EFFICACY OF COMMONLY USED ANTHELMINTICS IN SELECTED SHEEP FARMS IN ARUSHA

BY

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ABSTRACT

A study was conducted to determine the efficacy of albendazole, levamisole & oxyclozanide (Milsan^R) and ivermectin anthelmintics in selected sheep farms in Arusha, northern part of Tanzania. Nine study farms were screened, in each farm a total of 100 sheep were selected for faecal egg count reduction (FECR) test. Resistance was assumed to be present if egg count percent reduction was less than 90%. A study questionnaire was also administered in each farm to determine the worm control management practices. Albendazole resistance was found in the nine farms, the percent reduction ranged from 67% to 87%. Similarly Milsan^R resistance was detected in the nine farms with percent reduction ranged from 69% to 87%. Ivermectin resistance was also found in the nine farms, percent reduction ranged from 74% to 90%. Based on larvae identification from faecal cultures, the most predominant nematode species in the resistant population were *Haemonchus* and *Trichostrongylus*. The questionnaire survey revealed that all farmers examined use anthelmintics for the control of worm infection in sheep. Albendazole was used by 66.7% of the farmers, levamisole 22.2% and nitroxynil by 11.1%. Most farmers (88.9%) had used the same type of anthelmintic for five or more years and 66.7% administered anthelmintics themselves. 22.2% of farmers treated sheep twice per year while 22.2% of farmers dewormed every month, 11.1% of farmers dewormed every three months, 11.1% dewormed when animals fell sick and 33.3% of farmers dewormed when they got money. Worm infection was ranked the second most important constraint of productivity in sheep in most farms. It is concluded that anthelmintic resistance is widely spreading and becoming more serious than

previously anticipated. The magnitude of this problem in the country should be assessed and remedial solutions found in order to save the farmer from economic losses attributed to this problem.

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DECLARATION

I, JELLY SENYAGWA CHANG'A, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and has not been submitted for a degree award in any other university.

Signature.....

Date.....10/11/2006

SIGNATURE OF SUPERVISOR

Professor Kassuku, A. A.

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Date.....10/11/06.....

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DEDICATION

To my lovely husband Alex, my lovely kids Yvonne and Hans and my beloved parents.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Livestock production is an essential activity in many parts of the world, providing the nutritional and economic basis for local or even national welfare. The population of sheep in Tanzania is estimated to be 3.6 million (Anon, 1996), approximately one third the population of goats. Sheep are multipurpose animals; they produce meat, wool, milk and manure. The primary function is meat production, although in temperate countries wool has become of greater importance.

In Tanzania, animals are contributing below their optimal potential level because little effort have been made on the improvement of small ruminants. Diseases, poor nutrition, poor breeding policies and poor management are among the main constraints hindering the productivity of livestock in Tanzania (Mpelumbe, 1984; Mtenga *et al.*, 1986; Njombe, 1986; Kusiluka, 1995). Diseases contribute about 40-60% of small ruminant losses in Tanzania (Mtenga *et al.*, 1986) and among the diseases, helminthosis (worm infection) is considered to be the most important cause of reduced productivity in sheep in Tanzania (Connor, 1990; Fison, 1987).

Gastrointestinal nematode parasitism in sheep has been reported worldwide. In Tanzania, they have been reported by Ngomuo and Kassuku (1994), Ngomuo *et al.* (1994). Various worm parasites are widespread, which through their undermining activities, contribute to chronic wasting diseases or just to lowered livestock

productivity particularly when large worm burdens are present (Kelly and Hall, 1979). Gastrointestinal nematodes are by far the most important group of worms in cattle, sheep and goats. These animals constantly acquire large worm burdens while grazing permanent grassland pastures, therefore, worm control plays an essential part in improved livestock management.

For some decades, control of gastrointestinal nematode parasites has entirely been based on the use of anthelmintics. Routine and indiscriminate use of anthelmintics has resulted in the development of strains of nematodes resistant to the anthelmintics that have been in use over a long period (Kelly and Hall, 1979; Waller, 1994).

Sheep and goat farmers in Tanzania rely heavily on anthelmintics for the control of internal parasites. Significant problem due to helminth infection, other than improper treatment and the cost of treatment, is the growing parasite resistance to the frequently used deworming drugs. Currently there are several anthelmintics with different modes of action available in the market. There is a need to assess and continually monitor the efficacy of these compounds against worm populations in small ruminants in Tanzania.

Anthelmintic resistance is thought to exist when there is a greater frequency of individuals within a population able to tolerate therapeutic doses of the anthelmintic than in a normal population of the same species (Prichard *et al.*, 1980). Resistance to anthelmintics has been reported in various parts of the world where it has been looked for (Prichard *et al.*, 1980; Waller 1986). In Kenya anthelmintic resistance to

fenbendazole, levamisole and thiabendazole in small ruminants has been reported by Njanja *et al.* (1987) and Maingi (1991).

In Tanzania, there have been only few studies carried out to monitor the effectiveness of the most commercially available anthelmintics, although anthelmintics are commonly used. The few studies conducted in Tanzania have mainly involved Morogoro region which include resistance to thiabendazole and fenbendazole (Kassuku and Tibaijuka, 1987), resistance to oxfendazole (Msangi *et al.*, 1990) and resistance to thiophanate (Ngomuo *et al.*, 1990) in small ruminants. Also Monrad *et al.* (1987) have shown that, field strains of *Haemonchus contortus* at Sokoine University of Agriculture (SUA) are resistance to benzimidazole and probenzimidazole anthelmintics. These reports are of some interest because the emergence of resistant nematodes could pose problems of both economic and practical importance to sheep and goat enterprises. No reports of surveys on the extent of this problem in other parts of Tanzania are available. There is lack of information on the efficacy of anthelmintics available commercially in Tanzania against nematodes infections in other hosts as well, such as cattle, pigs and horses.

1.2 Objectives of the study

1.2.1 General objective

The general objective of this study was to determine the efficacy of commonly used anthelmintics against naturally occurring nematode burdens in sheep in selected farms of sheep in Arusha.

1.2.2 Specific objectives

The study had the following specific objectives:

(a) Administration of the questionnaire in order to determine the management practices for treatment and control of helminths in selected farms of sheep in Arusha.

(b) To determine the efficacy of albendazole in nine selected farms of sheep in Arusha.

(c) To determine the efficacy of Milsan^{R} in nine selected farms of sheep in Arusha.

(d) To determine the efficacy of ivermectin in nine selected farms of sheep in Arusha.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Gastrointestinal nematodes of sheep

2.1.1 Species spectrum

Gastrointestinal nematode parasites of sheep can be grouped according to their predilection sites. Abomasal nematodes include *Haemonchus contortus, Trichostrongylus axei, Ostertagia* species which include *O. circumcincta* and *O. trifurcata* (Dunn, 1978; Soulsby, 1982). The small intestinal nematodes are *Nematodirus* species include *N. battus, N. fillicolis* and *N. spathiger,* others are Bunostomum, *Cooperia curticei* and *Trichostrongylus colubriformis* (Dunn, 1978; Reinecke, 1989). The large intestinal nematodes are *Trichuris ovis, Oesophagostomum* species which include *Oe. columbianum* and *Oe. venulosum* (Dunn, 1978; Reinecke, 1989).

2.1.2. Life cycle

Many trichostrongyles, *Oesophagostomum* and *Bunostomum* have similar life cycle (Hansen and Perry, 1994). The life cycle is direct with no intermediate host. The original egg embryonate, eventually the larva is fully formed and ready to hatch. The hatched larva undergoes five stages of development, separated by four moults (Dunn, 1978). The first three larval stages are free living.

The first stage larva (L_1), hatches from the egg, feeds, grows and then moult into second stage larva (L_2). L_2 then grows, feeds, then moult to third stage larva (L_3). In

this form (L_3), the second moult is retained; the larva no longer feeds and is called a sheathed larva. This is usually the infective stage and must reach the definitive host to develop further (Hansen and Perry, 1994).

Ruminants are infected during grazing. Following ingestion of infective larvae, exsheathment of L_3 of *Haemonchus, Trichostrongylus, Ostertagia* and *Nematodirus* spp occurs in the rumen and parasitic larval stage migrate to the abomasums and penetrate between the gastric epithelial cells from which they emerge as L4 larvae (Soulsby, 1982).. The L_4 then emerge and moult into immature adults L_5. Matured female Trichostrongyles lay eggs which are carried down the gut and passed to the exterior in faeces. The prepatent period for *H. contortus* in sheep is an average of 15 days.

After ingestion, infective larvae of *Oesophagostomum* exsheath in the small intestine and larvae within one day after infection penetrate into the wall of the intestine, anywhere from the pylorus to the rectum, being coiled up against the muscularis mucosa. Normally they return to the rumen of the gut after five to seven days and pass to the colon, where they grow to adult after the fourth ecdysis (Soulsby, 1982).

Infection of *Bunostomum* to the host occurs through the mouth or skins, following skin penetration, the larvae pass to the lungs where the third ecdysis occurs. The fourth stage larvae reach intestine again after 11 days. In small intestine L_4 moult and mature. This takes about eight to nine weeks after infection (Hansen and Perry, 1994).

2.2 Diagnosis of gastrointestinal nematodes

Different methods can be used for the diagnosis of gastrointestinal nematodes; these include the use of epidemiology and clinical signs, parasitological methods, haematological methods, serological methods and molecular biology methods.

2.2.1 Epidemiology and clinical signs

History of the presence of gastrointestinal parasites and their seasonal incidence in a particular area are among the epidemiological data which are useful for the diagnosis of parasitic gastroenteritis. Clinical signs also aid in the diagnosis of helminthosis. Common clinical signs for helminthosis include loss of body condition, reduced feed intake and reduced productivity (Soulsby, 1982; Knox and Steel, 1997). Other signs are diarrhoea, dehydration, rough hair coat and in infections with blood sucking nematodes such as *Haemonchus* and *Bunostomum,* anaemia develops fairly rapidly, accompanied by hypoproteinaemia and oedema (i.e. bottle jaw) (Blood and Radostits, 1989).

