

**Nematode Prevalence, Helminth  
Management Practices and  
Anthelmintic Resistance  
in Small Ruminants in  
the Mid-Rift Valley of  
Ethiopia**

**BY**

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## ***Declaration***

I, Desalegn Lidetu Woldemariam, do hereby declare that the work on which this thesis is based is original work, except where acknowledgements indicate otherwise. Neither the full dissertation nor any part of it has been, is being, or is to be submitted for another degree at this or any other University.

Candidate: \_\_\_\_\_

Date: \_\_\_\_\_

Dedicated to

**Genet Getahun Mekoya**

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## Summary

Parasitic helminths, mainly nematodes, are the most important causes of diseases of sheep and goats in the relatively warmer climatic areas of Ethiopia. This thesis comprises five related studies on the gastro-intestinal parasites namely, (1) the intensity of gastro-intestinal nematodes and coccidia in a semi-arid area, (2) the fluctuation of parasitic diseases in arid and semi-arid environments, (3) a questionnaire survey to study the perception of farmers and animal health workers on the control of worms by using anthelmintics and implications to the development of resistance, (4) a survey on the occurrence of anthelmintic resistance in selected areas and (5) the evaluation of the effectiveness of selective anthelmintic treatment using the FAMACHA<sup>®</sup> system. Data that were collected both during the pilot and the main studies in 1998-1999 and 2002-2003, respectively, are presented here.

The overall results of the longitudinal study on gastro-intestinal parasites of sheep and goats indicate that nematode egg counts were higher at all sites after the rainy seasons and declined during the dry seasons. The mean egg counts during the long rainy seasons in the initial survey were 536, 554 and 483 eggs per gramme of faeces for young, juvenile and adult goats, respectively, while sheep of the same age group had 560, 487 and 637 eggs per gramme of faeces, respectively. The two most prevalent of worms recovered from the 48 lamb tracers were *Haemonchus contortus* (91-100%) and *Trichostrongylus colubriformis* (90-100%) followed by *Oesophagostomum columbianum* (33-83%) and *Trichuris ovis* (8-33%). Significant differences in worm counts were observed between seasons ( $P < 0.05$ ). The mean faecal nematode egg counts during the rainy seasons of 2002-2003 were 1 887, 2 085 and 2 273 for young, juvenile and adult goats, respectively, while sheep of the same age group had 2 000, 2 186 and 2 243 eggs per gramme of faeces, respectively. The overall nematode count was significantly higher than the nematode count during the initial study. The worms that were recovered during the different seasons of 2002-2003 from 57 lamb and 53 kid tracers showed *H. contortus* (91-100%) and *T. colubriformis* (90-100%) to be predominant, followed by *O. columbianum* (33-83%) and *Trichuris ovis* (8-33%). Similarly, *H. contortus* (95-100%) and *T. colubriformis* (83-100%) were predominant in the 53 kid tracers, followed by *O. columbianum* (58-83%) and *T. ovis* (41-74%). A significant difference in worm count was observed within seasons ( $P < 0.05$ ) and sites. The mean worm burden during this study was found to be much higher than the initial period (1998/1999) in almost all study sites during the worm seasons.

The species composition of nematodes does not vary between sites. Other helminths such as *Moniezia expansa*, *Taenia hydatigena* and *Echinococcus* spp. were found in sheep and goats in East Shewa.

This study also presents evidence that coccidiosis is a highly prevalent condition in lambs and kids in the Rift Valley areas. A total of 710 (83%) of sheep and 625 (74.2%) of goat faecal samples contained *Eimeria* oocysts. Lambs and kids had a significantly higher oocyst count than juvenile or adult sheep and goats ( $P < 0.05$ ).

Information on worm management practices with emphasis on the use of anthelmintics and their implications for the development of resistance was obtained through a questionnaire survey involving 100 smallholder farmers and 64 animal health workers in North and East Shewa zones. The main factors identified in this study, which may contribute significantly to the selection of worm resistance to anthelmintic according to Coles and Roush (1992) and Waller (1997) were failure to alternate classes of anthelmintics, under-dosing and the use of poor quality anthelmintics. The majority of farmers in North Shewa and East Shewa did not alternate anthelmintics and only 2% of the 64 animal health workers alternated anthelmintics.

The results obtained from faecal egg count reduction tests carried out in selected areas of East and North Shewa and on institutional farms indicate the presence of anthelmintic resistance of nematodes in sheep and goats in 4 out of 22 smallholder farms and on one institutional farm, where *H. contortus* was predominant. Resistance to levamisole was also detected on one smallholder farm and one institutional farm.

