects towards reduction of the frequency of treatments, interrupted treatments, overprotective treatments and prolonged use of the same anthelmintic. From the fact that animals shared the same pasture resources especially during the dry season, there is a need to link or coordinate dairy and traditional farmers at a community level. The lack of withdraw period in treated animals on traditional farms has indicated a need for education of farmers and village extension workers on recommended anthelmintics to use in lactating cows, as well as to abide on milk and meat clearance period for each anthelmintic. The high proportion (39%) of farmers that had at one time used traditional medicines to control worm infection in cattle has indicated a need to obtain more information on farmer's indigenous knowledge on anthelmintics, and to conduct some *in vitro* and *in vivo* efficacy studies on a number of herbal anthelmintics mentioned in this survey.

5.2 Abattoir survey of GI nematodes in indigenous Zebu cattle in the lowland zone

The study has indicated a high prevalence and a light to heavy worm burden of GI nematodes in indigenous Zebu cattle in the lowland zone, and therefore a need for preventive anthelmintic treatments especially in immature animals. The overall prevalence (97.2%) is in agreement with studies in other countries with a savannah type of climate (Kaufmann and Pfister, 1990). Most of the parasites recovered in the study have been reported in Tanzania (Ecimovic and Mahlau, 1973; Mellau, 1997) and in other tropical countries (Moyo *et al.*, 1996; Waruiru *et al.*, 1998). The study has indicated that *Haemonchus placei* and *O. radiatum* were the most prevalent species but the cattle harboured large numbers of *Cooperia* spp. This is in contrast to the situation in eastern Tanzania where *Haemonchus* spp predominated (Mellau, 1997).

The worm burden in most cattle was light, probably because the animals examined in our study were indigenous Zebu cattle (*Bos indicus*), which are relatively resistant to helminthosis (Williamson and Payne, 1978) compared to crossbred and exotic (*Bos taurus*) cattle. The low worm burden might also be due to the fact that only healthy animals are trekked long distances for sale in livestock markets leaving sick animals at the farm (Makundi *et al.*, 1998) or probably due to anthelmintic treatments given prior to sale in order to improve body condition score. Moreover, the worm burdens were only based on GI contents and washings of the gut with no digests of the GI mucosa to recover immature or arrested larvae; therefore, the worm burden might be underestimated.

The negative association between the grade of the live animal and worm burden in immature animals in our study suggested that immature animals have a high risk of clinical helminthosis. Therefore, anthelmintic treatments in Zebu cattle should be especially directed toward this age group. The high prevalence of *O. radiatum* observed and the presence of nematodes with a high pathogenic index (*B. phlebotomum* and *H. placei*) in the lowland zone necessitates institution of a sound worm control programme in Zebu cattle. Contrary to Lima (1998) and Waruiru and colleagues (1998), who reported the highest worm burden during the rainy season, the highest worm burden in this slaughterhouse survey occurred at the end of the rainy/ early dry season, persisting into the mid-dry season. However, the results concur with those reported in the Eastern zone of Tanzania by Mellau (1997), where the highest worm egg counts, clinical helminthosis and calf mortality on dairy farms occurred during the dry season. The high worm burden during the early dry and mid-dry season in our study might be due to the grazing cycle in the lowland zone during the dry season, whereby grazing is concentrated in flood plains, valleys and riverbanks for pasture and water. Such a grazing pattern extendeds the season during which the pastures on which the cattle actually graze are still favourable for survival and development of nematode larvae. The increased grazing pressure in those areas will have resulted in high pasture contamination with worm eggs. Moreover, the high worm burden might be the survivors from infections acquired during the late rainy season, because the animals rarely received anthelmintic treatment.

The highest worm burden at the end of the rainy/early dry season in our study has indicated that pasture contamination and acquisition of worm infection was slow and gradual and that the peak possibly occurred towards the end of the rainy season. Due to virtual absence of pasture during the dry season in semi-arid communal grazing lands, it usually takes time for pasture to flourish and become contaminated after commencement of the rainy season. The fact that the parasite load was mainly composed of *Cooperia* spp that characteristically has low fecundity (Honer *et al.*, 1992) might also have contributed significantly to a delayed peak in pasture infectivity. Peak pasture contamination at the end of the rainy season has also been reported in Nigeria (Chiejina and Emehelu, 1986) while the highest worm burden during the dry season has been reported in a semi- arid district in Kenya (Omara-Opyene, 1985). At the end of the dry season, the worm burden was very low because the rift valley and the flood plain were too dry for the survival and development of infective larvae.

Results from this study have indicated that the common practice whereby most treatments are concentrated during the rainy/wet season might not be optimal in the lowland zone. This is due to the likelihood of subclinical or clinical helminthosis that occur during the dry season, being exacerbated by inadequate and poor pasture and the fact that there is no supplementation in indigenous Zebu cattle. Treatment of nomadic and semi-nomadic herds might be difficult and time consuming due to continuous movement of animals during the dry season. However, the grazing cycle and pasture growth pattern make them easily accessible at certain times of the year. During the rainy season most herds are in nearby high grounds due to plenty of pasture and the fact that the lowland zone is mostly flooded.

The trend in worm burdens in slaughtered cattle has indicated that two strategic treatments might help to reduce morbidity and mortality especially in immature cattle in the zone. One anthelmintic treatment at the end of the rainy/early dry season (May/June), before nomadic herds move away, might help to prevent outbreaks of helminthosis during the dry season. A second treatment at the end of the dry/beginning of the rainy season (November/December) when most herds are back in nearby high grounds might help to reduce carry-over of infection into the next rainy season. However, the strategic treatment at the end of the dry season would be effective only if the drug used is efficient against inhibited larvae and that all animals on pasture are treated. An additional treatment might be necessary in young animals (calves/weaners) in the middle of the rainy season (February/March).

5.3 Cross sectional prevalence of helminth infections

The study has indicated a significant influence of management practices especially the grazing/feeding practice on the prevalence of flukes and GI nematodes. Moreover, the study indicated a high prevalence and widespread distribution of flukes and a moderate prevalence and low intensity of GI nematodes. The overall prevalence of *Fasciola* in traditional cattle (63.8%) is higher than reported previously in indigenous Zebu cattle in Iringa (Mahlau, 1970) and Mbeya (Ecimovic and Mahlau, 1973) regions in the Southern highlands of Tanzania. The high prevalence is in line with reports in other tropical countries (Ogambo-Ongoma, 1972; Silangwa, 1973; Schillhorn van Veen *et al.*, 1980; Tembely *et al.*, 1988; Heinonen *et al.*, 1995). The high and alarming prevalence of flukes in a number of farms has indicated that infection with flukes is a growing problem in the Southern highlands of Tanzania.

The moderate to high overall prevalence of *Fasciola* (28.4%) and amphistomes (41.1%) in adult zero-grazed cattle has given some indications for anthelmintic treatments in zero-grazed cattle fed pastures from contaminated areas. The 'cut and carry' system of pastures from valleys grazed by traditional cattle during the dry season might be responsible for infection of zero-grazed animals. Farmers should be educated on the importance of pasture plots and dry season feed reserves as means to ensure safe pasture for zero-grazed cattle. The high prevalence and intensity of flukes and GI nematodes in traditional cattle concur with studies in other tropical countries (Kaufmann and Pfister, 1990; Anene *et al.*, 1994; Moyo *et al.*, 1996). The high prevalence might be a reflection of management practices; dairy farms had improved management with routine anthelmintic treatments that may have reduced the prevalence and burdens unlike in traditional farms. The high stocking density in communal grazing and watering areas might have facilitated pasture contamination and ingestion of infective stages by grazing animals in traditional cattle. Under communal grazing, even if few farmers treated, the effectiveness in reducing pasture contamination and snail infection was negligible unless a high proportion of animals were treated.

The study has indicated an alarming prevalence of amphistomes in grazing animals. The higher prevalence of amphistomes than *Fasciola* concurs with other studies in Tanzania (Nyundo, 1994) and France (Szmidt-Adjidé *et al.*, 2000; Mage *et al.*, 2002). The high prevalence of amphistomes in the present study might be due to the high biological potential of the intermediate snail host (Dinnik, 1964), control measures being directed mainly against *Fasciola* and GI nematodes and the lack of effective drugs against amphistomes (Mage *et al.*, 2002). The higher prevalence of flukes in adults than young animals concurs with other studies in Tanzania (Mahlau, 1970), Zambia (Silangwa, 1973) and Nigeria (Schillhorn van Veen *et al.*, 1980) and might be related to longer exposure time during the animals' life for adults (cumulative worm burdens with age). It may also be due to the fact that adults rarely received anthelmintic treatments on traditional farms as shown in the study on anthelmintic usage. Moreover, it might be related to the management system, whereby adults were trekked long distances to valleys, flood plains or swampy areas during the dry season therefore exposing them to metacercariae contaminated pastures.

Most of nematode parasites recovered in faecal cultures have been reported previously in Tanzania (Ecimovic and Mahlau, 1973; Mellau, 1997) and in other tropical countries (Moyo *et al.*, 1996; Waruiru *et al.*, 2001) where *Haemonchus* spp *and Cooperia* spp were the main genera. The low FEC especially in adult cattle concurs with other studies (Stuedemann *et al.*, 1989; Couvillion *et al.*, 1996; Nodtvedt *et al.*, 2002). The higher FEC in calves than adults concurs with other studies (Snyder, 1993; Anene *et al.*, 1994). The high FEC in calves might be due limited previous exposure and immaturity of the immune system that resulted into a large proportion of ingested larvae to develop into adults.

5.4 Epidemiology of gastrointestinal nematodes

Results of faecal egg counts, pasture larval counts and tracer worm counts have indicated that GI nematode infection in the district has a seasonal pattern. The highest FEC, pasture larval counts and tracer worm counts during the rainy /late rainy season and lowest during the dry season concur with studies in other tropical countries with a distinct rainy and dry season (Agyei, 1991; Moyo *et al.*, 1996; Waruiru *et al.*, 2001). The difference in the time of peak FEC among production systems might be related to variation in management practices. The early peak of FEC in traditional cattle might be due to pasture contamination derived from the late dry season where grazing of animals was concentrated in flood plains, valleys and marshy areas. The late peak in FEC in large-scale dairy cattle and the highest pasture larval counts and worm counts toward the end of the rainy season in both management systems might be due to low fecundity of the dominant nematode specie e.g. *Cooperia* spp, 200 eggs/day (Honer *et al.*, 1992) or many treatments during the rainy season that suppressed pasture contamination.