2.2.2 Parasitological methods

Faecal egg counts (FEC), faecal culture and post mortem worm counts are among the parasitological methods used for the diagnosis of helminthosis.

2.2.2.1 Faecal egg counts

The demonstrations of the presence of worm eggs or larvae in the faeces provide positive evidence that an animal is infected. The development of quantitative methods for assessing the concentration of worm eggs in faeces was an important advance. In

this method, the procedure involves separation and concentration of eggs from faecal material. Nematode eggs are concentrated by flotation using saturated sodium chloride. McMaster counting chamber is used to examine and counting eggs microscopically (Anon, 1986).

2.2.2.2 Faecal cultures

The similarities in size and appearance of the egg of different species of trichostrongylid are such that their differentiation is extremely difficult. Their third stage larvae, however, are sufficiently different and therefore differentiation can be achieved by use of faecal cultures (Hansen and Perry, 1994). Faecal cultures provide a suitable environment for the hatching and development of helminth eggs into infective larvae (L_3) which can be differentiated to genus level. Different eggs require different conditions for development but mostly cultures are left at a temperature of about 25°C for 7 - 14 days (Anon, 1986). The L_3 are then recovered and examined under the microscope. L_3 are then identified and differentiated according to the Manual of Veterinary Parasitological Laboratory Techniques, MAFF (Anon, 1986).

2.2.2.3 Post mortem worm counts

Faecal egg and larva counts do not always give a reliable indication of worm burdens, whenever possible it is preferable to assess the worm burden directly. Where one of a group of affected animals had died or is slaughtered for post mortem examination, a worm count should be performed to determine the numbers of worms of different species that are present.

Recovery of nematodes from the alimentary tract can be done by decantation method. The abomasum is opened along the greater curvature and ingesta collected. The small intestine similarly opened and ingesta collected. Mucosal surface are washed under a jet of water. Worms and heavy debris are allowed to settle for 5 minutes, then the supernatant are decanted about two-thirds. Sediments are poured in Petri dish and examined under a stereoscope. Worms can be fished out with a needle and transferred onto a slide for closer examination using a stereo dissecting microscope or compound microscope. The large intestine is uncoiled and opened with scissors in a tray; worms of the large intestine can easily be recognized freely in the contents (*Oesophagostomum* spp) or attached to the intestinal wall (*Bunostomum, Chabertia* and *Trichuris* spp). A few drops of 45% iodine solution may be added to the sample to stain the worms and 2-3ml of 5% sodium thiosulphate to clear the background to facilitate worm counts.

Recovery of nematode larvae from the mucosa of the alimentary tract may be processed by either Williams technique or digestion method. Abomasal tissues are soaked, in physiological saline at 37-40°C overnight (larvae will migrate from the mucosa into the saline and sink), the mucosal surface will then be thoroughly washed in saline and washings then washed with a jet of water and the residue examined for larvae. Alternatively, the abomasal mucosa can be scrapped off with a knife and digested in a solution of 1% pepsin in 3% HCl at 37-40°C for 4-6 hours only, the digest may then be diluted as above.

2.2.3 Haematological methods of diagnosis

Haematological tests have been increasing in importance in diagnostic helminthology. Total white blood cell count (WBC), haemoglobin (Hb), haematocrit or packed cell volume (PCV) and the use of serum or plasma enzymes are common blood parameters used for detection of subclinical parasitism.

Studies have shown that, parasitic diseases cause blood loss, hepatopathies, gastroenteropathies, skeletal and cardiac myopathies which lead to lowered or elevated levels of haematological parameters, enzymes and biochemical metabolites compared with non-diseased individuals (Simesen and Nansen, 1974; Monrad *et al.*, 1982). Studies also have shown that, abomasal damage that is of parasitic origin in ruminants causes rise in plasma pepsinogen (Boa, 1995).

2.2.4 Molecular biological methods

Molecular biological methods are nucleic acid based diagnostic assays which rely on the demonstration of parasite nucleic acid sequences. DNA sequence analysis can be used for identification, diagnosis, molecular epidemiology, vaccine development and for studying the physiology and evolutionary biology of helminths (Prichard, 1997).

2.3 Control of gastrointestinal nematodes

Control of gastrointestinal nematodes can be divided into conventional and alternative methods. The conventional methods include management, anthelmintic treatment and integrated control (Soulsby, 1982), while alternative methods include development of

vaccines, biological control, nutritional control and breeding of resistant hosts (genetic control).

2.3.1 Control by use of anthelmintics (Curative/Suppressive)

The use of anthelmintics has been the most commonly used control method against clinical or subclinical parasitic diseases. Strategic, tactical and continuous treatments are the main classes in protective treatment.

2.3.1.1 Strategic treatments

Strategic treatment is a programme whereby anthelmintics are administered two to four time a year, at the same time each year or at the same stage with the specific purpose of reducing contamination (Blood and Radostits, 1989).

2.3.1.2 Tactical treatments

Tactical treatment is a programme whereby anthelmintic treatments done in a period of abnormally heavy rainfall and mild temperatures, when nutrition is unusually poor or when animals from a worm free environment are introduced to a danger area with the emphasis of prevention of the outbreaks of clinical parasitism (Blood and Radostits, 1989).

2.3.1.3 Continuous or intermittent treatment

Continuous or intermittent treatment is the method which involves continuous anthelmintic dosing at low levels calculated to inhibit egg production (Blood and Radostits, 1989), the aim is to reduce the establishment of worms and pasture contamination. The technique used in provision of anthelmintic in supplementary feed

blocks, intraruminal glass boluses and use of products which persist for long periods in the body and kill for extended periods.

2.3.2 Control by management (Grazing management)

Control based on management incorporates the knowledge of the life cycles, larval ecology and epidemiology of the gastrointestinal parasites. Rotational grazing, pasture resting and pasture sterilization, alternative grazing and adjustment of stocking density are common grazing management control methods.

2.3.2.1 Rotational grazing

The method involves grazing animals in different paddocks on rotational basis based on utilization of the survival time of infective larvae on pasture. The development of the nematode larvae in pasture is generally much faster, but their longevity is very low (Waller, 1997). Studies have shown that, peak larval concentrations occur on pasture within one week after contamination, but fall to barely detectable levels within four to six weeks (Banks *et al.*, 1990; Barger *et al.*, 1994). Barger *et al.* (1994) found that, in a ten paddock rotational grazing system in which each paddock was grazed for 3.5 days then spelled for 31.5 days, the set stocked animals required three additional anthelmintic treatments when mean egg counts exceeded 2000 egg per gram (epg), whereas the rotationally grazed animals required no additional treatments beyond their planned treatment at kidding.

2.3.2.2 Pasture resting and pasture sterilization

Pasture resting is seldom a suitable strategy. Stock would need to be withheld from pasture for at least six months under cool, moist conditions and two months under hot,

arid conditions for larval concentration to be reduced to a reasonably low level. Pasture sterilization is a method attempting to render a contaminated pasture helminthologically sterile in a short time. Ploughing, resowing and burning of pasture can reduce or markedly decrease levels of contamination, similarly heavy applications of some fertilizers may have some larvicidal effects.

2.3.2.3 Alternate grazing

Alternate grazing may be with the same animal species or with different animal species. In the same animal species, adult resistant stocks are grazed alongside young susceptible stock. This involves introduction of young susceptible animals into clean pastures and later adult animals are introduced in the same pasture when young animals are moved to the next clean pasture. Alternative grazing with different animal species, allow non-definitive host (dead end host) to pick the infective larvae on pasture, followed by introduction of definitive host in the same paddock. Cattle or sheep/ horse alternate grazing is highly effective with the exception of infection with *T. axei* (Waller, 1997)

2.3.2.4 Adjustment of stocking density

Increased stocking rate has numerous, often conflicting, effects on levels of infection on pasture. Increased stocking rate will increase contamination of the pasture. If the mass of herbage is reduced by the increased stocking rate, then infective stages will be more accessible (Larsen, 1991).

When animals are kept at a low stocking density, overgrazing is prevented. This results in dilution of infective larvae on pasture and animals will not be forced to feed

close to the faecal pat. The net effect is reduced number of infective larvae ingested by grazing animals.

2.3.3 Alternative control measures

Alternative control measures arise due to problems of resistance to anthelmintics, high cost of anthelmintics, also pressures from consumers for drug free animal products. These measures include vaccination, biological control, improved nutrition and breeding of resistant hosts.

2.3.3.1 Vaccination

Immunization is one way of controlling the spread of infectious diseases. However few vaccines against helminths have been produced due to poor host immune response to helminths, immune unresponsiveness in young animals and immunosuppression.

Mechanisms by which helminths escape the host immune system in immune competent animals are mimicry of host antigen, absorption of host antigens, antigenic variation, masking of antigens, shedding of glycocalyx as in *Fasciola*, blocking of antibodies as in schistosomiasis and tolerance (Soulsby, 1982; Weir, 1988; Tizard, 1994). The first vaccine against *Dictyocaulus viviparous* was developed in 1960s (Jarret *et al.*, 1960; Urquhart, 1985).

Richard *et al.* (1977), cultivated larvae of activated oncospheres of *Taenia ovis* in vitro in artificial media and collected antigen from culture medium. Ewes vaccinated four weeks before parturition conferred a high degree of immunity on their lambs via

the colostrums. Four months old lambs vaccinated with antigen collected from the culture medium had a high degree of immunity for a period of at least 12 months

2.3.3.2 Biological control

Biological control method relies on the use of living organisms which can utilize nematodes as source of food. A number of fungi have been demonstrated to be successful in controlling nematode (Larsen *et al.*, 1992; Gronvold *et al.*, 1993).These fungi act on the free living stages of nematodes on pasture. Gronvold *et al.* (1993) revealed two *Duddingtonia fragrans* isolates in Denmark that showed that the fungi were highly effective and they reduced herbage infectivity by 74% - 85%.