The alternative approach in the management of haemonchosis by selective anthelmintic treatment using the FAMACHA<sup>®</sup> method was studied using experimental sheep and goats. Correlations between the haematocrit values and FAMACHA<sup>®</sup> scores, faecal egg count and haematocrit values and worm and faecal egg counts were all significant ( $P < 0.05$ ) for both sheep and goats in the selective treatment group. Sheep and goats that were selectively treated gained significantly more weight than non-treated ( $P < 0.05$ ), or animals treated on single occasion ( $P < 0.05$ ). Animals treated monthly (Group I) had significantly higher weight gains than the other groups. The sensitivity of the FAMACHA<sup>®</sup> test to identify animals that fall into categories 3, 4 and 5 was 72.7% while the specificity was 94.9%. The FAMACHA<sup>®</sup> method was found to be a simple and cheap alternative to use in an integrated control programme for nematode parasites, particularly when *H. contortus* is the primary pathogen.

# **Chapter 1.**

## **General introduction**

One of the most important and immediate goals for Ethiopia is to become self-sufficient in food production, a goal that is clearly expressed in the National Food Policy and Strategy and in the Poverty Reduction Programme (Anonymous, 2003). The country has faced critical food shortages for decades and with the rapid growth in its population, it becomes crucial to maximize agricultural production through improved management. Therefore, the country needs to prioritize and improve agricultural production in various sectors, including the livestock industry.

An estimated 23 million sheep and 18 million goats are present in Ethiopia. They produce meat, milk, wool, skin and manure, and are, amongst others, kept as savings, which can easily be converted to cash if needed. Mutton and goat meat contribute 35% of the total meat consumption in the country (Anonymous, 1995). Goats' milk is also very important for pastoralists in the arid areas and the smallholders with their mixed farming systems in the semi-arid areas. In the eastern highlands it is an alternative and cheap source of milk. Sheep and goats, as well as their products, are regularly exported to neighbouring countries, thus contributing to the country's foreign earnings.

Losses due to diseases, including helminth infections, of livestock are estimated to be high, and have been studied by Graber (1975, 1978a) and Lemma, Gebre-ab and Tedla (1985). The epidemiology of nematode infections was extensively studied in the highlands in the north of the country, and in North Shewa (Tembely, Lahlou-Kassi, Rege, Sovani, Diedhiou and Baker, 1997; Mamo, Gebre-ab and Tedla, 1981; Mulugeta *et al.*, 1989). Results of these studies, conducted largely at research stations (Tembely *et al.*, 1997; Tembely, Lahlou-Kassi, Rege, Mukasa-Mugerwa, Anindo, Sovani and Baker, 1998; Bekele, Kasali and Woldeab Woldemariam, 1992a), clearly indicate a distinct seasonal availability of larvae on pastures. Examination of worm populations in tracer lambs at different periods of the year confirmed the occurrence of *Trichostrongylus*, *Haemonchus*, *Dictyocaulus* and *Longistrongylus elongata* (Tilahun, 1988; Tembely *et al.*, 1997). According to Tembely *et al.* (1997) transmission occurs during the wet seasons and infected hosts are the only important means as they carry the infection over from one season to another. However, little work has been done in the semi-arid and arid areas, which include the eastern and north-eastern parts of the country, including East Shewa.

In many sub-Saharan African countries, including Ethiopia, helminthoses adversely affect production and productivity of small ruminants (Troncy, 1989; Boomker, Horak and Ramsay, 1994). The productivity of these animals, however, is very low (Anonymous, 1993) and can be attributed to the various diseases, malnutrition and management practices. It is estimated that about 2 million cattle and 5-7 million sheep and goats die from various diseases each year. More significant, however, are losses resulting from inferior weight gains, condemnation of organs and carcasses, and lower milk yields (Jacob, 1979 cited by Tilahun, 1988). The economic loss to the Ethiopian meat industry and the export of livestock to foreign markets due to helminthoses is an estimated US\$ 400 million annually (Tilahun, 1988; Gezahegn, 1992).

The development of cost-effective and sustainable control programme to control helminth infections requires a thorough knowledge of the species of parasites present, the flock/herd structures, grazing management, seasonal availability of parasites and weather conditions in a particular area (Boomker, Horak and De Vos, 1989; Boomker *et al.*, 1994; Hansen and Perry, 1994).

Patterns of infection with gastro-intestinal nematodes and larval contamination on pastures in relation to weather conditions have not been investigated in the semi-arid and arid areas in the eastern and north-eastern regions of the country. The wide diversity of agro-ecological and environmental conditions in the country make studies carried out in one area almost non-applicable to other agro-ecological zones.