The study has indicated a remarkable difference between pasture infectivity and worm burden in tracers in communal grazing areas. The low worm burdens in tracers grazed on communal land might be due to abnormal grazing behaviour of tracers in these areas, as they usually went astray or even got lost for some days. Therefore, pasture larval counts probably provided more reliable information on pasture infectivity on traditional communal grazing areas than the worm burdens in tracers. Similar to the findings of Anene *et al.* (1994) and Waruiru *et al.* (2000), FEC in all management systems was generally low during most periods of the year. The low FEC in this study might have been exacerbated by the fact that farmers continued with their routine anthelmintic treatments during the study, and therefore the prevalence and intensity might be higher than obtained in the present study. Moreover, the shorter stay of tracers on pasture (30 days) and the abnormal grazing behaviour in communal areas probably contributed to low worm burdens in these animals. The high FEC in calves on large-scale dairy and traditional cattle are in line with most studies (Snyder, 1993; Anene *et al.*, 1994; Moyo *et al.*, 2003), and has indicated that strategic treatments might be especially important in young animals. This is even more important in developing countries where animals are always under a poor plane of nutrition, thus even low to moderate worm burdens can cause significant production losses. A feature worth to note in the study was the highest FEC in calves in grazing animals despite the fact that farmers continued with routine anthelmintic treatments and that calves were given a priority in case of limited anthelmintics/money. The results have provided an indication that the routine treatments given were not effective probably due to wrong timing; wrong drug choice, ineffective drugs, under-dosing or parasites have developed resistance to the drugs used.

The study has shown that routine treatment of adults and all age groups on small-scale dairy (zero-grazed) cattle might not be necessary, and should be based on clinical disease. A number of authors have commented that adults can also benefit from treatments because they are an important source of pasture contamination and infection to young animals (Craig, 1988; Kaufmann and Pfister, 1990; Lima, 1998). However, similar to a number of studies (Ciordia *et al.*, 1987; Stuedemann *et al.*, 1989; Couvillion *et al.*, 1996), the present study has indicated that adults had consistently low FEC throughout the year. Therefore, it appeared that adults had negligible contribution to overall pasture contamination when compared to young animals and the need to treat adult cattle remains in question. This is from the fact that production and parasitological responses for treatment have been shown to be more favourable in calves than adults (Ciordia *et al.*, 1987). Therefore, farmers' could probably save money and anthelmintics by excluding adults during routine treatments. One study by Michel *et al.* (1972) indicated that pasture contamination by calves born the previous fall was a more important source of infection for the next crop of calves than contamination from their dams. However, well-controlled studies are needed under different management systems in order to make the final recommendation, because the outcome of excluding adults may be different between environments depending on the predominant nematode species.

Most nematodes recovered in tracer calves have been reported previously in Tanzania (Ecimovic and Mahlau, 1973; Mellau, 1997). However, *Ostertagia ostertagi* is reported and confirmed for the first time in Tanzania, and that *Nematodirus* spp is reported for the fist time in Tanzania based on egg morphology. It is suspected that these parasites have been introduced in Tanzania through imported heifers through dairy development projects in the Southern highlands of Tanzania. The decline in PCV towards the end of the dry season might be due to inadequate nutrition.

The epidemiological study has shown that farmers could greatly save money through two strategic anthelmintic treatments per year as opposed to the previous shotgun approach of at least four treatments per year. One treatment at the end of the rainy/early dry season (May/June) will terminate the late rainy peak in worm burden and also prevent outbreaks of helminthosis during the dry season while another treatment at the end of the dry/ early rainy season (November/December) will prevent carry over of infection into the next rainy season. An additional treatment during the mid rainy season (February/March) will reduce pasture contamination and prevent clinical helminthosis especially in calves and weaners/yearlings.

5.5 Epidemiology of *Fasciola* and amphistomes

The study has shown a significant influence of the type of management and season on the prevalence of fluke infections in cattle. The annual prevalence of *Fasciola* compares with previous reports in the Southern highlands of Tanzania

(Mahlau, 1970; Ecimovic and Mahlau; 1973); and is lower than that of a previous cross-sectional study probably because farmers continued with their routine anthelmintic treatment programmes during the study. The study has clearly indicated a seasonal pattern of *Fasciola* infection, despite the fact that the proportion of animals excreting fluke eggs was affected by anthelmintic treatments in dairy farms and probably self-cure in traditional cattle. The proportion of animals excreting fluke eggs increased gradually from the early dry season and peaked towards the end of the dry/early rainy season. The pattern appeared to overlap that of snail transmission dynamics in the area described by Kassuku *et al.* (1986) and Makundi (2001) that indicated a high proportion of infected snails from the end of the rainy season into the dry season. The high proportion of traditional cattle excreting *Fasciola* eggs during the rainy season concurs with studies in other tropical countries (Schillhorn van Veen *et al.*, 1980; Roberts and Suhardono, 1996; Vassilev, 1999), and considering the three months prepatent period of flukes it implies that most animals were infected towards the end of the dry season. The low proportion of egg positive animals during the rainy season in dairy farms might be due to more treatments with drugs effective against nematodes and flukes during that period. The overall high proportion of animals excreting fluke eggs towards the end of the dry season concurs with findings of Mahlau (1970) and might be related to the grazing practice during the dry season, whereby cattle were grazed in marshy areas, valleys and flood plains thus exposing them to contaminated pastures. Also, it might be due to few anthelmintic treatments in most farms during the dry season on the basis that helminthosis is a wet season disease and the types of anthelmintics used only kill mature flukes.

Small-scale dairy farms had consistently low proportion of animals passing fluke eggs all year-round. The higher proportion of egg positive animals in traditional farms compared to other management systems concurs with studies in traditional migratory cattle in Mali (Tembely *et al.*, 1988) and communal cattle in Ethiopia (Lemma *et al.*, 1985). The high prevalence might be due heavy contamination of snail habitats and ingestion of metacercariae as a result of high stocking density and local overcrowding around watering points. Contrary to a number of studies (Asanji, 1989; Rolfe *et al.*, 1991; Szmidt-Adjidé *et al.*, 2000), there was no seasonal pattern of amphistome infection in the present study. Though difficult to comment on the observation, the lack of seasonal pattern might be due to a high proportion of infected animals throughout the year. The high prevalence might be due to lack of specific drugs effective against amphistomes (Mage *et al.*, 2002). Most drugs that were used in the district were specific for nematodes and/or *Fasciola*; and the dosage has to be increased in order to attain some activity against amphistomes, which was not the case. Therefore, the proportion of egg positive animals was constantly high throughout the year especially in traditional farms and it was rare to find a non-infected animal. Amphistomosis is a neglected disease with unknown effects in most areas despite regular recovery of amphistomes in slaughtered cattle. The higher prevalence in adults than young animals concurs with other studies (Silangwa, 1973; Holland *et al.*, 2000; Rubaire-Akiiki *et al.*, 2001). The high prevalence in adults might be due to a long exposure time and partly due to some management practices, whereby adults were trekked long distances to graze in valleys, flood plains and swampy areas leaving calves around farms.

The study has indicated a high prevalence of *Fasciola* in grazing animals despite the fact that farmers continued with routine anthelmintic treatments during the study. The implication might be inappropriate timing of treatments; inappropriate drugs, under-dosing or flukes have developed resistance to the commonly used anthelmintics. Based on the local climatic conditions and management systems, routine treatments against *Fasciola* and amphistomes might be not necessary in zero-grazed small-scale dairy cattle if farmers can provide them safe pastures, anthelmintic treatments in this management system probably should be based on clinical disease. The study has indicated that strategic treatments against flukes might be important only in grazing animals. However, the present results should be interpreted with caution as the variation in prevalence among management systems might have been influenced by anthelmintic treatments during the study. Therefore controlled studies are deemed necessary in order to reach realistic conclusions.

The epidemiological study has indicated that communal grazing and watering management practices appeared to be responsible for enzootic fasciolosis and amphistomosis in Iringa. Basing on the seasonal epidemiological pattern observed, two strategic treatments might be appropriate instead of the prevailing situation of routine treatments based on availability of money/drugs. One strategic treatment with drugs effective against both flukes and GI nematodes at the beginning of the dry season (May/June) will eliminate nematodes acquired during the rainy season and immature flukes and therefore prevent acute fasciolosis/ amphistomosis especially in sheep kept in dairy farms. Another treatment at the end of the dry/early rainy season (November/December) will prevent chronic fasciolosis, pasture contamination with fluke eggs and infection of snails during the rainy season.

5.6 Efficacy of drugs against *Fasciola* and amphistomes

Based on the presence of eggs in faeces, the study has indicated that triclabendazole; nitroxynil and ivermectin-clorsulon were 100% effective in terminating *Fasciola* egg excretion. All animals treated with triclabendazole were negative for *Fasciola* eggs for a prolonged period reflecting the efficacy of the drug against all stages of *Fasciola*. The high efficacy of triclabendazole in the present study concurs with other efficacy studies in cattle, sheep and goats (Wolff *et al.*, 1983; Boray *et al.*, 1983; Rapic *et al.*, 1988; Richards *et al.*, 1990; Waruiru *et al.*, 1994; Makundi and Kassuku, 1996; Martinez-Moreno *et al.*, 1997). The high efficacy of ivermectin-clorsulon compares well with the findings of Fettere *et al.* (1985). The three formulations (triclabendazole, ivermectin-clorsulon and nitroxynil) have shown good prospects of reducing pasture contamination with *Fasciola* eggs in the district. However, ivermectin-clorsulon and nitroxynil are effective only against adult flukes (Rapic *et al.*, 1988; Richards *et al.*, 1990). As a result, egg positive animals re-appeared earlier than in those treated with triclabendazole and therefore the dosing frequency has to be increased in order to maintain low pasture contamination. It is from this ground that triclabendazole is recommended in the district because under field conditions animals harbour both immature and mature stages of *Fasciola*. The use of triclabendazole can improve liver fluke control considerably (Wolf *et al*., 1983) and reduce the number of treatments per year. However, the drug is not recommended in lactating/dairy animals due to its long withdraw period.

The study has shown a reduced activity of levamisole-oxyclozanide (Milsan®, Levoxy®) and albendazole formulations against liver flukes and there was a quick re-appearance of fluke eggs in faeces soon after treatment. The rapid detection of fluke eggs in faeces soon after treatment was due to lack of activity of the formulations against immature flukes. Also, it might have been exacerbated by marginal effects of albendazole against adult flukes even at increased doses (Boray *et al*., 1983). Therefore, immature flukes subsequently matured to shed eggs in faeces two to three weeks after treatment, and results concur with those of Rapic *et al*. (1988). The reduced efficacy of Milsan® contrasts with the 100% efficacy obtained by Makundi (2001) in the district about eight years ago. The reduced efficacy of Milsan® and albendazole bolus might be due to continuous use of these anthelmintics among farms in Iringa for the past fifteen years. The results have provided more evidence on a "reported" reduced efficacy of the two anthelmintics at Ihimbo village during the survey on anthelmintic usage.