Other studies have shown that when the nematophagus fungus *Arthrobotrys oligospora* was mixed in cattle faeces, it reduced the number of infective larvae of *Ostertagia ostertagi* and *Cooperia oncophora* in the dung and the surrounding herbage and subsequently in grazing calves on pasture (Gronvold *et al.*, 1987; Gronvold, 1989). Studies by Larsen *et al.* (1992) showed that two fungi of the genus *Duddingtonia* reduced the development of *Ostertagia ostertagi* third stage larvae by 85%.

2.3.3.3 Nutritional control

Nutritional status of the host has been shown to have effect on the infection of the nematode (Coop, 1995; Knox and Steel, 1996; Israf *et al.*, 1996). Protein supply to the host may influence the course of helminth infections by limiting the pathogenic effect of helminth and by improving the development of immunity to helminths (Kyvsgaard

et al., 1995). Improved nutrition has been shown to increase resistance to nematode parasite infections in sheep (Abbot *et al.*, 1985; Coop, 1995; Van Houtert *et al.*, 1995) and this has been found to be associated with enhanced cellular immune response in the gastrointestinal mucosa (Coop and Holmes, 1996).

2.3.3.4 Genetic control (Breeding for resistance)

Breeding for genetic resistance to diseases has long been recognized as a valid strategy for disease prevention. It avoids the use of drugs and vaccines and once achieved, may provide long term protection. This alternative control measure against helminth parasites is being sought owing to current anthelmintic resistance of some nematodes and the increasing awareness on the drug residues in the treated animals' meat, milk and eggs (Owen and Axford, 1991; Gray, 1991; Eady *et al.*, 1996; Stear and Murray, 1994).

Studies have been done to observe the differences in susceptibility to gastrointestinal nematodes between breeds of sheep, in Scotland (Altaif and Degree, 1978), in Kenya (Preston and Allonby, 1979; Baker *et al.*, 1994; Mugambi *et al.*, 1997; Wanyagu *et al.*, 1997) and in North America (Loggins *et al.*, 1965). Some breeds of sheep in Africa (Djallonke and Sabi), in North America (Navajo) and in India (Garole) appear to be relatively resistant or tolerant to gastrointestinal nematodes (Yadar *et al.*, 1993; Stear *et al.*, 1995).

2.4 Anthelmintic resistance

2.4.1 General review

Anthelmintic resistance is one of the most important problems confronting the successful control of gastrointestinal nematodes of farm animals. Anthelmintic resistance in nematodes can be a problem in sheep, goats, horses, cattle and pigs. Development of anthelmintic resistance is based on genetic selection of parasite strains capable of tolerating anthelmintic drug concentrations which are otherwise lethal to the majority of individuals in a normal population of the same species. Once acquired by a parasite strain, anthelmintic resistance appears to be a more or less irreversible property (Kelly and Hall, 1979).

Resistance is defined as a significant increase in the ability of individuals within a strain to tolerate doses of a compound which would prove lethal to the majority of individuals in a normal population of the same species. (Prichard *et al.*, 1980). Prichard *et al.* (1980) also described side resistance phenomenon, where resistance to a compound is the result of selection by another compound of similar structure and activity and the same mode of action, while cross resistance resembles side resistance but involves compounds with different modes of action. Multiple resistances occur when individuals are resistant to two or more different anthelmintic groups, as a result of selection of each group independently. Reversion is a decrease in the frequency of resistant individuals in a population following removal of the selecting agent. LD_{50} is the dose of a compound required to kill 50% of a population of test organisms. Resistance factor is the ratio between LD_{50} for a resistant strain of parasites and LD_{50} for a susceptible strain of parasite of the same species.

Anthelmintic resistance is now widespread, mainly due to over use which selects for genetically resistant populations. Genes are passed from generation to generation and the more a population of worms is exposed to the drugs the faster it develops resistance to those drugs (Rege et al., 2002). In many parts of the world, nematode control has been based on the extensive use of anthelmintics. In Australia, New Zealand, USA and South Africa, it was observed at an early stage that some of the pathogenic worms readily developed resistance to commonly used anthelmintics (Drudge *et al.,* 1964). Since then *H. contortus* strains resistant to various anthelmintics have been reported from a number of countries (Table 1).

Reports of anthelmintic resistance have emanated from Australia, Africa, Europe, North and South America, wherever animals are regularly treated with anthelmintics and investigation have been made. The first anthelmintic (phenothiazine) resistant parasite was reported in Australia in 1957. In addition, in 1988 the first case of *Haemonchus contortus* strain resistant to the drug ivermectin was reported in South Africa, along with a few other anthelmintics (Van Wyk and Malan, 1988). The first case of resistance to ivermectin in the United State was *H. contortus* in Angora goats as well as cattle in Texas (Bowman, 2003). Anthelmintic resistance has also been described in Kenya (Njanja *et al.,* 1987; Waruiru *et al.,* 1991a). Waller (1986) found a high level of benzimidazole resistance in gastrointestinal nematodes in sheep in Australia. In Europe an increasing incidence of anthelmintic resistance in sheep was reported in Great Britain (Coles and Rush, 1992), in France (Guerin, 1996) and in Denmark (Maingi *et al.,* 1997). In USA benzimidazole resistance of gastrointestinal nematodes in sheep was found in North Carolina (Uhlinger *et al.*, 1992) and in Eastern region (Lyons *et al.*, 1992).

Table 1: The occurrence of anthelmintic resistance in *H.contortus* of sheep and goats, anthelmintic and geographical distribution

Host	Nematode species	Anthelmintic	Country
Sheep	*H.contortus*	phenothiazine	USA (1957)
		TBZ	USA (1964) Australia (1968) Chile (1967) South Africa (1975) India (1975) Brazil (1978) New Zealand (1980) Switzerland (1980) England (1983)
sheep	"	CBZ	Australia (1983)
		(PBZ+MBZ+FBZ+ OBZ+CBZ+TP)	Australia (1978)
		(TBZ+CBZ+MBZ+ FBZ+OBZ)	South Africa (1980)
		FBZ	Tanzania (1987)
Sheep	"	TBZ/CBZ/PBZ	Netherlands (1982)
		LEV	Brazil (1979)
		BZ+LEV+MO	Australia (1981)
		TP	New Zealand (1985)
Goats	"	TBZ	Texas (1970) Kenya (1987)
		FBZ/TBZ/OXF/ ALB	France (1985)

Key note;
TBZ- thiabendazole, LEV- levamisole, BZ- all benzimidazole, ALB- albendazole OBZ- oxibendazole, TP- thiophanate, CBZ- canbendazole, MO- morantel PBZ- pebendazole, FBZ - fenbendazole , MBZ- mebendazole, OXF- oxfendazole.

2.4.2 Genetics of drug resistance

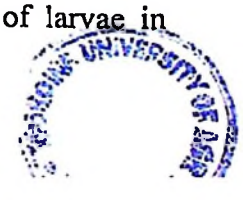
It is well known that the rate at which drug resistance develops in a given helminth population depends on many factors, among them the frequency of resistance alleles in the initial untreated population. Usually this frequency is estimated at very low level (10^{-6} to 10^{-10}). However in untreated *H. contortus* populations the initial frequencies of alleles responsible for resistance to benzimidazoles at the isotype 1 and 2 beta tubulin loci were reported to be 46 and 12% respectively, which is surprisingly high (Beech *et al.*, 1994). Similarly high (10-20%) frequencies of alleles for ivermectin resistance have been reported in unexposed *H. contortus* (Anderson *et al.*, 1998) and in feral horses, which had never been treated with anthelmintics, the subpopulation of ivermectin resistant Cyanthostomes was estimated at 4% (Young *et al.*, 1999).

The numbers of genes involved in resistance and their dominance or recessiveness are other factors with an important impact on the rate at which drug resistance spreads. Although contradictory reports have been published on *H. contortus*, there is consensus that, resistance to benzimidazole is polygenic with at least two, possibly three, genes with recessive alleles involved. Levamisole resistance seems to be caused by one gene or gene cluster, the alleles of which are autosomal recessive and ivermectin resistance appears to be mediated by a single gene or gene complex with primarily dominant effects (Anderson *et al.*, 1998). Although some argument is still going on about the number of genes involved in resistance to these different anthelmintics, there is a general agreement that reversion to susceptibility is rare once drug resistance has developed in livestock helminths, even when other drugs with

completely different working mechanisms are used. This is supported by many field and experimental data, reviewed by Conder and Campbell (1995).

2.4.3 The proportion of worms in refugia and development of resistance

The unexposed proportion of the population, to an anthelmintic considered to be in refugia, could delay the development of resistance through a dilution of selected alleles by unselected ones. Some idea of the size of the worm population in refugia can be estimated from calculations which show that during outbreaks of worm infections, when a very large proportion of the population is in the host, more than 95% of the population may be in refugia as free living stages on pasture (Le Jambre, 1978). The proportion in refugia of a worm population declines when seasonal conditions are bad for development and survival. However relatively few of the larvae on pasture are ultimately successful in finding a host and when they do can only contribute to delay in the development of resistance if they mate and leave offspring before further anthelmintic treatment take place. Continuous use of anthelmintics (e.g. slow release devices) could minimize a delaying effect because larvae moving from refugia would be exposed to selection. Likewise, if an anthelmintic is used infrequently then it is possible for larvae to produce offsprings without ever getting in contact with the chemical. Martin *et al.* (1981) did an experiment, started with a strain of *H. contortus* containing 5% thiabendazole resistant and 95% thiabendazole susceptible worms. This strain was divided into five lines based on the percentages which were 100, 90, 70, 25 and 0%. Over six generations of selection the egg hatch assay indicated that refugia delayed the development of resistance. When none or 10% were in refugia resistance developed rapidly. As the proportion of larvae in



refugia increased, there was a corresponding reduction in the development of resistance.

2.5 Contributing factors to the development of drug resistance

Treatment frequency is certainly one of the most important factors in the selection of drug resistance. Although treatment frequencies of 10-15 times a year have been reported in livestock (Dorny *et al.*, 1994), most often the number of treatments is limited to 1-3 per year. Even at these lower treatment frequencies, many cases of drug resistance have been reported in sheep and goat helminths (Geerts *et al.*, 1990; Bauer and Failung, 1992; Burger and Bauer, 1994; Coles *et al.*, 1995; Boudsocq *et al.*, 1999). Computer models have shown that the development of drug resistance in livestock helminths is delayed if drugs with different working mechanism are used in combination and on condition that the initial frequency of resistance alleles is low. Annual rotation of two drugs is the second best choice, because it postpones resistance for a longer period than in case of rotation of drugs at each treatment or rotations at 5 or 10 year intervals (Barnes *et al.*, 1995).