The control of gastro-intestinal parasites of livestock in Ethiopia is based only on the use of anthelmintics. Pasture management is either unknown or not practiced by peasant farmers and/or smallholders. In general, due to lack of information about the epidemiology of helminths in ruminants in the country, anthelmintics are imported in bulk by government, private companies and individuals, and distributed all over the country. Due to the high cost of drugs, peasant farmers do not deworm regularly but rather treat selectively according to clinical signs.

Strategic worm control programmes for sheep, based on the epidemiology of their gastro-intestinal nematodes have been successfully evaluated in the UK (Taylor, Hunt, Wilson and Quick, 1991) and Australia (Dash and Waller, 1987). Numerous field trials have confirmed that strategic drenching can be highly effective and computer simulation studies have predicted almost without exception that preventive strategic drenching is superior for worm control than most schedules of suppressive drenching that are applied at a time when the

worm challenge on pasture is high (Michel, 1969, 1976; Brunndon, 1980; Lloyd, Smith, Connan, Hatcher, Hedges, Humphrey and Jones, 2000). Selection for anthelmintic resistance, however, needs to be considered. Field trials and mathematical models have indicated that to prevent a build-up of parasites on pastures, drenching at the beginning of the worm season, or immediately before the long, dry and hot summers, when few refugia are present, can be highly effective in managing worms. Once again, the greater the success of the strategy, the greater the degree of selection for anthelmintic resistance is likely to be (Waller, 1995).

The use of clinical anaemia as an aid in the control of haemonchosis in sheep is a new concept developed for selective treatment (Bath, Malan and Van Wyk, 1997; Malan and Van Wyk, 1992; Vatta, Letty, Van der Linde, Van Wijk, Hansen and Krecek, 2001) and has also successfully been used in goats (Vatta, Krecek, Letty, Van der Linde, Grimbeck, De Villiers, Motswatswe, Molebiemang, Boshoff and Hansen, 2002). Progress is being made with various forms of integrated worm management using anthelmintics as well as alternative methods of control, as reviewed by Waller (1997) and Van Wyk, Malan and Bath (1997a). Anthelmintics remain the cornerstone of worm management/control, but need to be supported by methods such as drenching only a proportion of the flock to reduce selection for resistance (Besier, 1997). Farmers also accept this, because they themselves do it often by treating selectively those animals that show symptoms of worm infection.

The use of anthelmintics at regular intervals for an extended period has resulted in the development of resistance of nematodes to one or more available drugs in many countries, for instance against the benzimidazoles, probenzimidazoles, levamisole, avermectins, closantel and organophosphates (Prichard, Hall, Kelly, Martin and Donald, 1980; Prichard, 1990). Indiscriminate and overuse of anthelmintics (Van Wyk, Stenson, Van der Merwe, Vorster and Viljoen, 1999), under-dosage (Atanasio, 2000) and the introduction of breeding stock from other countries (Varady, Praslicka, Corba and Veseley, 1993; Maingi, Bjørn, Thamsborg, Bogh and Nansen, 1996a; Mwamachi, Audho, Thrope and Baker, 1995) are responsible for the development of anthelmintic resistance. Waller (1997) reported that resistance of nematodes to the commonly used groups of anthelmintics is an increasing problem, with variation between and within countries and farming systems. He further stated that anthelmintic resistance has been exacerbated by the proliferation in the number of generic anthelmintic products often of dubious quality offered to farmers at reduced prices, together with the unethical practices of drug substitution and adulteration (Waller, 1997). These practices take place in Ethiopia, which again suggest the need to investigate the occurrence of anthelmintic resistance.

Several studies have been undertaken in various countries to examine existing worm control practices. This has laid the basis for recommendations on the control of helminths, depending on the local strategies to prevent the development of resistance to anthelmintics. Neither anthelmintic resistance on sheep and goat farms in East and North Shewa and the factors associated with its occurrence have been investigated before, nor is information recorded on worm control practices on these farms. Two questionnaires of different formats, one for peasant farmers and the other for animal health workers, were developed to obtain information on the use anthelmintics and worm control practices. Reliable information on the occurrence of anthelmintic resistance is required and standardized tests have to be followed (Coles, Bauer, Borgsteede, Geerts, Klei, Taylor and Waller, 1992). Thus, the most recommended and commonly used faecal egg count reduction test (Coles *et al.*, 1992) was employed in this study.