A feature worth to note in the study was a reduced efficacy of most flukicides commonly used by farmers in the district. The extent of anthelmintic failure at a farm/village level might be even higher than obtained because most farmers drench their animals themselves. Therefore, there is a need to replace levamisole-oxyclozanide and albendazole formulations with triclabendazole in the treatment of *Fasciola*.

Based on the presence of amphistome eggs, the study has shown very low efficacy of levamisole-oxyclozanide formulations against amphistomes (29-43%). A reduced efficacy has been suspected and probably the study provided more evidence on a remarkable variation of the efficacy of drugs against immature and mature flukes (Whitehead, 1976; Soulsby, 1982). The fact that effective drugs against *Fasciola* (triclabendazole, nitroxynil and ivermectin-clorsulon) had shown no activity against amphistomes is a challenge to the control of flukes because most animals in the area harbour both *Fasciola* and amphistomes. The very low efficacy of levamisole-oxyclozanide formulations against amphistomes needs considerable attention because these are the most available drugs in Tanzania. Moreover, amphistomes are more prevalent and widespread than *Fasciola* (Nyundo, 1994) and yet there are no drugs specific against amphistomes (Mage *et al.*, 2002). The fact that farmers think one anthelmintic can eliminate all helminths in an animal implies that drugs effective against both *Fasciola* and amphistomes will be more appropriate in the district.

5.7 Strategic treatment of flukes and GI nematodes at the end of the rainy/early dry season

Strategic treatment with triclabendazole greatly reduced the proportion of animals excreting *Fasciola* eggs in faeces and eliminated clinical fasciolosis. The results concur with other strategic treatment trials in sheep and cattle (Armour *et al.*, 1973; Whitelaw and Fawcett, 1981; Fawcett, 1990; Parr and Gray, 2000; Maingi *et al.*, 2002). Following treatment with triclabendazole, the incidence of fluke infection was slow and it took about six months for 33% of treated animals to be positive for *Fasciola* eggs. The findings are in agreement with those of Makundi (2001) on a gradual acquisition of *Fasciola* and *Schistosoma bovis* infection after treatment with triclabendazole and praziquantel respectively.

Based on the proportion of egg positive animals, the efficacy of albendazole against *Fasciola* at 11.25 mg/kg was 88.2% and a reduced efficacy of albendazole was suspected. The reduced efficacy might be due to continuous use of benzimidazoles especially albendazole bolus in most farms for more than fifteen years. Compared to triclabendazole, a rapid re-infection or re-appearance of *Fasciola* eggs in faeces occurred in animals treated with albendazole. The results are in line with those of Mahlau (1976) in animals treated with tetramisole-oxyclozanide preparation (Nilzan®). The rapid re-appearance of animals excreting *Fasciola* eggs might be due to presence of all stages of flukes including those less than six weeks old at the time of treatment that is common in natural field infections. The lack of activity of albendazole against immature flukes (Boray *et al.*, 1983) might have resulted in subsequent maturation and shedding of eggs in faeces three weeks after treatment. In view of a rapid onset of egg excretion after treatment with drugs effective only against mature flukes in the district, Mahlau (1976) recommended six anthelmintic treatments per year in an attempt to keep the proportion of animals passing fluke eggs below 20%. However, the recommended frequency appears too costly and beyond the ability of a resource poor farmer, and can potentially lead to rapid development of resistance to flukicides (Parr and Gray, 2000). The high efficacy of triclabendazole against immature and mature flukes coupled with the delayed re-appearance of animals excreting fluke eggs offers an opportunity to reduce the number of treatments in the district.

The high proportion of animals passing fluke eggs during the rainy season concurs with studies in other tropical countries (Schillhorn van Veen *et al.*, 1980; Asanji and Williams, 1984; Vassilev, 1999). Based on the prepatent period of flukes, the high prevalence during the rainy season has provided more evidence that peak infection rate occurred at the end of the dry season as has been reported by Mahlau (1970). Therefore, there is a need for a second anthelmintic treatment at the end of the dry/early rainy season. The treatment might help to suppress faecal egg output in animals and limit infection of intermediate snail host, and therefore reduce pasture contamination with metacercariae (Parr and Gray, 2000).

Strategic treatment with albendazole towards the end of the rainy season maintained strongyle egg counts low (<200 EPG) up to the next rainy season. The results are in agreement with early dry season strategic treatments in Gambia (Ndao *et al.*, 1995) and Nigeria (Chiejina and Emehelu, 1986). The elimination of infections acquired during the rainy season and the absence of new infections from pasture during the dry season was probably responsible for maintenance of low FEC until the next rainy season. The high prevalence of GI nematodes, the enzootic nature of flukes and the fact that a high proportion of animals had both flukes and nematodes calls for the control of both flukes and nematodes in the same host.

The pattern of *Fasciola* and nematodes exhibited in animals treated with triclabendazole, albendazole and results from the longitudinal study on GI nematodes offer prospects for strategic use of anthelmintics effective against both *Fasciola* and nematodes at the same time (combined anthelmintics) in the district. Generally, combined anthelmintics against both *Fasciola* and nematodes would be appropriate in the district due to inadequate knowledge by farmers and some extension workers on helminths and anthelmintic spectrum. For combined anthelmintics, results from animals treated with albendazole have indicated that an additional treatment against nematodes only might be necessary in the mid rainy season (February/March). Control of amphistomes in Iringa and Tanzania in general will probably remain problematic because there is no drug specific against amphistomes (Mage *et al.*, 2002) and the commonly used drugs (with oxyclozanide) had been shown to have a reduced efficacy.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Worm control in Iringa District was mainly based on routine use of anthelmintics and that poor quality control of anthelmintics, low income and education among farmers, high price of anthelmintics, inadequate animal health extension workers and a multitude of drug vendors, service providers and anthelmintic brands contributed significantly to irrational worm control and anthelmintic usage.
2. Gastrointestinal nematodes infection in the district has a seasonal pattern and that high faecal egg counts, pasture larval counts and worm burdens occurred towards the end of the rainy season. The intensity of infection was high only in calves and weaners on large-scale dairy and traditional farms; all age groups on small-scale dairy farms had low intensity of GI nematodes.
3. *Fasciola gigantica* and amphistomes infections were highly prevalent and widespread in traditional and large-scale dairy cattle throughout the year especially in adults and yearlings. The prevalence was low in all age groups on small-scale dairy farms in most part of the year. The prevalence of flukes was highest towards the end of the dry season and the early part of the rainy season.
4. Triclabendazole (Fasinex®), nitroxynil (Trodax®) and ivermectin-clorsulon

(Ivomec-Super®) were highly effective against *Fasciola* and that triclabendazole significantly delayed onset of egg excretion in faeces for a long period. A reduced efficacy of albendazole and levamisole-oxyclozanide formulations (Milsan® and Levoxy®) was suspected.

5. Strategic treatment with triclabendazole towards the early dry season significantly reduced the proportion of animals excreting *Fasciola* eggs in faeces and prevented acute fasciolosis through elimination of immature flukes; strategic treatment with albendazole towards the early dry season significantly maintained low FEC until the next rainy season.

6.2 Recommendations

1. In order to have effective and sustainable helminth control in the area, farmers' education on the **Right anthelmintic** to use, the **Right time** to treat, the **Right dosage** to use and **the Right category** of animals to treat (**'4R'**), appears to be the only solution in each agro-climatic zone and management system. This should be coupled with a significant improvement in livestock nutrition and management in order to achieve some economic benefits at a farm level.
2. Community based worm control programmes might be a turning point towards rational worm control strategies at a community level as they offer good prospects towards correct/programmed treatments, reduced frequency of treatments, reduced interrupted treatments, reduced overprotective/ unnecessary treatments and reduced prolonged use of the same anthelmintic

for a long time.

3. Two strategic treatments against both GI nematodes and flukes are recommended in the district as opposed to the previous routine of at least four treatments per year. The first treatment is recommended at the end of the rainy/early dry season (May/June) while the second one should be at the end of the dry/early rainy season (November/December). Strategic treatment at the end of the rainy/early dry season will have few interrupted treatments because it will coincide with times when farmers have available cash. Drugs effective against immature flukes are highly recommended during the early dry season treatment. An additional third treatment against GI nematodes might be necessary in the middle of the rainy season (February/March) especially in calves and weanlings.
4. Due to high activity of triclabendazole (Fasinex®) against both immature and mature flukes coupled with the high safety index and the delayed onset of egg excretion in treated animals; it is recommended that triclabendazole replace other Fasciolicides in the district in order to reduce the number of treatments per year as well as to slow down the impending threat of resistance to flukicides. However, the drug is not recommended in lactating/dairy animals due to its long withdraw period.
5. Due to resistance to common anthelmintics, increasing consumer pressure for no or little chemicals in animal products and concerns on the effects of some anthelmintics on the environment on fauna and flora, it is high time for farmers in Tanzania to move for alternative control measures that do not

rely entirely on the use of anthelmintics. (e.g. improved nutrition, grazing management, use of bioactive forages, biological control and breeding for worm resistant hosts) in order to meet the global demand with regard to livestock products.

6.3 Areas for further studies

1. Controlled tests are required to confirm the suspected resistance of flukes to oxyclozanide and albendazole preparations.
2. Impact studies are required to demonstrate the importance of anthelmintic treatments in small-scale dairy (zero-grazed) cattle and the benefits of better targeting anthelmintic treatments among management systems.
3. The effect of a single strategic treatment at the end of the dry/early rainy season alone or in combination with the late rainy/early dry season treatment are required in order to design an effective control programme.
4. Economic and epidemiological consequences of excluding adult cattle in treatments against GI nematodes and that of excluding calves in treatments against flukes need to be evaluated under local conditions in various management practices. This is because in areas where transmission of flukes is high, weaned calves may be infected before they attain the yearling age especially if they are weaned during the dry season.
5. There is a need to asses the effects of using drugs effective against both flukes and GI nematodes in strategic treatments in the district.