The target group of the anthelmintic treatment is another important parameter which influences the development of drug resistance. In the past, strategic or prophylactic mass treatments of livestock have been the rule and this practice is certainly responsible for many of the drug resistance problems veterinarians are facing now especially the drench and move system, in which all animals in a flock are treated before they are moved to clean pastures containing few or no worms in refugia, is a strong selector of drug resistance. The phenomenon of refugia, i.e. the proportion of

the helminth population that is not exposed to drugs and thus escapes selection for resistance, is a very important factor, whose impact on the development of drug resistance is often overlooked (Van Wyk, 2001). Only recently have veterinary parasitologists realized that a balance has to be found between treatment efficacy and delaying the development of drug resistance.

Models have shown that it is possible to delay the development of drug resistance by not treating part (e.g. 20%) of the herd or flock, although this might have some consequences on productivity (Barnes *et al.*, 1995). It is well known that there is an over dispersion of the helminth population, with a few individuals carrying a heavy worm burden, whereas the majority only lightly infected. In South Africa, this principle has been applied in the development of the Famacha, a colour card, which allows the farmer to identify anaemic sheep that need to be dewormed, on the basis of coloured drawings of the eye mucosa. In areas of South Africa where anaemia is mainly caused by *H. contortus,* this system has allowed to decrease significantly the number of animals treated and the treatment frequency without reducing productivity too much (Van Wyk *et al.*, 1997a).

In control programmes of human helminths, indiscriminate mass treatments are no longer advocated by most of the medical parasitologists. Treatment is usually limited to target groups such as school children. This considerably reduces the selection pressure, which is even further reduced because the compliance is often less than 80% contrary to livestock where the coverage usually approaches 100% (Chitsulo *et al.*, 2000).

Besides the target group, the timing of the treatment might also influence the development of drug resistance. This can be explained by the fact that the helminths generation which develops after treatment in dry environments will almost completely consist of resistant worms, whereas in wetter parts preparasitic stages of susceptible worms might survive on pasture and dilute the resistant genes in the next worm generation.

Finally, under dosing might also constitute an important risk factor for the development of drug resistance. As is shown by the models developed by Smith *et al.* (1999), here again the impact depends on the initial (before exposure to a given anthelmintic) and the resultant (after treatment) frequency of resistance alleles in the helminth population. Depending on their ability to kill all or part of the susceptible homozygote, heterozygote and/or homozygote resistant helminths, there are dose levels where under dosing promotes resistance and others where resistance is impeded. Assuming that resistance is determined by a single major gene comprising two alleles at a single autosomal locus and low initial frequency of the allele for resistance, the most dangerous dose is the one that kills all susceptible homozygotes but none of the other genotypes. On the contrary, when the initial frequency of the allele for resistance is high, the dose which promotes resistance most strongly is that which kills all susceptible homozygotes and all heterozygotes, but none of the resistant homozygotes (Smith *et al.*, 1999). The most important factors resulting in under dosing are underestimation of weight, dilution of the drug for economic reasons and the use of substandard drugs (Shakoor *et al.*, 1997; Van Wyk *et al.*, 1997b; Monteiro *et al.*, 1998). However, lower drug doses are sometimes advocated for the

treatment of human helminths in order to reduce side-effects or when the objective is only morbidity control (Warren *et al.*, 1993). Taking into account these restrictions, this approach might select for resistance under certain conditions.

2.6 Mode of action of anthelmintics

There are three main categories of dewormers commonly used. Nicotinics such as levamisole, benzimidazoles such as albendazole and macrolides such as ivermectin (Besier and Love, 2003). However there are other chemical groups e.g salicylanilides, piperazine and organophosphate which could also be used for the helminths treatments. Table 2 shows a summary of some commonly used anthelmintic agents, their effects and mode of action.

Ivermectin creates a chloride influx in the parasite neuron. This influx then hyperpolarizes the parasite neuron and inhibits any potential action. This results in paralysis and death of the parasite (Gogolewski *et al.*, 1997).

The benzimidazoles attack the glucose catabolism at two points. Mebendazole inhibits the glucose uptake (Van den Bossche and De Nollin, 1973). The second attack point is the inhibition of fumarate reductase. Prichard (1973) found that thiabendazole, oxfenbendazole and fenbendazole inhibited fumerate reductase in benzimidazole resistant *Haemonchus contortus*. Inhibition of the fumerate reductase system is less pronounced in benzimidazole resistant strains of *H. contortus* (Prichard, 1978). In resistant *H. contortus*, Rew (1978) found that, ethanol production, which does not involve fumerate reductase activity doubled in presence of thiabendazole. This ethanol production would assist the resistant parasite to maintain energy production.

The possession of these attributes is consistent with the reported polygenic nature of benzimidazole resistance in *H. contortus* (Le Jambre *et al.*, 1979). It has also been observed that, the free living aerobic stages are not susceptible to the benzimidazoles (Le Jambre *et al.*, 1976). These findings strongly suggest that the benzimidazoles primarily inhibit the electron transport at the level of fumarate reductase. Borgers et al. (1975) (cited by Rew, 1978) found that mebendazole disrupted the microtubules. Benzimidazoles inhibit tubulin polymerization. Inhibition of cellular transport and energy metabolism are consequences of the depolymerization of microtubules. Inhibition of these secondary events play an essential role in the lethal effect on worms. Side-resistance can exist among all members of this group because they act on the same receptor protein, *B*-tubulin, which is altered in resistant organisms such that none of the benzimidazoles can bind to the receptor with high affinity.

Levamisole has been found to be cholinergic agonist in nematodes at the ganglion level (Coles *et al.*, 1974, cited by Prichard *et al.*, 1980). The paralysis is due to sustained muscle contraction. Levamisole act as a ganglion stimulant. High concentrations of levamisole inhibit the fumerate reductase system (Prichard, 1973) and this effect may directly contribute to anthelmintic action or be linked to paralyzing effects. The paralysis they produce may be reversible. Coles *et al.* (1975) found that *Nippostrongylus brasiliensis* and *Nematospiroidea dubius* recovered from paralysis after a certain time of exposure to levamisole. The rate of return increased with increasing concentrations of levamisole. Prichard (1973) found that *Ostertagia* species regained motility after prolonged exposure to levamisole.

Table 2: Chemical groups and mechanisms of action of commonly used anthelmintic agents.

Group	chemical name	Mode of action in parasite	Effect
Benzimidazole	thiabendazole parabendazole cambendazole oxibendazole fenbendazole	Interfere with energy production. Inhibit fumarate reductase. Block tubulin synthesis. Inhibit glucose transport.	Starvation of Parasite (slow Process) ovicidal
probenzimidazole	thiophanate febantel	metabolized in vivo to benzimodazole carbamates	starvation of parasite
Imidothiazoles	tetramisole levamisole	ganglionic stimulants	spastic paralysis
Pyrimidines	pyrantel morantel	cholinergic agonists (ganglionic)	spastic paralysis
Salicylanilides	rafoxanide oxyclozanide niclosamide	uncouple oxidative phosphorylation	energy depletion
Substituted phenols	nitroxynil niclofolan bithionol hexachlorophene	uncouple oxidative phosphorylation	energy depletion
Organophosphate	trichlorphon dichlorovos haloxon	cholinesterase inhibition	spastic paralysis
Piperazine	piperazine diethylcarbamazine	neuromuscular hyperpolarizers	flaccid paralysis
Ivermectin	ivermectin	GABA potentiation	flaccid paralysis

Source: Barragry, T. (1984)

2.7 Biochemical mechanisms by which parasites may develop resistance to anthelmintics.

The following points must be considered stepwise investigating the biochemical mechanisms behind changes in susceptibility of a parasite to anthelmintics.

(a) Uptake of the drugs

Before any drug can act in an organism it must be absorbed and diffuse to the site of action. The uptake of anthelmintic in resistant worms has not been demonstrated to be reduced. There is some evidence that resistant *Trichostrongylus* species take up more thiabendazole than non resistant strains (Sangster *et al.*, 1980).

(b) Change in the site of action

The action of any drug depends on its ability to combine with the receptors at the binding site. There is some evidence that a change at the binding site can be one of the ways of a resistant worm to avoid the anthelmintic (Sangster *et al.*, 1980).

(c) Metabolism

One possible way for an organism to handle a toxicant is to include it in the metabolism and/or excrete it. It is known that DDT resistant insects are able to metabolise DDT. Similar changes in nematodes has up to now not been demonstrated.

(d) Avoiding the toxicant

A parasite can possibly avoid toxic products by possibly switching to an alternative biochemical pathway. Most adult parasitic nematodes have an anaerobic fermentation of glucose as energy supply, which involved fumerate reductase activity (Rew, 1978). Rew (1978) found that canbendazole resistant

Haemonchus contortus doubled ethanol production, which does not involve fumerate reductase activity, possibly a way to maintain a favorable energetic state until the drug is metabolized.

2.8 Detection of drug resistance

Martin *et al.* (1989) did prove that the faecal egg count reduction test (FECRT) and the egg hatch assay are able to detect anthelmintic resistance only when at least 25% of the helminth population carries resistance genes. For the larval development assay it is assumed, although not proved, that its sensitivity is slightly better, resistance being detected when 10% of the worm population is resistant (Dobson *et al.*, 1996). As modeling exercises have shown that reversion to susceptibility might only be possible when less than 5% of the helminths carry resistance genes (Roos and Kwa, 1994), these tests will obviously detect resistance when it is too late. Nevertheless a standardized FECRT is of crucial importance in order to allow comparison of data on a spatial and temporal basis. The World Association for the Advancement of Veterinary Parasitology (WAAVP) has developed standardized guidelines for the detection of drug resistance in helminths of livestock (Coles *et al.*, 1992).

2.8.1 Faecal egg count reduction test (FECRT)

The failure of the anthelmintic drug to depress faecal egg output of a group of animals following treatment is a direct indication of anthelmintic resistance. A group of animals harbouring worms suspected of being resistant is selected for the trial, and the relevant anthelmintic is administered according to the instructions for use. Egg counts (egg per gram of faeces = EPG) are performed according to the modified McMaster counting technique. (Anon, 1986) using faecal samples from individual

animals on day zero and ten days after treatment. Evaluation of anthelmintic resistance is done based on the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations for detecting anthelmintic resistance (Coles *et al.*, 1992).

2.8.2 In vitro egg hatch assay

This method was originally developed by Le Jambre (1976) for measuring thiabendazole resistance in sheep strongylid worms. The method is based on the fact that benzimidazoles prevent embryonation and hatching of strongylid eggs. Eggs from benzimidazole resistant roundworm are not susceptible to the ovicidal activity of this group of drugs. The test consists of two main steps:

a) Recovery of eggs from faeces:

Faeces revealing high counts of strongylid eggs are collected directly from the rectum and placed in water at $4^{O}C$. All the following steps are performed at $4^{o}C$. the faecal suspension is washed through a sieve, the eggs are sedmented and resuspended in saturated sugar solution in a flat sided flask. While flask is placed on its flat side for 15 minutes, all the eggs adhere to the inner upper surface of the flask. In this way an almost pure suspension containing 1000-1200 eggs per ml of water is eventually produced.

b) The egg hatch assay

A serial of 24 test solutions containing thiabendazole concentrations from 10ug per ml to 0.094ug per ml is used for testing the thiabendazole susceptibility of the worm eggs. 0.1ml of each test solution is placed in each of the wells of a flat

bottomed microtitre plate and to each well is added 0.1ml of the egg suspension. The plate is covered and agitated on a plate shaker, incubated at 26°C for 48 hours, and eventually one drop of iodine is added to each well in order to stop larval development in unhatched but not fully embryonated eggs. The hatched first stage larvae and the unhatched eggs are counted and the percentage of unhatched eggs is calculated and adjusted for natural egg death rate. A log concentration - probit line is produced and tested statistically against a log concentration - probit line of a known susceptible strain.

2.8.3 Direct controlled critical test

This is the most reliable test in which the actual percentage of worms surviving anthelmintic treatment is estimated in a controlled trial involving comparison of the suspected resistant strain to a known susceptible strain of the same worm species. One group of controlled worm free animals is infected with the suspected resistant strain and another group is infected with a known susceptible strain. When adult worm populations have been established, one half of each group is treated with the anthelmintic in question. The rest of the animals are kept as untreated controls. Ten days after the day of treatment all animals are slaughtered and total worm counts are performed by washing all the bowel contents. Worm recoveries from treated animals are compared to those of untreated animals.

The percent efficacy (PE), defined as the difference between the geometric mean worm counts in the control and treated group expressed as a percentage of the geometric mean worm counts in the control group (Presidente, 1986), is then

calculated for each anthelmintic. Anthelmintics showing less than 90% PE are considered to be ineffective.

2.9 Delay or prevention of development of anthelmintic resistance

Where anthelmintic is not a problem already it is desirable to delay its development. The first principle is to use anthelmintics sparingly and if possible to augment with other methods of control (mixed, alternate grazing etc). If a narrow spectrum anthelmintic can be used it will reduce the selection pressure to one of the broad spectrum groups. However, when drenching prophylactically against a broad range of parasites anthelmintics of group I or II will be required (Prichard *et al.*, 1980).

Prichard (1978) proposed a slow rotation in which group1 and group 2 anthelmintics are used between, but not within a single generation. The maximum generation interval of common ruminant trichostrongylid is about one year. Within this interval only broad spectrum anthelmintics from one group should be used. The theoretical advantages of slow rotation are that no individual worm would be subjected to multiple selections with anthelmintics from both broad spectrum groups, so that selection for multiple resistances, which occurs with rapid alternation, would be less likely (Prichard *et al.*, 1980).

2.10 Control in the presence of resistance

When resistance to an anthelmintic is diagnosed the use of that group in drenching programmes should be discontinued. Another anthelmintic of the same group may give reasonable efficacy at first, but the evidence of Hall *et al.* (1978, 1980) indicated that initial low level of side resistance may increase rapidly after only a few

generations of selection. Therefore, replacing an anthelmintic to which resistance has developed with another from the same group cannot be recommended.

When the level of resistance to a broad spectrum anthelmintic group precludes its use, drugs in other broad spectrum group have to be used. In a population of oxfendazole resistant *Ostertagia* species in sheep, the survivors of levamisole treatment have shown a substantial fall in the level of oxfendazole resistance after both a single treatment in penned sheep or 3 doses at 4 weekly intervals in grazing sheep. The level of oxfendazole resistance in the latter case was significantly less that when grazing sheep were not drenched at all for six months. These results suggested that levamisole selected positively against benzimidazole resistance (Donald *et al.*, 1980). In this circumstance alternation in the use of different groups of anthelmintics could delay high level of resistance.

Le Jambre (1981) reported that a levamisole resistant strain of *Ostertagia* species from sheep could be eradicated by using double doses of oxfendazole and albendazole. If the use of an anthelmintic group has been suspended for a period following detection of resistance to that group, its reintroduction in a slow rotation program can be considered, if specific tests have shown that the population has reverted to susceptibility. Reintroduction in a slow rotation program should assist to maintain the effectiveness of the alternate broad spectrum group (Prichard *et al.*, 1980). If multiple resistance to both broad spectrum groups should occur narrow spectrum anthelmintics should be tried.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in peri urban areas in Arusha municipality and in Hai district, between September and October 2005. Arusha is located in Northern part of Tanzania at latitude 03° South and longitude 36° East. Its altitude is 1387m above sea level. The average annual rainfall is 888mm spread over three months of short rains, October, November and December and three months of long rains March, April and May, the rest of months form the dry season. The minimum temperature is 14.0°C (July) and maximum temperature is 25.5°C (February). The average monthly relative humidity is 46% for the dry season and 68% for the rainy season. The study was carried out in nine farms, including a total of 900 sheep. The farms were selected randomly and the only requirements were that a minimum of 100 sheep of three months of age and above be available for examination of faecal samples on each farm and that the animals had not been treated with anthelmintic for at least 12 weeks prior to the study.

3.2 Questionnaire administration

On each farm, a standard questionnaire (Appendix I) was administered by the researcher to determine the management practices for the treatments and control of helminths in their farms. Farm identities were recorded included name of the enterprise, location and owner. Type and breeds of the animals were recorded.

Management practices, pasture types and grazing areas were asked and recorded. Types of anthelmintics used for treatment and control of helminths were recorded too. Duration of anthelmintic usage and the interval of deworming were also asked and recorded. The problems/diseases hindering productivity of sheep in their areas were asked and noted in the questionnaire.

3.3 Samples and sample processing

In each farm a total of 100 sheep above three months of age was tagged. Faecal samples were collected directly from the rectum and stored in a cool box using ice packs until laboratory examination on the same day. Egg counts were done at Arusha VIC parasitological laboratory. A modified McMaster technique was used for the detection of strongyle eggs in faecal samples (Anon, 1986). Briefly, 3g of faeces were homogenized in 42ml of flotation fluid. The faecal suspension was passed through a mesh sieve to remove coarse materials. After thorough mixing, two chambers of the Universal McMaster slide were filled and all eggs under the two ruled grids (total volume 0.3ml) counted at x40 magnification. The number of eggs obtained was multiplied by 50 to give the epg in the faecal sample.

3.4 Treatment regimen

In each farm animals were allocated into four groups according to the faecal egg count to give approximately the same average egg count, each group with 25 animals. The minimum required number is n = 15 (Coles *et al.*, 1992), the excess sheep were to allow for losses due to various causes during the course of the experiment. Three groups in each farm were treated with three different anthelmintics according to

manufacturer recommended doses, while the fourth groups were left as untreated control. The first group received albendazole (Tramazole(R) UNIVET LTD) at the single dose of 5mg/kg body weight, administered orally. The second group received a combination of levamisole and oxyclozanide (Milsan(R) INTERCHEM PHARMA LTD) at a dose of 7.5mg/kg body weight per os and the third group received ivermectin (Ivermectin(R) Product of UK) at a dose of 0.2mg/kg body weight, administered subcutaneously. The heaviest weights in each group were used for all dose calculations. The farms were visited again 10 days after treatment and all animals in the experiment resampled.

3.5 Laboratory work

Samples collected before treatment and 10 days after treatment were processed and feacal egg counts were done at Arusha Veterinary Investigation Centre (VIC), Parasitology laboratory. Faeces were cultured, third stage larvae harvested after six days and stored for identification. Third stage larvae identification was done at Sokoine University of Agriculture, Department of Veterinary Microbiology and Parasitology laboratory.

3.6 Faecal culture and third stage larvae identification

From each farm pre-treatment faecal samples were pooled and then processed for faecal culturing. Similarly, post-treatment faecal samples for each treatment group in each farm were pooled and processed for faecal culturing. Faecal samples were ground in a mortar, mixed with sterile vermiculite to form a consistency of horse faeces. The mixture was transferred into plastic cups covered with muslin cloth and

incubated at 25°C for 7 days. The third stage larvae (L_3) were recovered by partially immersing the plastic cups upside down into water in conical flasks, to allow the larvae to swim and sediment to the bottom of the conical flasks. The L_3 were then recovered using a pipette, transferred into clean petri dishes and examined under stereo microscope. Samples of the L_3 were drawn from the petri dishes using a Pasteur pipette, placed on a microscope slide and a cover slip placed on top. The larvae were then identified using microscope at X40 magnification and identified and differentiated according to the Manual of Veterinary Parasitological Laboratory Techniques, MAFF (Anon, 1986).