REFERENCES

Abbot, E.M.; Parkins, J.J.; Holmes, P.H. (1985). Influence of dietary protein on parasite establishment and pathogenesis in Finn Dorset and Blackface lambs given a single moderate infection of *Haemonchus contortus*. *Research in Veterinary Science* 38: 6-13.

Abdel-Rahman, S.M.; O'Reilly, K.L.; Malone, J.B. (1998). Evaluation of a diagnostic monoclonal antibody-based capture enzyme-linked immunosorbent assay for detection of a 26- to 28-kd *Fasciola hepatica* coproantigen in cattle. *American Journal of Veterinary Research* 59: 533-537.

Agyei, A.D. (1991). Epidemiological observations on helminth infections of calves in southern Ghana. *Tropical Animal Health and Production* 23: 134-140.

Akkaro, F.M.N. and Maro, J.K. (1992). Animal health constraints to cattle production in the Southern highlands of Tanzania. In: *Proceedings of an International Conference on Agricultural Research, Training and Technology Transfer in the Southern Highlands of Tanzania*: past and future prospects. Uyole Agricultural Centre, Mbeya, Tanzania, pp 317-323.

Al-khshali, M. and Altaif, K.I. (1979). The response of Awassi and Merino sheep to primary infection with *Haemonchus contortus*. *Tropical Animal Health and Production* 11 (3): 164-170.

Anderson, N.; Luong, T.T.; Vo, N.G.; Bui, K.L.; Smooker, P.M.; Spithill, T.W. (1999). The sensitivity and specificity of two methods for detecting *Fasciola* infections in cattle. *Veterinary Parasitology* 83: 15-24.

Anene, B.M.; Onyekwodiri, E.O.; Chime, A.B.; Anika, S.M. (1994). A survey of gastrointestinal parasites in cattle in Southeast Nigeria. *Preventive Veterinary Medicine* 20: 297-306.

Anonymous (1983). *Manual of Laboratory Techniques*. British Small Animal Veterinary Association. (Edited by Hawken, M.L.), 2nd edition. 5 St Georges Terrace, Cheltenham, UK. pp. 108.

Armour, J.; Corba, J.; Bruce, R.G. (1973). The prophylaxis of ovine fascioliasis by strategic use of rafoxanide. *Veterinary Record* 92: 83-89.

Asanji, M.F. (1989). Paramphistomosis of cattle in Sierra Leone and seasonal fluctuations in its prevalence. *Bulletin of Animal Health and Production in Africa* 37: 327-331.

Asanji, M.F. and Williams, M.O. (1984). The effect of sex on seasonal variation in single and double infection of cattle in Sierra Leone by *Dicrocoelium hospes* and *Fasciola gigantica*. *Veterinary Parasitology* 15: 247-255.

Baker, R.L.; Mwamachi, D.M.; Audho, J.O. and Thorpe, W. (1994) Genetic resistance to gastrointestinal parasites in Red Maasai, Dorper and Red Maasai x Dorper ewes in coastal Kenya. In: *Proceedings of the 12th Small Ruminant Collaborative Research Support Project (SR-CRSP) Scientific workshop*, 2-3 March, Nairobi, Kenya. pp. 100-109.

Banks, D.J.D.; Singh, R.; Barger, I.A.; Pratap, B.; Le Jambre, L.F. (1990). Development and survival of infective larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis* in tropical environment. *International Journal for Parasitology* 19: 539-546.

Barger, I.A. (1989). Genetic resistance of the host and its influence on epidemiology. *Veterinary Parasitology* 15: 645-649.

Barger, I.A. (1999). The role of epidemiological knowledge and grazing management for helminth control in small ruminants. *International Journal for Parasitology* 29: 41-47

Barger, I.A. and Southcott, W.H. (1978). Parasitism and production in weaner sheep grazing alternatively with cattle. *Australian Journal of Experimental Agriculture and Animal Husbandry* 18: 340-346.

Barger, I.A.; Siale, K.; Banks, D.J.; Le Jambre, L.F. (1994). Rotational grazing for control of gastrointestinal nematodes of goats in a wet tropical environment. *Veterinary Parasitology* 53: 109-116.

Baudena, M.A.; Chapman, M.E.; Larsen, M.; Klei, T.R. (2000). Efficacy of the nematophagous fungus *Duddingtonia flagrans* in reducing equine cyanthostome larvae on pasture in Southern Louisiana. *Veterinary Parasitology* 89: 219-230.

Berghen, P.; Hilderson, H.; Vercruysse, J.; Dorny, P. (1993). Evaluation of pepsinogen, gastrin and antibody response in diagnosing ostertagiosis. *Veterinary Parasitology* 46: 175-195.

Bitakamire, P.K. (1973). The incidence of fasciolosis in different breeds of cattle in Kenya. *Bulletin of Epizootic Diseases in Africa* 21: 145-152.

Blood, D.C., Radostits, O.M. (1989). *Veterinary Medicine*, A textbook of diseases of cattle, sheep, pigs and horses. 7th edition. English Language Book Society (ELBS), Pitman Press, Bailliere Tindal, 10 Greycoat Place, London. 809pp.

Boag, B. and Thomas, R.J. (1989). Epidemiological studies on gastrointestinal nematode parasites of sheep. The seasonal number of generations and succession of species. *Research in Veterinary Science* 22: 62-67.

Boray, J.C. (1967). The effect of host reaction to experimental *Fasciola hepatica* infections in sheep and cattle: the reaction of the host to parasitism. In: *Proceedings of the 3rd International Conference of the World Association for the Advancement of Veterinary Parasitology* (WAAVP), (Edited by Soulsby, E.L.J), Lyons, Elwert, Marburg, Germany. pp. 119-122.

Boray, F.C.; Crowfoot, P.D.; Strong, M.B.; Allison, J.R.; Schellenhaum, M.; Sarasin, G.O. (1983). Treatment of immature and mature *Fasciola hepatica* infections in sheep with triclabendazole. *Veterinary Record* 113: 315-317.

Boyd, J.W. (1988). Serum enzymes in the diagnosis of diseases in man and animals. *Journal of Comparative Pathology* 98: 381-404.

Charles, T.P. and Furlong, J. (1996). A survey of dairy cattle worm control practices in Southeast Brazil. *Veterinary Parasitology* 67: 65-73.

Chauvin, A.; Moreau, E.; Boulard, C. (1997). Diagnosis of bovine fasciolosis using serology on pools of sera. *Veterinary Research* 28: 37-43.

Chiejina, S.N. (1994). Epidemiology of some helminth infections of domestic animals in the tropics with emphasis on fasciolosis and parasitic gastroenteritis. In: *Helminthology* (Edited by Chowdhury, N. and Tada, I.). Springer-Verlag, Narosa Publishing House, Delhi, India. pp. 34-71.

Chiejina, S.N. and Fakae, B.B. (1984). Development and survival of infective larvae of gastrointestinal nematode parasites of cattle on pasture in Eastern

Nigeria. *Research in Veterinary Science* 37: 148-153.

Chiejina, S.N. and Emehelu, C.O. (1986). Evaluation of three strategic anthelmintic programmes for the prophylaxis of parasitic gastroenteritis in cattle in Nigeria. *Tropical Animal Health and Production* 18: 55-63

Ciordia, H.; Plue, R.E.; Calvert, G.V.; McCampbell, H.C. (1987). Evaluation of the parasitological and production response of a cow-calf operation to an anthelmintic treatment programme with ivermectin. *Veterinary Parasitology* 23: 265-271.

Coop, R.L. (1995). Interactions between helminth infections and nutrition in sheep. In: *Proceedings of the 6th Symposium on Tropical Animal Health and Production*, 6th October, Utrecht University, The Netherlands. pp. 28-33.

Coop, R.L. and Holmes, P.H. (1996). Nutrition and parasite interactions. *International Journal for Parasitology* 26: 951-962.

Coop, R.L. and Kyriazakis, I. (1999). Nutrition-parasite interaction. *Veterinary Parasitology* 84: 187-204.

Cornellisen, J.B.; Gaasenbeek, C.P.H.; Boersma, W.; Borgsteede, F.H.M.; van Milligen, F.J. (1999). Use of a pre-selected epitope of cathepsin-L-1 in a highly specific peptide based immunoassay for the diagnosis of *Fasciola hepatica* infections in cattle. *International Journal for Parasitology* 29: 685-696.

Corwin, R.M and Stromberg, B.E. (1995). Internal parasitism and deworming of beef-cattle-status in the 1990. *Veterinary Medicine* 90: 486.

Couvillion, C.E.; Siefker, C.; Evans, R.R. (1996). Epidemiological study of nematode infections in a grazing beef cow-calf herd in Mississippi. *Veterinary Parasitology* 64: 207-218.

Craig, T.M. (1988). Impact of internal parasites on beef cattle. *Journal of Animal Science* 66: 1565-1569.

Dalton, J.P. and Mulcahy, G. (2001). Parasite vaccines- a reality? *Veterinary Parasitology* 98: 149-167.

Dimander, S.O.; Hoglund, J.; Sporndly, E.; Waller, P.J. (2000). The impact of internal parasites on the productivity of young cattle organically reared on semi-natural pasture in Sweden. *Veterinary Parasitology* 90: 271-284.

Dinnik, J.A. (1964). Intestinal paramphistomiasis and *Calicophoron microbothrium* Fischoeder in Africa. *Bulletin of Epizootic Diseases in Africa* 12: 439-454.

Dobson, C. (1972). Immune response to gastrointestinal helminths. In: *Immunity to Animal Parasites,* (Edited by Soulsby, E.L.J.), New York and London, Academic Press. pp. 191-222.

Doenhoff, M.; Bickle, Q.; Long, E.; Bain, J.; McGregor, A. (1978). Factors affecting the acquisition of resistance against *Schistosoma mansoni* in the mouse.1. Demonstration of resistance to reinfection using a model system that involves perfusion of mice within three weeks post infection. 2. The time at which resistance develops. *Journal of Helminthology* 52: 173-191.

Donald, A.D.; Morey, F.H.W.; Axelsen, A.; Donnelly, J.B.; Waller, P.J. (1987). Integration of grazing management and anthelmintic treatment for the control of nematode infections in young sheep. In: *Temperate pasture, their production, use and management.* (Edited by Wheeler, J.L., Pearson C.J.

and Robards, G.E.). *Australian Wool Corporation*, CSIRO, Melbourne, Australia. pp. 567-569.

Dumenigo, B.E.; Espino, A.M.; Finlay, C.M. (1996). Detection of *Fasciola hepatica* antigen in cattle faeces by a monoclonal antibody-based sandwich immunoassays. *Research in Veterinary Science* 60: 278-279.

Eady, S.J.; Woolaston, R.R.; Mortimer, S.I.; Lewer, R.P.; Raadsma, H.W.; Swan, A.A. and Ponzoni, R.W. (1996). Resistance to nematode parasites in Merino sheep: sources of genetic variation. *Australian Journal of Agricultural Research* 47: 895-915.

Ecimovic, T. J. and Mahlau, E.A. (1973). Animal Health Survey in cattle in Mbeya Region of Tanzania. *Bulletin of Epizootic Diseases in Africa* 21: 187-192.

El-Bahi, M.M.; Malone, J.B.; Todd, W.J.; Schnorr, K.L. (1992). Detection of stable diagnostic antigen from bile and faeces of *Fasciola hepatica* infected cattle. *Veterinary Parasitology* 45: 157-167.

Faedo, M.; Larsen, M.; Gronvold, J. (2002). Predacious activity of *Duddingtonia flagrans* within the cattle faecal pat. *Journal of Helminthology* 76 (4): 295-302.

Fagbemi, B.O. and Guobadia, E.E. (1995). Immunodiagnosis of fasciolosis in ruminants using 28-kDa cysteine protease of *Fasciola gigantica* adult worms. *Veterinary Parasitology* 57: 309-318.

FAO (1994). *Diseases of Domestic Animals Caused by Flukes*. Food and Agriculture Organization of the United Nations, Rome, Italy, 49pp.

Fawcett, A.R. (1990). A study of a restricted programme of strategic dosing against *Fasciola hepatica* with triclabendazole. *Veterinary Record* 127 (20): 492-493.

Fernandez, A.S.; Larsen, M.; Henningsen, E.; Nansen, P.; Gronvold, J.; Bjorn, H.; Wolstrup, J. (1999). Effect of *Duddingtonia flagrans* against *Ostertagia ostertagi* in cattle grazing under different stocking rate. *Parasitology* 119: 105-111.

Fettere, R.H.; Rew, R.S.; Gasbarre, L.C.; Oslind, D.A. (1985). Prophylactic efficacy of clorsulon against *Fasciola hepatica* in calves and sheep. *Veterinary Parasitology* 18: 21-27.

Fox, M.T.; Gerreli, D.; Pitt, S.R.; Jacobs, D.E. (1989). *Ostertagia ostertagi* infection in the calf: effects of tricle challenge on appetite, digestibility, rate of passage of digesta and live weight gain. *Research in Veterinary Science* 47: 294-298.

Gallie, G.J. (1973). The development of acquired resistance and age resistance to *Nematodirus battus* in laboratory rabbit. *Journal of Helminthology* 57: 369-376.

Gasser, R.B. (1999). PCR-based technology in Veterinary Parasitology. *Veterinary Parasitology* 84: 229-358.

Gray, D.G. (1995). Worm control in Australia - where now? In: *Parasitology Research in Africa: Proceedings of an International Foundation for Science* (IFS) Workshop, Bobo-Dioulasso, Burkina Faso, 6-10 November. pp. 155-168.

Gray, G.D. (1997). The use of genetically resistant sheep to control nematode parasitism. *Veterinary Parasitology* 72: 345-366.

Gray, G.D., (1991). Breeding for resistance to trichostrongyle nematodes in sheep.

In: *Breeding for Resistance in Farm Animals*. (Edited by Axford, R.F.E. and Owens, J.B.). CAB international, Wallingford. pp. 139-161.

Gronvold, J. (1989). Interaction of bovine gastrointestinal nematode parasites and nematode destroying fungi. In: Transmission of infective larvae of *Ostertagia ostertagi* and *Cooperia oncophora* (Trichostrongylidae: Nematoda). PhD thesis. The Royal Veterinary and Agricultural University, Copenhagen, Denmark, 93pp.

Gronvold, J.; Wolstrup, J.; Larsen, M.; Henriksen, Sv.Aa.; Nansen, P. (1993). Biological control of *Ostertagia ostertagi* by feeding selected nematode-trapping fungi to calves. *Journal of Helminthology* 67: 31-36.

Hammond, J.A. (1965). Observations of fascioliasis in Tanganyika. *Bulletin of Epizootic Diseases in Africa* 13: 55-65.

Hanna, R.E.B. and Jura, W. (1977). Antibody response of calves to a single infection of *Fasciola gigantica* determined by Indirect Fluorescent Antibody Test (IFAT). *Research in Veterinary Science* 22: 339-342.

Hansen, J. and Perry, B. (1994). *The Epidemiology, Diagnosis and Control of Helminth Parasites of Ruminants*. 2nd edition, ILRAD, Nairobi, Kenya, 171pp.

Haroun, E.M. and Hillyer, G.V. (1986). Resistance to fasciolosis: A review. *Veterinary Parasitology* 20: 63-93.

Heinonen, M.; Rufe, G.; Kabede, S. (1995). The effect of ant parasitic treatment against *Fasciola* on crossbred and Zebu cows in Ethiopia. *War/RMS* 82 (1): 90-92.

Hilderson, H.; Berghen, P.; Vercruysse, J.; Dorny, P.; Braem, L. (1989). Diagnostic value of gastrin for clinical bovine ostertagiosis. *Veterinary*

Record 125: 376-377.

Holland, W.G.; Luong, T.T.; Nguyen, L.A.; Do, T.T.; Vercruysse, J. (2000). The epidemiology of nematode and fluke infections in cattle in the Red River Delta in Vietnam. *Veterinary Parasitology* 93: 141-147.

Holmes, P.H. (1987). Pathophysiology of parasitic infections. *Parasitology* 94: 529-551.

Honer, M.R.; Branchin, I.; Nascimento, Y.A. (1992). The interpretation of the population dynamics of bovine gastrointestinal nematodes with the use of tracer animals. *Review of Brazilian Veterinary Parasitology* 1: 97-103.

Horak, I.G. (1971). Paramphistomosis in domestic ruminants. In: *Advances in Parasitology* (Edited by Dawes, B). Academic press, London, Volume 9, pp. 33-72.

Howell, M.J. and Boray, J.C. (1994). Host immune to Fasciolosis. In: *Immunology, Pathology and Control of Fasciolosis, Proceedings of the 8th International Congress of Parasitology* (ICOPA 8). (Edited by Boray, J.C.). 10-14 October, Izmir, Turkey. pp. 21-28.

Ibarra, F.; Montenegro, N.; Vera, Y.; Boulard, C.; Quiroz, H.; Flores, J.; Ochoa, P. (1998). Comparison of three ELISA for seroepidemiology of bovine fasciolosis. *Veterinary Parasitology* 77: 229-236.

Itty, P.; Ankers, P.; Zinsstag, T.; Trawally, S.; Pfister, K. (1997). Productivity and profitability of sheep production in the Gambia and implications for livestock development in West Africa. *Quarterly Journal of International Agriculture* 36: 153-172.

Jarrett, J.W.H.; Jennings, F.W.; McIntyre, W.I.M.; Mulligan, W.; Urquhart, G.M. (1960). Immunological studies on *Dictyocaulus viviparus* infection. Immunity produced by the administration of irradiated larvae. *Immunology* 3: 145-151.

Kahn, L.P.; Knox, M.R.; Walkden-Brown, S.W.; Lea, J.M. (2003). Regulation of the resistance to nematode parasites of single- and twin-bearing Merino ewes through nutrition and genetic selection. *Veterinary Parasitology* 114: 15-31.

Kassai, T. (1999). *Veterinary Helminthology*. Butter worth-Heinemann. Linacre House, Jordan Hill, Oxford, OX2 8DP, 225 Wildwood Avenue, Woburn, 260pp.

Kassuku, A.; Christensen, N.Ø.; Monrad, J.; Nansen, P.; Knudsen, J. (1986). Epidemiological studies on *Schistosoma bovis* in Iringa region, Tanzania. *Acta Tropica* 43: 153-163.

Kaufmann, J. (1996). *Parasitic Infection of Domestic Animals*. A diagnostic manual. Birkhauser Verlag, Switzerland, 423pp.

Kaufmann, J. and Pfister, K. (1990). The seasonal epidemiology of gastrointestinal nematodes in N'dama cattle in the Gambia. *Veterinary Parasitology* 37: 45-54.

Kendall, S.B. (1965) Relationships between the species of *Fasciola* and their molluscan hosts. *Advances in Parasitology* 3: 59-98.

Kendall, S.B.; Sinclair, I.J.; Everett, G.; Parfitt, J.W. (1978). Resistance to *Fasciola hepatica* in cattle. 1. Parasitological and serological observations. *Journal of Comparative Pathology* 88: 115-122.

Keyyu, J.D.; Kassuku, A.A.; Kyvsgaard, N.C.; Willingham, A.L. (1998). Genetic resistance of small East African goats to mixed gastrointestinal nematode parasites. *Tanzania Veterinary Journal* 18 (3): 208-223.

Keyyu, J.D.; Kassuku, A.A.; Willingham, A.L.; Kyvsgaard, N.C. (2001). Peri-parturient helminthosis in strains of East African goats in Tanzania. *Preventive Veterinary Medicine* 50: 177-182.

Kifaro, G.C. and Temba, E.A. (1990). Calf mortality and culling rate in two dairy farms in Iringa region, Tanzania. In: *Proceedings of the Tanzania Society for Animal Production* (TSAP), Volume 1: 138-148.

Kinoti, G.K.; Maingi, N.; Coles, C.G. (1994). Anthelmintic use in Kenya and its implications. *Bulletin of Animal Health and Production in Africa* 42: 71-73.

Knox, D.P.; Redmond, D.R.; Skuce, P.J.; Newlands, G.F.J. (2001). The contribution of molecular biology to the development of vaccines against nematode and trematode parasites of domestic ruminants. *Veterinary Parasitology* 101: 311-335.

Knox, M.R. and Steel, J.W. (1997). Effect of Urea Molasses Blocks on parasitised sheep. In: *Proceedings of the 16th International Conference of the Word Association for the Advancement of Veterinary Parasitology* (WAAVP), Abstracts, 10-15 August, Sun City, South Africa. 49pp.

Lang, B.Z. (1967). Host parasite relationships of *Fasciola hepatica* in the white mouse. II. Studies on acquired immunity. *Journal of Parasitology* 53: 21-30.

Larsen, M. (2002). Biological control in a global perspective- A review with emphasis on *Duddingtonia flagrans*. In: *Biological control of nematode parasites of small ruminants in Asia, FAO Animal Production and Health Paper*. pp. 19- 39.