3.7 Data analysis

The criteria used to evaluate anthelmintic resistance were based on the WAAVP Recommendations for detecting anthelmintic resistance (Coles *et al.*, 1992). Resistance is present when the faecal egg count reduction post-treatment is less than 95% and the lower limit of the 95% confidence interval for the percentage reduction is equal to or less than 90%. Resistance is suspected when only one of these two criteria is satisfied (Coles *et al.*, 1992). Data from the questionnaire study was coded to facilitate data entry in the computer. Data analysis was conducted using Statistical Package for Social Science (SPSS) computer software. The analysis involved use of descriptive statistics and content analysis.

CHAPTER FOUR

4.0 RESULTS

4.1 Questionnaire survey to determine the worm control management practices.

Observations on animal management were obtained from all nine farms. Most farms (66.7%) had between 101-200 sheep, while 3 farms (33.3%) had more than 300 sheep. 66.7% of farms kept only Black Head Persian sheep (figure 1, 2 and 3) while 33.3% of farms had mixed breeds.

Figure 1: Flock of Black Head Persian sheep at Manyara ranch being given supplementary feeds.

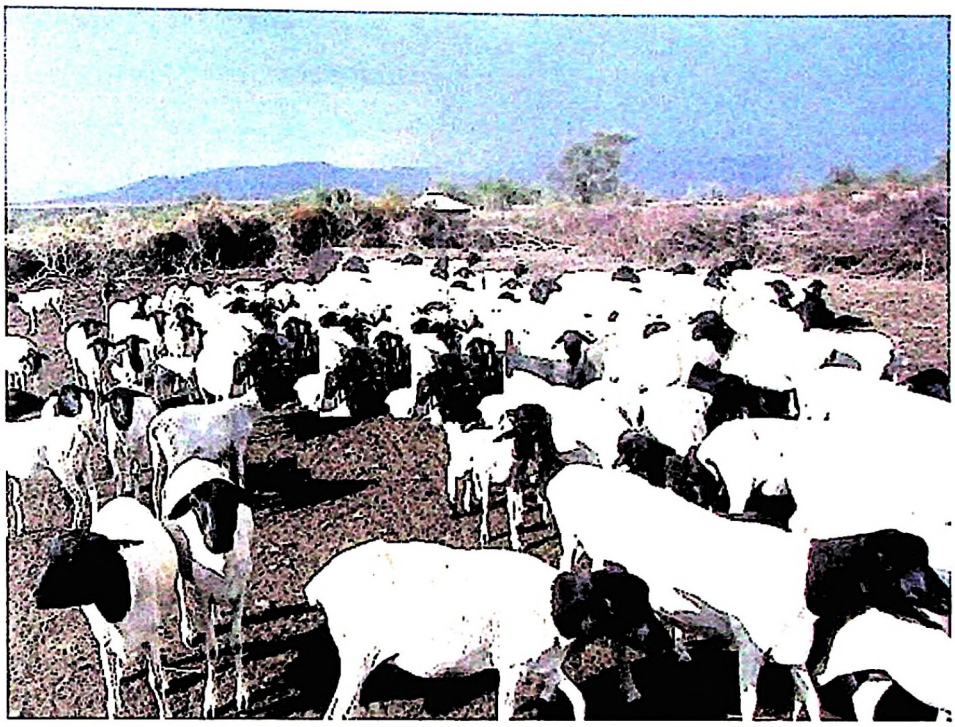


Figure 2: Flock of Black Head Persian sheep at Gomba estate.

Extensive system was found to be the most common management practice in which they use mostly natural pastures. Lambs start to graze at the age of 2 months and most farmers (77.8%) mix them with yearling or adults. Some farmers (33.3%) have a tendency to introduce animals from another farm or buying from market without deworming them, so might introduce new strains of helminths in their locality.

Figure 3: Flock of Black Head Persian sheep at Tengeru flowers farm.

All farmers (100%) used anthelmintics for the control of worm infection in sheep. The drugs used were albendazole, levamisole and nitroxynil (Trodax$^{(R)}$). Table 3 shows types of anthelmintics used in each farm. Generally albendazole was used in 66.7% of the farms and levamisole in 22.2%. About 88.9% of farmers used the same anthelmintics for more than 5 years. 77.8% of farmers continued with the same anthelmintics. One farmer (11.1%) changed the type of anthelmintic due to lack of improvement after treatment, (from levamisole (Milsan$^{(R)}$) to nitroxynil (Trodax$^{(R)}$) against trematodes.

Table 3: Types of anthelmintics used by farmers

Anthelmintic	Number of farms	Percentage
Albendazole	6	66.7
Levamisole	2	2.2
Nitroxynil	1	11.1
Total	9	100.0

About 66.7% of farmers administered anthelmintics themselves and all of them selected dosage based on weight estimates. The frequency of treatments in different farms is indicated in table 4. Only 2 farms (22.2%) treated sheep twice per year (before and after the rain season) while 33.3% of farmers dewormed when they got money, 22.2% of farmers dewormed every month, 11.1% dewormed when animals fell sick and 11.1% dewormed every three months.

Table 4: Frequency of anthelmintics treatments

Number of treatments	number of farms	percentage
Every three months	1	11.1
Before and after the rain season	2	22.2
Every month	2	22.2
When get money	3	33.3
When animals fall sick	1	11.1
Total	9	100.0

The most important constraints hindering health and productivity of sheep were diarrhoea, helminthosis, paralysis, and coughing, feed shortage during dry season, loss (death) of animals during heavy rain season, sheep pox and other skin conditions.

4.2 Efficacy of anthelmintics

Percent Faecal Egg Count Reductions of albendazole, Milsan^{R} and ivermectin in each farm 10 days after treatment are shown in table 5 and 6.

A total of 225 sheep from nine farms were treated with albendazole, 25 sheep in each farm. The percentage reduction and approximate 95% lower confidence limits respectively in each farm were as follows:- Njoolay (73%, 43%), Tengeru flowers (77%, 62%), Solomon Paul (67%, 45%), Manyara ranch (87%, 78%), West Kilimanjaro (83%, 68%), Livestock Research Centre (LRC) (73%, 57%), Gomba estate (78%, 57%), Ndoibo (68%, 48%) and Lamaiyan (71%, 50%). Given the criteria that resistance is indicated if the percentage reduction is <95% and 95% lower confidence level is <90%, albendazole resistance was detected in all nine farms examined.

225 sheep from nine farms were also treated with Milsan^{R}, 25 sheep in each farm. The percentage reduction and approximate 95% lower confidence limits respectively in each farm were as follows:- Njoolay (69%, 37%), Tengeru (85%, 69%), Solomon Paul (78%, 63%), Manyara ranch (87%, 78%), W/Kilimanjaro (85%, 74%), LRC (83%, 69%), Gomba estate (85%, 71%), Ndoibo (82%, 66%) and Lamaiyan (75%,

59%). Using the same criteria, Milsan^{R} resistance was also detected in the nine farms examined.

Similarly 225 sheep from nine farms were treated with ivermectin, 25 sheep in each farm. The percentage reduction and approximate 95% lower confidence limits respectively in each farm were as follows:- Njoolay (74%, 46%), Tengeru flowers (85%, 78%), Solomon Paul (83%, 70%), Manyara ranch (90%, 76%), W/Kilimanjaro (85%, 78%), Livestock Research Centre (86%, 70%), Gomba estate (84%, 70%), Ndoibo (78%, 60%), and Lamaiyan (81%, 68%). Ivermectin resistance was detected in the nine farms examined.

Table 5: Faecal egg count reduction (10 days post treatment) from five farms

Farm name	Anthelmintic	Percentage Reduction	Upper confidence Limit	Lower Confidence Limit	Remarks
Njoolay	Albendazole	73	88	43	resistance
	Milsan	69	85	37	resistance
	Ivermectin	74	88	46	resistance
Tengeru	Albendazole	77	80	62	resistance
	Milsan	85	93	69	resistance
	Ivermectin	85	90	78	resistance
Solomon Paul	Albendazole	67	80	45	resistance
	Milsan	78	87	63	resistance
	Ivermectin	83	91	70	resistance
Manyara ranch	Albendazole	87	92	78	resistance
	Milsan	87	92	78	resistance
	Ivermectin	90	96	76	resistance
W/Kilimanjaro	Albendazole	83	91	68	resistance
	Milsan	85	91	74	resistance
	Ivermectin	85	91	78	resistance

Table 6: Faecal egg count reduction (10 days post treatment) from four farms

Farm Name	anthelmintic	percentage reduction	upper confidence limit	lower confidence limit	Remarks
LRC	Albendazole	73	83	57	resistance
	Milsan	83	91	69	resistance
	Ivermectin	86	93	70	resistance
Gomba estate	Albendazole	78	88	57	resistance
	Milsan	85	92	71	resistance
	Ivermectin	84	92	70	resistance
Ndoibo	Albendazole	68	80	48	resistance
	Milsan	82	90	66	resistance
	Ivermectin	78	88	60	resistance
Lamaiyan	Albendazole	71	83	50	resistance
	Milsan	75	85	59	resistance
	Ivermectin	81	89	68	resistance

4.3 Larvae culture and L_3 identification

Third stage larvae (L_3) identification before and after treatments are shown in table 7 and 8 respectively. Larval identification before treatment revealed *Trichostrongylus* and *Haemonchus* larvae as being predominant. In faecal cultures from Solomon Paul, Manyara ranch and Gomba estate farms only *Trichostrongylus* (45%, 49%, 61%) and *Haemonchus* (55%, 51%, 39%) respectively were present. Faecal culture from Njoolay and W/Kilimanjaro farms consisted of three species: *Trichostrongylus* (39.7%, 37.5%), *Haemonchus* (53.4%, 60.4%) and *Bunostomum* (6.8%, 2.1%) respectively. In Tengeru flowers, Livestock Research Centre, Ndoibo and Lamaiyan farms the composition were as follows: *Trichostrongylus* (55.3%, 32.4%, 52.7%, 48.3%), *Haemonchus* (38.2%, 64.9%, 42.9%, 48.3%) and *Ostertagia* (6.6%, 2.7%, 4.4%, 3.5%) respectively.