Lemma, B.; Gabre-A.B.F.; Tedla, S. (1985). Studies on fascioliasis in four selected farms in Ethiopia. *Veterinary Parasitology* 18: 29-37.

Lima, W.S. (1998). Seasonal infection of gastrointestinal nematodes of beef cattle in Minas Gerais State-Brazil. *Veterinary Parasitology* 74: 203-214.

MAFF (1986). Ministry of Agriculture, Fisheries and Food. *Manual of Parasitological Laboratory Techniques*. Reference book number 418. 3rd edition, Her Majesty's Stationery Office, London, pp. 160.

Mage, C.; Bourgne, H.; Toullien, J.M.; Rondelaud, D.; Dreyfuss, G. (2002). *Fasciola hepatica* and *Paramphistomum daubneyi*: changes in prevalences of natural infections in cattle and in *Lymnaea truncatula* from central France over 12 years. *Veterinary Research* 33: 439-447.

Mahlau, E.A. (1970). Liver fluke survey in Zebu cattle of Iringa region of Tanzania and the first finding of the small fluke *Dicrocoelium hospes* /Looss 1907. *Bulletin of Epizootic Diseases in Africa* 18: 21-28.

Mahlau, E.A. (1976). Liverfluke and Dicrocoelium infections. In: *Annual report, Veterinary Investigation Centre, Iringa*. Ministry of Agriculture, Dar es Salaam, Tanzania, pp 10-13.

Maingi, N.; Bjørn, H.; Thamsborg, S.M.; Munyua, W.K.; Gathuma, J.M.; Dangolla, A.; Kyvsgaard, N.C. (1996). Worm control practices on sheep farms in Denmark and implications for the development of anthelmintic resistance. *Veterinary Parasitology* 66: 39-52.

Maingi, N.; Bjørn, H.; Thamsborg, S.M.; Munyua, W.K.; Gathuma, J.M.; Dangolla, A. (1997). Worm control practices on sheep farms in Nyandarua district of Kenya. *Acta Tropica* 68: 1-9.

Maingi, N.; Otieno, R.O.; Weda, E.H.; Gichohi, V.M. (2002). Effects of three anthelmintic regimes against *Fasciola* and nematodes on the performance of ewes and lambs on pasture in the highland of Kenya. *Veterinary Research Communications* 26 (7): 543-552.

Makundi, A.E. (2001). *Epidemiology and control of Bovine fasciolosis and schistosomosis in Tanzania.* A Thesis submitted in fulfilment for the degree of Doctor of Philosophy (PhD) at Sokoine University of Agriculture (SUA), Morogoro, Tanzania, 159pp.

Makundi, A.E. and Kassuku, A.A. (1996). Efficacy of praziquantel and triclabendazole in cattle naturally infected with *Schistosoma bovis* and *Fasciola gigantica. Tanzanian Veterinary Journal* 16 (Supplement 1): 197-204.

Makundi, A.E.; Kassuku, A.A.; Maselle, R.M.; Boa, M.E. (1998). Distribution, prevalence and intensity of *Schistosoma bovis* in cattle in Iringa district, Tanzania. *Veterinary Parasitology* 75: 59-69.

Makundi, A.E.; Namuba, F.V.; Kassuku, A.A. (2004). The finding of *Fasciola hepatica* for the first time in the southern highlands of Tanzania. In: *Proceedings of the 13th Annual Meeting of the ENRECA Livestock Helminths Research Project in Eastern and Southern Africa.* 26-28th April 2004, University of Nairobi, Nairobi, Kenya. Abstracts. p 31.

Martinez-Moreno, A.; Jimenez, V.; Martinez-Cruz, M.S.; Martinez-Moreno, F.J.;

Berecca, C.; Hernandez, S. (1997). Triclabendazole treatment in experimental goat fasciolosis: anthelmintic efficacy and influence in antibody response and pathology of the disease. *Veterinary Parasitology* 68: 57-67.

McKenna, P.B. (1981). The diagnostic and interpretation of faecal egg counts in sheep. *New Zealand Veterinary Journal* 29: 129-132.

Meeusen, E.N.T. (1999). Immunology of helminth infections, with special reference to immunopathology. *Veterinary Parasitology* 84: 259-273.

Mellau, L.B.S. (1997). *Factors Associated with Diseases and Mortality of Calves in Morogoro.* A dissertation submitted in partial fulfilment for the degree of Master of Veterinary Medicine (MVM), Sokoine University of Agriculture, Morogoro, Tanzania, Un published, 133pp.

Michel, J.F., Lancaster, M.B., Hong, C. (1972). The epidemiology of gastrointestinal nematode infection in the single-suckled calf. *Veterinary Record* 91: 301-306.

Monrad, J.; Christensen, N.O.; Nansen, P.; Frandsen, F. (1981). Resistance to *Fasciola hepatica* in sheep harbouring primary *Schistosoma bovis* infection. *Journal of Helminthology* 55: 261-271.

Morris, R.S. and Marsh, W.E. (1994). The relationships between infections, diseases and their economic effects. In: *Modelling vector borne and other parasitic diseases, Proceedings of the ILRAD/FAO Workshop.* (Edited by Perry, B.D. and Hansen, J.W.). November 1992, Nairobi, Kenya, pp 199-213.

Morris, R.S. and Meek, A.H. (1980). Costs of major parasites of the Australian Livestock industries. *International Journal for Parasitology* 25: 1363-1367.

Mostofa, M.; McKellar, Q.A.; Alam, M.N.; Le Jambre, L.F.; Know (1997). Sustainable parasite control in small ruminants. *An International Workshop Sponsored by ACIAR*, 22-25 April, Bogor, Indonesia. pp. 105-108.

Moyo, D.Z.; Bwangamoi, O.; Hendrikx, W.M.L.; Eysker, M. (1996). The epidemiology of gastrointestinal nematode infections in communal cattle and commercial beef cattle on the highveld of Zimbabwe. *Veterinary Parasitology* 67: 105-120.

Moyo, D.Z.; Hendrikx, W.M.; Obwolo, M.J.; Eysker, M. (2003). The effect of treatment with 1% injectable formulation of moxidectin during the rainy season and at the beginning of the dry season on gastrointestinal nematodes in cattle from communal area in Zimbabwe. *Tropical Animal Health and Production* 35: 1-16.

Mzembe, S.A.T. and Chaudhry, M.A. (1979). The epidemiology of fascioliasis in Malawi: I. The epidemiology in the intermediate host. *Tropical Animal Health and Production* 11: 246-250.

Nansen, P. (1985) Importance of immunology in relation to parasitism and production. In: *Parasites, Pests and Predators*. (Edited by S.M. Gaafar, W.E. Howard and R.E. Marsh). pp. 372-386.

Nansen, P.; Foldager, J.; Hansen, J.W.; Henriksen, S.A.; Jørgensen, R.J. (1988). Grazing pasture and acquisition of *Ostertagia ostertagi* in calves. *Veterinary Parasitology* 27: 325-335.

Nansen, P.; Larsen, M.; Roepstorff, A.; Gronvold, J.; Wolstrup, J.; Henriksen, S.A. (1996). Control of *Oesophagostomum dentatum* and *Hyostrongylus rubidus* in outdoor reared pigs through daily feeding with the microfungus

Duddingtonia flagrans. *Parasitology Research* 82: 580-584.

Ndao, M.; Pandey, V.S.; Zinsstag, J.; Pfister, K. (1995). Effect of a single dry season anthelmintic treatment on N'dama cattle on communal pastures in the Gambia. *Veterinary Research Communications* 19: 205-213.

Nodtvedt, A.; Dohoo, I.; Sanchez, J.; Conboy, G.; DesCjteaux, L.; Keefe, G.; Leslie, K.; Campbell, J. (2002). The use of negative binomial modelling in a longitudinal study of gastrointestinal parasite burdens in Canadian dairy cows. *Canadian Journal of Veterinary Research* 66 (4): 249-457.

Nyundo, B.A. (1994). *Studies on amphistomes of domestic ruminants in Kasulu district, Tanzania*. MSc Dissertation, University of Dar es Salaam, Tanzania, 94pp.

Ogambo-Ongoma, A.H. (1972). Fasciolosis survey in Uganda. *Bulletin of Epizootic Diseases in Africa* 20: 35-41.

Ogunsussi, R.A. and Eysker, M. (1979). Inhibited development of trichostongylids of sheep in Northern Nigeria. *Research in Veterinary Science* 26: 108-110.

Okon, E.D. (1988). Causes and control measures to improve increased productivity. In: *Proceedings of the Workshop on the Improvement of Small Ruminants in West and Central Africa*, (Edited by Adeniji, K.O.). 21-25 November, Ibadan, Nigeria, pp 191-200.

Omara-Opyene (1985). A survey of gastrointestinal nematode parasitism in cattle under nomadic management in Marsabit district of Northern Kenya. *Bulletin of Animal Health and Production in Africa* 33: 107-112.

Over, H.J. (1982) Ecological basis of parasite control: Trematodes with special reference to fascioliasis. *Veterinary Parasitology* 11: 85-97.

Owen, J.B. and Axford, R.F.E. (1991). *Breeding for Disease resistance in farm animals*. CAB International, Wallingford, United Kingdom, 368pp.

Paraud, C. and Chartier, C. (2003). Biological control of infective larvae of gastrointestinal nematode (*Teladosargia circumcincta*) and a small lungworm (*Mullerius capillaries*) by *Duddingtonia flagrans* in goat faeces. *Parasitology Research* 89: 102-106.

Parr, S.L. and Gray, J.S. (2000). Strategic dosing scheme for the control of fasciolosis in cattle and sheep in Ireland. *Veterinary Parasitology* 88 (3&4): 187-197.

Pena, M.T.; Miller, J.E.; Fontenot, M.E.; Gillespie, A.; Larsen, M. (2002). Evaluation of *Duddingtonia flagrans* in reducing infective larvae of *Haemonchus contortus* in faeces of sheep. *Veterinary Parasitology* 103 (3): 250-265.

Perry, B.D., Randolph, T.F., McDermott, J.J., Sones, K.R., Thornton, P.K. (2002). *Investing in Animal Health Research to Alleviate Poverty.* International Livestock Research Institute (ILRI), Nairobi, Kenya. pp. 140.

Petkevicius, S.; Larsen, M.; Bach-Knudsen, K.E.; Nansen, P.; Gronvold, J.; Henriksen, S.Aa.; Wolstrup, J. (1998). The effect of nematode destroying fungus *Duddingtonia flagrans* against *Oesophagostomum dentatum* larvae in faeces from pigs fed different diets. *Helminthologia* 35: 111-116.