Table 7: Percentage of third stage larval identification before treatment

Farm Name	Helminths			
	Haemonchus	*Trichostrongylus*	*Ostertagia*	*Bunostomum*
Njoolay	53.4	39.7	0.0	6.9
Tengeru	38.2	55.3	6.5	0.0
Solomon	55.0	45.0	0.0	0.0
Manyara	51.0	49.0	0.0	0.0
W/Kilimanjaro	60.4	37.5	0.0	2.1
LRC	64.9	32.4	2.7	0.0
Gomba	39.0	61.0	0.0	0.0
Ndoibo	42.9	52.7	4.4	0.0
Lamaiyan	48.3	48.3	3.4	0.0

Post-treatment cultures consisted solely of *Trichostrongylus* and *Haemonchus* larvae. The proportional percentage of larvae identified after treatment in each farm are shown in table 8. After treatment with albendazole, *Trichostrongylus* species larvae were encountered in all nine farms. The percentage in each farm were as follows:- Njoolay 71%, Tengeru 75%, Solomon Paul 75%, Manyara ranch 100%, W/Kilimanjaro 100%, Livestock Research Centre 100%, Gomba estate 60%, Ndoibo 18% and Lamaiyan 33%. *Haemonchus* spp larvae encountered in six farms, Njoolay 29%, Tengeru flowers 25%, Solomon Paul 25%, Gomba estate 40%, Ndoibo 82% and Lamaiyan 67%. No *Haemonchus* spp larvae were encountered after treatment with albendazole in Manyara ranch, West Kilimanjaro and Livestock Research Centre farms.

Larvae identified after treatment with Milsan^{R} revealed *Trichostrongylus* spp in seven farms, with the following percentages; Njoolay 67%, Tengeru flowers 100%, Solomon Paul 100%, Manyara ranch 100%, West Kilimanjaro 100%, Livestock Research Centre 100% and Gomba estate 50%, while in Ndoibo and Lamaiyan farms no *Trichostrongylus* spp encountered after treatment with Milsan^{R}. *Haemonchus* spp larvae encountered in Njoolay 33% and Gomba estate 50% farms after treatment with Milsan^{R} whereas in Tengeru flowers, Solomon Paul, Manyara ranch, W/Kilimanjaro, Livestock Research Centre, Ndoibo and Lamaiyan farms no *Haemonchus* spp encountered.

After treatment with ivermectin, larvae of *Trichostrongylus* spp had been found in five farms; Njoolay 100%, Tengeru flowers 100%, Solomon Paul 100%, Livestock Research Centre and Ndoibo 100%, while in Manyara ranch, West Kilimanjaro ranch, Gomba estate and Lamaiyan farms no *Trichostrongylus* spp encountered. *Haemonchus* spp larvae was identified only in Gomba estate farm, after ivermectin treatment while in the rest of the farms no *Haemonchus* spp larvae encountered.

Table 8: Percentage of larval identification 10 days after treatments.

Farm name	Larvae	Albendazole	Milsan	Ivermectin
Njoolay	*Haemonchus*	28.6	33.3	-
	Trichostrongylus	71.4	66.7	100.0
Tengeru flower	*Haemonchus*	25.0	-	-
	Trichostrongylus	75.0	100.0	100.0
Solomon Paul	*Haemonchus*	25.0	-	-
	Trichostrongylus	75.0	100.0	100.0
Manyara ranch	*Haemonchus*	-	-	-
	Trichostrongylus	100.0	100.0	-
W/Kilimanjaro	*Haemonchus*	-	-	-
	Trichostrongylus	100.0	100.0	-
LRC	*Haemonchus*	-	-	-
	Trichostrongylus	100.0	100.0	100.0
Gomba	*Haemonchus*	40.0	50.0	100.0
	Trichostrongylus	60.0	50.0	-
Ndoibo	*Haemonchus*	81.8	-	-
	Trichostrongylus	18.2	-	
				100.0
Lamaiyan	*Haemonchus*	66.7	100.0	-
	Trichostrongylus	33.3	-	-

CHAPTER FIVE

5.0 DISCUSSION

5.1 Questionnaire survey

The questionnaire study revealed that worm control was mostly based on the use of anthelmintics. Results obtained in this study are similar to studies elsewhere like Kenya (Kinoti *et al.*, 1994; Maingi *et al.*, 1997) and Brazil (Charles and Furlong, 1996). Anthelmintic treatments in most farms surveyed were performed irregularly, depending on the availability of drugs and money and not according to the epidemiology of the parasites. Epidemiological studies indicated that, the burden of gastrointestinal nematodes was lowest at the end of the dry season, increased gradually through the rainy season to reach peak at the end of the rainy season (Keyyu *et al.*, 2003). Therefore there is a great potential for utilization of the epidemiological knowledge in better targeting of anthelmintic treatments as opposed to the observed situation.

The use of epidemiological knowledge may also reduce the rate of development of drug resistance. Studies have shown that, the timing of the treatment might also influence the development of drug resistance. The helminth generation which develops after treatment in dry environments will almost consist of worms arising from faecal egg contamination from treated animals hence these worms have survived treatment (regarded as resistant worms), whereas in wetter parts pre-parasitic stages of susceptible worms might survive on pasture longer and dilute the resistant genes in the next worm generation.

Similar to results in Brazil by Charles and Furlong (1996), the number of anthelmintic treatments was surprisingly high. Most farmers deworm animals irregularly at least 4-5 times a year. Results from this survey have indicated that, such frequency is not justifiable and may increase the possibility of development of anthelmintic resistance. There is a high opportunity for farmers to save money by deleting unnecessary treatments at the same time reducing the chances of worms developing resistant strains. Studies have shown that, the higher the treatment pressure, the faster the selection of the resistant helminth strains (Dorney *et al.*, 1994).

In some farms, animals shared the same pasture especially during the dry season, so mixing of different worm populations of trichostrongylid worms is likely. There is a need to link or coordinate farmers on worm control strategies at a community or village level. Some farmers have a tendency to introduce animals from another farm or buying from market without deworming them, so they may introduce new strain of helminths in their locality. In order to reduce the risk of transporting resistant strain from one area to another, sheep must be checked for helminthosis and treated with an effective anthelmintic before being introduced to a new farm.

Most farmers used the same drug for more than 5 years. This also increased the chances of development of drug resistance. Annual rotation (slow) between different classes of anthelmintics is considered as an appropriate method to avoid or delay development of anthelmintic resistance in parasites (Coles and Roush, 1992). Although slow rotation is generally accepted as the best approach for delaying resistance, most effective approach is to treat simultaneously with two chemically

distinct anthelmintics (Kaplan, 2002). Models have shown that, the development of drug resistance in livestock helminths is delayed if drugs with different working mechanisms are used in combination and on condition that the initial frequency of resistance alleles is low. Barnes *et al.* (1995) found that, annual rotation of two drugs, postpones resistance for a longer period than in case of rotation of drugs at each treatment or rotations at 5 or 10 years intervals.

Most farmers administered anthelmintics themselves and all of them selected dosage based on weight estimates. However none of the farmer performed faecal egg count before treatment. The main reason for self administration was unavailability of village extension officers because they are involved in other development projects. Farmers said that, fellow farmers had shown them how to estimate dosage and administer anthelmintics.. This may cause under dosing which might also contribute to the development of drug resistance (Smith *et al.*, 1999). Expired anthelmintics were also encountered in one farm. Shakoor *et al.* (1997) found that the most important factors resulting in under dosing are underestimation of weight, dilution of the drug for economic reasons and the use of substandard drugs.

5.2 Anthelmintics efficacy

In this study, the results of the faecal egg count reduction test indicated that, albendazole, Milsan^{R} and ivermectin resistance is present in the nine farms screened.

Resistance to albendazole was detected in the nine farms, with faecal egg count reduction ranging from 67% to 87% and 95% lower confidence limit ranging from

43% to 78% (Table 5 & 6). This indicating the high level of resistance. Similarly Keyyu et al. 2002 revealed the presence of albendazole resistant *Haemonchus* in Morogoro, supporting the results obtained by Bjorn *et al.* (1991), Kassuku and Tibaijuka (1987) and Ngomuo *et al.* (1987). Albendazole, fenbendazole and thiophanate have the same mode of action, so there is also a possibility of development of side resistance. Benzimidazoles have been in the market for a long time. It is thus expected that resistant strains of nematodes would have developed over the years.

Benzimidazole resistant trichostrongylid worms problem in Africa and in the world may be widespread and serious than previously anticipated. This is due to the regular use of benzimidazole anthelmintics to control trichostrongylid worm infections in domestic ruminants for the last 4-5 decades.

Benzimidazole resistant nematodes of sheep have been reported from Australia, Africa, Europe, North and South America, wherever animals are regularly treated with anthelmintics. Maingi (1991) and Waruiru *et al.* (1991b) found high level of benzimidazole resistance in gastrointestinal nematodes in sheep in Kenya. Similarly, Beveridge *et al.* (1990), Eady *et al.* (1998), Rolfe (1993) and Waller (1986) found high levels of benzimidazole resistance in gastrointestinal nematodes in sheep in Australia. In Europe, an increasing incidence of anthelmintic resistance in sheep was reported in Great Britain (Coles, 1997; Hunt *et al.*, 1992), in France (Guerin, 1996) and in Denmark (Maingi *et al.*, 1997). In USA benzimidazole resistance of

gastrointestinal nematodes in sheep were found in North Carolina (Uhlinger *et al.*, 1992) and in Eastern region (Lyons *et al.*, 1992).

In this study, the faecal egg count reduction indicated resistance to Milsan^{R} in the nine farms ranging from 69% to 87% and 95% lower confidence limit ranging from 37% to 78%. This also indicate high level of resistance but slightly lower than in albendazole.

Ngomuo et al. (1990) noted an increasing trend in the incidence of resistant strains of strongylid parasites to anthelmintics in East Africa and it is possible that levamisole and tetramisole resistant worms may be present in other areas. Levamisole resistance has also been reported in Kenya (Waruiru *et al.*, 1991b; Maingi, 1991).