Rapic, D.; Dzakula, N.; Sakar, D.; Richards, R.J. (1988). Comparative efficacy of triclabendazole, nitroxynil and rafoxanide against immature and mature *Fasciola hepatica* in naturally infected cattle. *Veterinary Record* 122: 59-62.

Reinecke, R.K. (1989) *Veterinary Helminthology*, Butterworth, Durban, 392pp.

Richards, R.J.; Bowen, F.L.; Essenwein, F.; Steiger, R.F.; Büscher, G. (1990). The efficacy of triclabendazole and other anthelmintics against *Fasciola hepatica* in controlled studies in cattle. *Veterinary Record* 126: 213-216.

Roberts, J.A. and Suhardono (1996). Approaches to the control of fasciolosis in ruminants. *International Journal for Parasitology* 26: 971-981.

Rolfe, P.F., Boray, J.C., Nichols, J.C., Collins, G.H. (1991) Epidemiology of paramphistomosis in cattle. *International Journal for Parasitology* 21: 813-819.

Ross, J.G. (1967). Studies on immunity to *Fasciola hepatica*: Acquired immunity in cattle, sheep and rabbits following natural infections and vaccine procedures. *Journal of Helminthology* 41: 393-399.

Ross, J.G. and Armour, J. (1960). The significant of faecal egg counts and the use of albumin levels and packed cell volume percentage to asses pathogenicity of helminthosis. *Veterinary Record* 8: 201-206.

Rubaire-Akiiki, C.; Okello-Onen, J.; Nasinyama, G.; Vaarst, M.; Mwayi, W. (2001). Dairy farm circumstances and helminthosis in Mbale district, Uganda. *In: Abstracts of the 18th International Conference of the World Association for the Advancement of Veterinary Parasitology* (WAAVP), 26-30 August, Stresa, Italy, Abstracts, pp 52.

SAS® (1990). Statistical Analysis system Institute Inc. Introductory Guide for Personal Computers, Release 6.03 Edition, Carry, NC, USA. pp. 111.

Schillhorn van Veen, T.W.; Folaranmi, D.O.; Usman, S.; Ishaya, T. (1980). Incidence of liver fluke infection (*Fasciola gigantica* and *Dicrocoelium* *hospes*) in ruminants in Northern Nigeria. *Tropical Animal Health and Production* 12: 97-104.

Scott, I.; Stear, M.J.; McKellar, Q.A. (1995). Comparison of four methods for the determination of plasma pepsinogen concentrations. *Research in Veterinary Science* 59: 234-237.

Silangwa, S.M. (1973). Incidence of liver flukes (*Fasciola gigantica*) in Barotse cattle, Zambia. *Bulletin of Epizootic Disease in Africa* 21: 11-17.

Silangwa, S.M. (1974). Epizootioloy of bovine fasciolosis and problems of control in the major flood plain region of Zambia. In: *Proceedings of the Annual Scientific Conference of East African Medical Research Council*, Dar es Salaam, Tanzania, pp 325-335.

Silangwa, S.M. and Todd, A.C. (1964). Vertical migration of trichostrongylid larvae. *Journal of Parasitology* 50: 278-285.

Simesen, M.G. and Nansen, P. (1974). Serum Gamma Glutamyl Transpeptidase (GGT) and Aspartate Aminotransferase (AspAT) in adult cattle with chronic *Fasciola hepatica* infection. *Acta Veterinaria Scandinavica* 15: 239-243.

Sirag, S.B.; Christensen, N.O.; Nansen, P.; Monrad, J.; Frandsen, F. (1981). Resistance to *Fasciola hepatica* in calves harbouring primary patent *Schistosoma bovis* infections. *Journal of Helminthology* 55: 63-70.

Smith, W.D. (1999). Prospects for vaccines of helminth parasites of grazing ruminats. *International Journal for Parasitology* 29: 17-24.

Smithers, S.R.; Terry, R.J.; Hockley, D.J. (1969). Host antigens in

Schistosomiasis. *Proceedings of the Royal Society* (London) 171: 483-494.

Snyder, D.E. (1993). Epidemiology of *Ostertagia ostertagi* in cow-calf herds in the southeast United States. *Veterinary Parasitology* 46: 277-288.

Soulsby, E.L.J (1982). Helminths, arthropods and protozoa of domestic animals. 7th edition, Bailliere Tindal, London, UK, 809pp.

Statistix® (1994). Statistix® 4.1. *Analytical software*. User's manual. Tallahassee, USA, 329pp.

Stear, M.J. and Murray, M. (1994). Genetic resistance to parasitic disease: particularly of resistance in ruminants to gastrointestinal nematodes. *Veterinary Parasitology* 54: 161-176.

Stear, M.J.; Bairden, K.; Bishop, S.C.; Buitkamp, J.; Duncan, J.L.; Gettinby, G.; McKellar, Q.A.; Park, M.; Parkins, J.J.; Reid, S.W.J.; Strain, S. and Murray, M. (1997). The genetic basis of resistance to *Ostertagia circumcincta* in lambs. *Veterinary Journal* 154: 111-119.

Stevenson, C.R.; Mahoney, R.H.; Fisara, P.; Strehlau, G.; Reiche, M.P. (2002). The efficacy of formulations of triclabendazole and ivermectin in combination against Liverfluke (*Fasciola hepatica*) and gastrointestinal nematodes in cattle and sheep and suckling lice species in cattle. *Australian Veterinary Journal* 80 (11): 798-701.

Stuedemann, J.A.; Ciordia, H.; Myers, G.H.; McCampbell, H.C. (1989). Effect of a single strategically timed dose of fenbendazole on cow and calf performance. *Veterinary Parasitology* 34: 77-86.

Stromberg, B.E.; Vatthauer, R.J.; Schlotthauer, J.C.; Myers, G.H.; Haggard, D.L.; King, V.L.; Hanke, H. (1997). Production responses following strategic parasite control in a beef cow/calf herd.. *Veterinary Parasitology* 68: 315-322.

Sykes, A.R. (1982). Nutritional and physiological aspects of helminthiasis in sheep. In: *Biology and Control of Endoparasites*. (Edited by Donald, L.E.A. and Dineen, J.K.). pp 217-551.

Szmidt-Adjidé, V.; Abrous, M.; Adjidé, C.C.; Dreyfuss, G. (2000). Prevalence of *Paramphistomum daubneyi* infection in cattle. *Veterinary Parasitology* 87: 133-138.

Tembely, S.; Galvin, T.J.; Craig, T.M.; Traore, S. (1988). Liver fluke infections of cattle in Mali. An abattoir survey on prevalence and geographic distribution. *Tropical Animal Health and Production* 20: 117-121.

Tizard, I. (1994). *Veterinary Immunology*, an introduction. 4th edition. W.B. Saunders, Philadephia, pp 261-276

Urquhart, G.M. (1985). Field experience with the bovine lungworm vaccine. *Developments in Biological Standardization* 62: 109-112.

Urquhart, G.M.; Jarret, W.F.H.; Mulligan, W. (1962). Helminth immunity. *Advances in Veterinary Science* 7: 87-129.

Van Houtert; Barger, I.A.; Steel, J.W., Windon, R.G.; Emery, D.L. (1995). Effects of dietary protein intake on responses of young sheep to infection with *Trichostrongylus colubriformis*. *Veterinary Parasitology* 56: 163-180.

Vassilev, G.D. (1999). Prevalence of internal parasite infections of cattle in communal farming areas of Mashonaland East Province. *Zimbabwe Veterinary Journal* 30(1): 1-17.

Vassilev, G.D. and Jooste, R. (1991). Production losses and control of fasciolosis in cattle in Zimbabwe. *Zimbabwe Veterinary Journal* 22: 45-56.

Vercruysse, J. and Claerebout, E. (2001). Treatment vs. non-treatment of helminth infections in cattle: defining the thresholds. *Veterinary Parasitology* 98: 195-214.

Waller, P.J. (1997). Sustainable helminth control of ruminants in developing countries. *Veterinary Parasitology* 71: 195-207.

Waller, P.J. (2002). Global perspectives on nematode parasites control in ruminant livestock: The need to adopt alternatives to chemotherapy, with emphasis on biological control. In: *Biological control of nematode parasites of small ruminants in Asia, FAO Animal Production and Health Paper,* pp 5- 18.

Waller, P.J.; Dash, K.M.; Barger, I.A.; Le Jambre, L.F.; Plant, J. (1995). Anthelmintic resistance in nematode parasites of sheep: Learning from Australian sheep experience. *Veterinary Record* 136: 411-413.

Waller, P.J.; Larsen, M.; Faedo, M.; Hennesy, D.R. (1994). The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: *In vitro* and *in vivo* studies. *Veterinary Parasitology* 51: 289-299.

Waruiru, R.M.; Kyvsgaard, N.C.; Thamsborg, S.M.; Nansen, P.; Bøgh, H.O.; Munyua, W.K. and Gathuma, J.M. (2000). The prevalence and intensity of helminth and coccidial infections in dairy cattle in central Kenya. *Veterinary Research Communications* 24: 39-53.

Waruiru, R.M.; Thamsborg, S.M.; Nansen, P.; Kyvsgaard, N.C.; Bøgh, H.O.; Munyua, W.K.; Gathuma, J.M. (2001). The epidemiology of gastrointestinal nematodes of dairy cattle in central Kenya. *Tropical Animal Health and Production* 33: 173-187.

Waruiru, R.M.; Weda, E.H.; Munyua, W.K. (1994). The efficacy of triclabendazole and oxyclozanide against *Fasciola gigantica* in naturally infected dairy cattle in Kenya. *Bulletin of Animal Health and Production in Africa* 42: 205-209.

Waruiru, R.M.; Nansen, P.; Kyvsgaard, N.C.; Thamsborg, S.M.; Munyua, W.K., Gathuma, J.M.; Bøgh, H.O. (1998). An abattoir survey of gastrointestinal nematode infections in cattle in the central highlands of Kenya. *Veterinary Research Communications*, 22: 325-334.

Weir, D.M. (1988). *Veterinary Immunology*. Students notes. 6th edition, Churchill Livingstone, 312pp.

Whitehead, J.D. (1976). Observations on the repeated treatment for fascioliasis of stock on a farm in South-west England. *Veterinary Record* 98: 5-9.

Whitelaw, A. and Fawcett, A.R. (1981). Further studies in the control of ovine fascioliasis by strategic dosing. *Veterinary Record* 109: 118-119.