Results in this study differ with those obtained in earlier studies conducted at SUA Morogoro which revealed that levamisole was effective against the gastrointestinal nematodes of sheep and goats at Sokoine University of Agriculture (Nyangi *et al.*, 1990; Ngomuo *et al.*, 1994). At that time levamisole had not been used extensively as compared to the benzimidazoles in the control of gastrointestinal worms in small ruminants. It would be of great interest to test the efficacy of levamisole at this time.

The present study showed that, ivermectin at its recommended dose was not effective against gastrointestinal nematodes with the faecal egg count percentage reduction ranging from 74% to 90% and 95% lower confidence limit ranging from 46% to 78%. This show slightly lower level of resistance as compared to albendazole and

levamisole. This observation is contrary to the findings that ivermectin was not one of the anthelmintics used by the farmers for the last five years (Table 3). Although ivermectin had not been used by the farmers, resistance was detected. This possibly arose as a result of previous use of the anthemintics at the farm or may be the resistant strains may have been introduced from other areas. However further study could be done using a different test such as Larvae Development Assay (LDA) or carry out a dose and slaughter method to confirm this result.

However our results differ from those of Ngomuo *et al.* (1987) who found that, ivermectin was 100% effective in suppressing faecal egg output in sheep and goats at Faculty of Veterinary Medicine - SUA. Similarly Barragry (1987) revealed that, ivermectin was highly effective against nematodes resistant to the benzimidazole.

The first case of *H. contortus* strain resistant to ivermectin was reported in South Africa in 1988, along with a few other anthelmintics (Van Wyk and Malan, 1988). Ivermectin resistant strain of *Haemonchus contortus* in sheep was also reported in Brazil (Echevarria and Trindade, 1989) and ivermectin resistant strain of *Trichostrongylus colubriformis* was also reported in Brazil (Giordano *et al.*, 1988). The first case of resistance to ivermectin in the United State was *H. contortus* in Angora goats as well as cattle in Texas (Bowman, 2003).

This study has indicated multiple resistances to albendazole, Milsan^{R} and ivermectin, which have different modes of action. Multiple resistance were also reported by Echevarria *et al.* (1991), who found that ivermectin resistant strain of *H.contortus* in

sheep in Brazil were also resistant to albendazole. Similarly Van Wyk and Malan (1988) found *Haemonchus contortus* resistant to ivermectin, rafoxanide, closantel and benzimidazole in South Africa. Maingi (1991) also reported resistant strains of *Haemonchus* and *Trichostrongylus* species to thiabendazole, fenbendazole and levamisole in sheep on a Kenyan farm.

5.3 Faecal cultures

Albendazole, Milsan^{R} and ivermectin had not been able to clear worms in naturally parasitized sheep in several different farms indicating worms developed resistance to these anthelmintics in those farms. This effect was seen in *Haemonchus* and *Trichostrongylus*. *Trichostrongylus* spp had been encountered in all nine farms and *Haemonchus* spp in six farms after treatment with albendazole (Table 8). This revealed that *Trichostrongylus* population were highly resistant to albendazole in all nine farms screened, whereas *Haemonchus* population were resistant to albendazole in Njoolay, Tengeru flowers, Solomon, Gomba estate, Ndoibo and Lamaiyan farms.

The results concur with those obtained by Keyyu *et al.* (2002) who observed the presence of albendazole resistant *Haemonchus* in Morogoro. Similarly Maingi *et al.* (1997) found resistant *Haemonchus* and *Trichostrongylus* nematode to albendazole in Kenya. Promroy *et al.* (1985) reported a field strain of *H. contortus* which was resistant to albendazole in New Zealand. Ndamukong and Sewell (1986), in Cameroon observed that local strains of trichostrongylid worms in sheep had developed resistance to albendazole.

Trichostrongylus spp larvae resistant to Milsan^R were encountered in Njoolay, Tengeru flowers, Solomon, Manyara, West Kilimanjaro, Livestock Research Centre and Gomba estate farms and *Haemonchus* spp in Njoolay, Gomba estate and Lamaiyan farms. This revealed development of resistant strains of *Trichostrongylus* and *Haemonchus* spp for Milsan^R in those farms. Levamisole resistant to *Trichostrongylus* and *Haemonchus* has also been reported in Kenya (Waruiru *et al.*, 1991a; Maingi, 1991).

After ivermectin treatment, *Trichostrongylus* spp larvae were encountered in Njoolay, Tengeru flowers, Solomon Paul, Livestock Research Centre and Ndoibo farms while *Haemonchus* spp encountered in Gomba estate farm. *Trichostrongylus* strain resistant to ivermectin was reported in Brazil (Giordano *et al.*, 1988). Ivermectin resistant strain of *Haemonchus* was reported in South Africa (Van Wyk and Malan, 1988), in Brazil (Echevarria and Trindade, 1989) and in United State (Bowman, 2003).

In Manyara ranch, W/Kilimanjaro and Livestock Research Centre farms, *Haemonchus* spp were found to be susceptible to all drugs used. The explanation might be that in Manyara ranch and W/Kilimanjaro deworming were done before and after rain season while Livestock Research Centre dewormed their animals after every three months. Under such conditions the rate of selection for anthelmintic resistance in *Haemonchus* and other strongyles would be reduced. *Bunostomum* and *Ostertagia* were found to be susceptible to all anthelmintics used in all farms examined (Table 8).

Haemonchosis caused by *Haemonchus contortus* was found to be the major cause of parasitic gastroenteritis among small herbivores in developing countries (FAO, 1991)

and in Africa it is considered to be the most important worm of sheep and goats (Monrad *et al.*, 1987; Bjorn *et al.*, 1991). This is due to its adaptability to climatic conditions and its ability to survive prolonged periods of hot and dry conditions by undergoing hypobiosis.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

It has been established that, albendazole, Milsan^{R} and ivermectin anthelmintics are not effective against some nematode strains of sheep at the nine farms studied in Arusha. The most predominant species in the resistant population were *Haemonchus* and *Trichostrongylus*, two of the most important gastrointestinal nematodes of sheep in Tanzania.

Anthelmintic resistance problem may be more widespread and serious than previously anticipated. The magnitude of this problem in this country and other African countries should be assessed and remedial solutions found in order to save the farmer from economic losses attributed to this problem.

It is essential to formulate strategic programmes for the control of gastrointestinal nematodes, which do not rely entirely on the use of anthelmintics. There is also a need to regularly monitor the efficacy of commercially available anthelmintics on sheep farms so as to avoid losses due to anthelmintic failure. Effective solutions to this problem would play a significant role in improving the African food situation.

6.2 Recommendations

a) Similar studies should be carried out in other farms in other regions in the country where anthelmintics have been used for many years in the past in strategic anthelmintic control programmes. This will help to show whether these anthelmintics are still effective or not.

b) Anthelmintics currently in use in the country should have their efficacy monitored regularly and should be withdrawn as soon as it is established that they are no longer effective.

c) Research on other methods of control of nematodes in ruminants should be encouraged, e.g. methods geared towards reduction of pasture contamination by prevention of translation of L_3 from faecal pats/pellets to pastures such as using nematode trapping fungi, *Arthrobotrys oligospora*.

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APPENDIX

Appendix 1: Questionnaire on farmer's management of sheep and helminths control practices in Arusha, Tanzania.

Questionnaire number.....

A: Farm Data/ Farm Particulars;

1. Name of the farm/farmer.....

2. Number of animals (sheep)/farm size

   i) 1-100

   ii) 101-200

   iii) 201-300

   iv) > 300

3. Type of sheep

   i) Black Head Persian

   ii) Red Maasai

   iii) Mixed breeds

   iv) Local (define)

B: Data on farm/animal management

1. What type of grazing management do you use?

   i) Zero grazing (intensive)

   ii) Farm paddocks (semi-intensive)

   iii) Communal grazing (extensive)

   iv) Rotational grazing (extensive)

2. What type of pastures do you use?

   i) Natural pastures

   ii) Improved pastures

   iii) Others (specify)

3. When do lambs/kids start grazing with adult animals?

   i) 1-2 months

   ii) 3-4 months

   iii) 5-6 months

   iv) Over 6 months

4. How long do animals graze in their grazing area?

   i) Throughout the year

   ii) During the rain season

   iii) During the dry season

5. Where do you graze animals during the dry seasons?.....

6. Where do you graze animals during the rainy seasons?.....

7. Where do animals have access for drinking water?.....

8. How do you graze lambs/kids?

   i) Grazed separately

   ii) Mixed with yearlings

   iii) Mixed with adults

9. Which pasture grazing practice do you use?

   i) Rotational grazing in paddocks

   ii) Permanent paddocks

   iii) No paddocks

10. Have you ever introduced/ bought animals in your farm from another farm?

i) Yes

ii) No

11. If Yes, were they dewormed before being introduced?

i) Yes

ii) No

C: Data on helmiths control practices

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

i) Helminthosis

ii) Diarrhoea

iii) CCPP

iv) Poor pasture/feed shortage

v) Others (specify)

2. How do you control helminths infection in your farm?

i) Anthelmintics

ii) Pasture management i.e. rotational grazing/pasture spelling

iii) None

iv) Others (specify)

3. Which types of anthelmintics were you using the previous years/season?

i) Benzimidazole

ii) Levamisole

iii) Ivermectin

iv) Others (specify)

4. Which anthelmintic are you using this season/year?

   i) Benzimidazole

   ii) Levamisole

   iii) Ivermectin

   iv) Others (specify)

5. For how long have you been using this anthelmintic?

   i) 1-2 years

   ii) 3-4 years

   iii) More than 5 years

   iv) Don't know

6. Have you been using it continuously without interruption with other kind of anthelmintics?

   i) Yes

   ii) No

7. How do you deworming/ interval of deworming

   i) Every three months

   ii) Before & after rain seasons

   iii) When they fall sick

   iv) Others (specify)

8. How do you choose the dosage before deworming the group?

   i) By estimation

   ii) Using weighing machine

   iii) Using average body weight

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

i) Local veterinarians

ii) Local field officers

iii) Drug seller

iv) Another experienced farmer

v) None