Williams, J.C. and Bilkovick, F.R. (1973). Distribution of *Ostertagia ostertagi* infective larvae on pasture herbage. *American Journal of Veterinary Research* 34: 1337-1344.

Williamson, G. and Payne, W.J.A. (1978). *An introductory to animal husbandry in the tropics*. 2nd edition. Longmans, London, 171pp.

Wolff, K.; Eckert, J.; Scheneiter, G.; Lutz, H. (1983). Efficacy of triclabendazole against *Fasciola hepatica* in sheep and goats. *Veterinary Parasitology* 13: 145-150.

Woolaston, R.R. and Baker, R.L. (1996). Prospects for breeding for parasite resistance. *International Journal for Parasitology* 26: 845-855.

Yagi, A.I.; Younis, S.A.; Haroun, E.M.; Gameel, A.A.; Bushara, H.O.; Taylor, M.G. (1986). Studies on heterologous resistance between *Schistosoma bovis* and *Fasciola gigantica* in Sudanese cattle. *Journal of Helminthology* 60 (1): 55-59.

APPENDICES

APPENDIX 1: A QUESTIONNAIRE ON CATTLE MANAGEMENT SYSTEMS AND WORM CONTROL PRACTICES IN IRINGA DISTRICT

QUESTIONNAIRE No:-----------

A: FARM PARTICULARS

1. Name of the farm/farmer……………………..

2. Farm/ farmer category

   1.Small dairy holder 2.Large scale dairy farmer 3.Traditional holder

3. Type/breed of cattle

   1. Local 2. Exotic 3. Crosses 4.Crosses and exotic

   5. Crosses and local

4. Location of the farm

   1. Rural 2. Municipal

5. Total number of animals..................

6. Number of calves.................

7. Number of male animals............

8. Average daily milk production per cow......................

9.Area of land..........................

10. Source of manpower 1. Relative 2. Non-relative

11. Type of breeding 1. Natural (own bull) 2. Natural (outside bull) 3. Artificial insemination

B: FARM MANAGEMENT (Circle the answer)

1. What type of grazing system do you use?

    1. Zero grazing (intensive) 2. In farm paddocks (semi-intensive)

    3. Communal grazing areas (extensive)

2. Do you supplement your animals?

    1. Yes 2. No

3. What groups of animals do you supplement ?

    1.Calves 2.Heifers

    3.Adults 4.All 5.Other, specify

4. What supplement do you use?

    1. ………….. 2. ……………….. 3. ……………………..

5. What type of pastures do you use?

    1. Natural pasture 2. Improved pasture 3. Other (specify)

6. How do you feed milk to young calves ?

    1. Natural suckling 2. Bucket feeding

7. Where do you obtain grasses/graze animals during the dry season?

…………………………………………………………………

8. Where do you obtain grasses/graze animals during the rain season?

………………………………………………………………………………

9. How do you house calves?

    1. independent pens/housing 2. Mixed with adults 3. No housing

10. Do you have your own pasture plot?

    1.Yes 2. No

11. How do you solve the problem of pasture shortage during the dry season?

....

12. Where do you graze calves after weaning?

1.Grazed separately 2. Mixed with heifers 3. Mixed with adults

13. At what age do calves start grazing?

1. 1-2 months 2. 2-3 months 3. 5-6 months 4. > 6 months

C: <u>WORM CONTROL PRACTICES (Circle the correct answer)</u>

1. Which is the most important constraint hindering health and productivity of your animals?

1. ECF
2. Trypanosomosis
3. Helminthosis
4.Diarrhoea
5. Poor pasture/ feed shortage
6.Mastitis
7. Other (specify)

2. How do you find helminth (worm) infection in your farm?

1. High 2. Moderate 3. Very low 4. Not present

3. How do you control helminth infection in the farm?

1. Anthelmintics/drugs 2. Management
3. None 4. Other (specify)

4. What type of anthelmintic were you using the previous season/years ?

1.Benzimidazoles 2. Levamisole
3. Ivermectin 4. Trodax 5.Other (specify)

5. What anthelmintic are you using this season/year ?

1.Benzimidazoles 2. Levamisole

3. Ivermectin 4. Trodax 5.Other (specify)

6. How long have you been using this anthelmintic?

1. 1-2 years 2. 3-4 years 3. More than 4 years 4. Don't know

7. Will you continue using the same anthelmintic?

1. Yes (why?) 2. No (why?)

8. How do you choose the dosage?

1. According to body weight/actual weighing 2. By using a fixed dose eg. 20 cc

3. By individual animal estimation 4.Other (Specify)

9. Where do you get information on worm control/anthelmintic usage?

1. Veterinarians /Animal scientist/field officers 2. Drug sales men

3. Seminars/Course/advertisements 4. Fellow animal owners/other farmers

10.Where do you buy anthelmintics/drugs?

1. Livestock offices 2. Private veterinary drugs shops

3. Livestock officers/vets come with them 4. Veterinary clinics

11. When do you drench your animals?

1. After clinical signs/theraupetically 2. Prophylactically

3. Any time 4. Other (specify)

12. When do you treat animals with anthelmintics oftenly?

1.During the dry season 2.During the rain season

3.All year round (both seasons) 4. Other (specify)

13. How many times do you drench your animals per year?

1. Once 2. Twice 3. Thrice

4. More than 3 5.Other (specify)

14. Do you recall any interruption/failure in following your treatment schedule?

1. Yes 2.No (Go to Qn 16)

15. What was the cause of treatment schedule interruption?

1. Increased cost of the drug 2. Adverse reactions

3. No improvement after drenching 4. Product not available

5. Decision to change/with reasons

16. Have you ever changed your anthelmintic?

1. Yes 2. No (Go to Qn 18)

17. Why did you change the anthelmintic?

1. Increased cost of the drug 2. Adverse reactions

3. No improvement after treatment 4. Product not available

18. How do you evaluate the efficacy of an anthelmintic?

1. By checking worm eggs in faeces post treatment

2. Improvement of body condition 3. Other (specify)

19. What factors do you consider in choosing anthelmintic product?

1. Commercial advertisement 2. Advice from veterinarians

3. Advice from feed meal 4. Withdrawal period

5. Price of the drug 6. Route of administration

7. Other farmers advice 8. Other (Specify)

20. Have you or colleagues tried to use medicinal plants in controlling helminthosis?

1. Yes (what medicinal plants) ... 2. No

21. How many times have you attended seminars on dairy husbandry/diseases control in livestock?

1.None 2. 1-2 times 3.Three times 4. More than three times

22. How do you know that this animal is infected with worms?

....

23. How do animals get infected with worms ?(rank his/her knowledge on life cycle)

1. Very good 2. Good 3. Satisfactory 4. Poor

24. Personal observation of the animal house (cleanliness, floor type)/animal feed/animal condition

etc...

25. Comments from the farmer with regard to dairy cattle keeping (problems, needs etc)

Name of interviewer .... Date ....

APPENDIX 2

DETERMINATION OF NEMATODE FAECAL EGG COUNTS (FEC) BY THE MODIFIED MACMASTER METHOD (MAFF, 1986)

1. 3 gm of faeces were weighed and placed in a plastic container.

2. 42 ml of saturated sodium chloride (Nacl) solution was added into the plastic container.

3. The plastic container was closed, mixed thoroughly by shaking.

4. The mixture was poured through a tea strainer with an aperture of about 0.15 mm and the strained fluid caught in a plastic container. The debris left on the strainer was discarded.

5. The filtrate of faeces was well stirred and a sufficient quantity was withdrawn with a Pasteur pipette and carefully run into one counting chamber of the McMaster slide.

6. After further stirring, a second sample was withdrawn and run into the second chamber of the McMaster slide.

7. All eggs under the two separate grids were counted. The number of eggs per gram (EPG) of faeces was obtained by multiplying the total number of eggs under the two grids chambers by 50.

APPENDIX 3

QUALITATIVE DETERMINATION OF FLUKE EGGS BY THE SEDIMENTATION TECHNIQUE (MAFF, 1986; Hansen and Perry, 1994)

1. Five grams of faeces from each sample was measured on an electrical balance and placed into a 100 ml plastic container.
2. The plastic container was half filled with clean tap water, closed and mixed thoroughly by shaking.
3. The solution of the sample was filtered into a 250 ml conical flask through a tea strainer.
4. The filtrate in the conical flask was allowed to stand for 20 minutes and the supernatant was carefully decanted without disturbing the sediment.
5. The conical flask with the sediment was refilled with water and the solution allowed to stand for another 20 minutes before the supernatant was decanted. The exercise was repeated until the supernatant was clear.
6. Three drops of malachite green solution was added and allowed to stand for at least three minutes.
7. The sediment was poured onto a plastic petri dish with ruled parallel lines and with the aid of a stereomicroscope fluke eggs were examined.
8. Fresh *Fasciola* eggs were differentiated from those of amphistomes by their yellowish appearance, an eccentrically placed nucleus toward the operculated side and small yolk cells, while amphistome eggs appeared colourless, with an eccentrically placed nucleus and large yolk cells.

APPENDIX 4

METHOD FOR PREPARATION OF FAECAL CULTURE (Hansen and Perry, 1994)

Equipments: Spatula, Water, Plastic containers, charcoal, cheesecloth

Procedure:

1. The collected faeces were broken and mixed well using a stirring device.
2. If faeces were too dry, water was added and if they were too wet, charcoal was added until the consistent (crumbly and moist) was obtained.
3. The mixture was transferred to plastic container.
4. The cultures were left at room temperature for seven days, by which time all larvae reached the infective stage.
5. Water was added to cultures regularly to maintain moisture content.

   Larvae were recovered using the Baermann technique and identified to genus level using identification keys.

APPENDIX 5

DETERMINATION OF PACKED CELL VOLUME (PCV) BY THE MICRO-HAEMATOCRIT CENTRIFUGE TECHNIQUE (Anony, 1983)

Materials: Micro haematocrit centrifuge, micro haematocrit reader and capillary tubes.

Method:

1. An EDTA stabilized blood in vacutainer® was mixed thoroughly.
2. A capillary tube was taken and one end was dipped into the blood, allowing the tube to fill by capillary attraction.
3. The outside of the capillary tube was wiped and cleaned.
4. The unfilled end of the capillary tube was sealed with a specific sealant (Hawskley).
5. The capillary tube was placed in a micro haematocrit centrifuge (Hawskley & Sons Ltd.) with the sealed end pointing outwards.
6. Capillary tubes were centrifuged at 12 000 rotations per minute (rpm) for 5 minutes.
7. PCV (%) was then determined by placing the capillary tube on the micro haematocrit reader.