The objectives of the study were to:

- determine the prevalence and intensity of infection with gastro-intestinal parasites in sheep and goats in the Rift Valley areas of East Shewa zone in relation to seasonal weather conditions, age and sex of the host;
- evaluate the effectiveness of selective anthelmintic treatment in sheep and goats by clinically identifying individual animals using FAMACHA<sup>®</sup>, body condition scores and haematocrit levels;
- determine the prevalence of anthelmintic resistance and the factors contributing to the development of resistance on sheep and goat farms in East and North Shewa zones and
- determine worm control practices in East and North Shewa zones using questionnaire responses on the management practices and use of anthelmintics in veterinary clinics and on sheep and goat farms.

## **Chapter 2.**

### **Review of the literature**

#### **2.1. BACKGROUND**

Infection with gastro-intestinal helminths has been studied in many countries and appropriate times for intervention with anthelmintic drugs have been determined. This is not so for many developing countries in tropical Africa. Helminthoses in small ruminants are of considerable significance in a wide range of agro-ecological zones of the continent (Tembely *et al.*, 1997). In Ethiopia, where livestock is kept on pasture throughout the year and climatic conditions favour the development and survival of free-living stages, helminth parasites are major causes of economic loss (Tilahun, 1988; Mamo *et al.*, 1981).

Sheep and goats are usually infected with a range of different species of nematodes. The economically most important and widely prevalent gastro-intestinal nematodes are the Trichostrongyloidea that include genera such as *Haemonchus*, *Trichostrongylus*, *Mecistocirrus*, *Cooperia*, and *Nematodirus*, and the Strongyloidea and Ancylostomatoidea with *Oesophagostomum* and *Bunostomum*, respectively, as representatives. Graber (1978b) and Graber, Delavenay and Tesfa Mariam (1978c) have reported a wide-spread occurrence of the metacestodes *Cysticercus ovis* and *C. tenuicollis*. Fasciolosis in sheep and goats is considered to be of great economic importance and both *Fasciola hepatica* and *Fasciola gigantica* occur in the country (Gall and Scott, 1978; Lemma *et al.*, 1985). Amphistome infections were reported by Graber *et al.* (1978c).

These helminth parasites, among others, are responsible for a considerable amount of pathology in small ruminants. Gastro-intestinal nematode infection is associated with effects on feed intake, gastro-intestinal function and protein turn over (Holmes, 1987). These results in many of the other changes were associated with helminth infections such as poor growth, loss of weight and mortality (Holmes, 1987).

Since different helminth species have different pathogenic effects, it is important to know which groups are present in a flock or herd in an area or region, and the factors that influence their life-cycles and epidemiology. Furthermore, some of these parasites have different development times, both outside and inside the host, the knowledge of which is important for effective control measures. The factors, which affect the development and survival, are mainly environmental, especially seasonal climatic change and certain

management practices (Hansen and Perry, 1994; Urquhart, Armour, Duncan, Dunn and Jennings, 1994).

## 2.2. ENVIRONMENTAL FACTORS

From an epidemiological viewpoint the infective stages, which eventually become available to the host, depend on the independent and interactive influences of several factors in the macro- and micro-environment. An excellent summary of the ecological requirements and epidemiological factors that apply to helminth infections is given by Urquhart *et al.* (1994).

The free-living stages of nematode parasites of grazing animals have two basic environmental requirements, namely high temperature and high moisture. High moisture levels, particularly surface soil moisture are determined by the amount and distribution of rain and the rate of evaporation from the soil. The latter is to a large extent dependant on the soil type, as well as by the vegetation and the micro-habitat. The third larval stages (L<sub>3</sub>) and embryonated eggs are the least susceptible to adverse environmental conditions, while unembryonated eggs, and the first (L<sub>1</sub>) and second (L<sub>2</sub>) larval stages, in that order, are more susceptible.

A seasonal pattern of infection of pastures occurs in the tropics where transmission of gastro-intestinal nematodes is mainly restricted to the wet seasons. The only means to carry the infection over from one rainy season to another is through animals harbouring adult worms and/or arrested (hypobiotic) larvae (Chiejina, 1994; Hansen and Perry, 1994; Tembely *et al.*, 1997; Vlassoff and Bisset, 1991). Urquhart *et al.* (1994) similarly described that in certain areas in the tropics and subtropics, survival of *H. contortus* is associated with the ability of the larvae to undergo hypobiosis, which usually starts at the beginning of a prolonged dry season and permits the parasites to survive in the host as arrested L<sub>4</sub>. Unlike *Haemonchus*, however, hypobiosis in *Trichostrongylus* in temperate areas occurs as the L<sub>3</sub> stage (Urquhart *et al.*, 1994).

The survival of *H. contortus* on tropical pastures is variable but the infective larvae are relatively resistant to desiccation and some may survive for 1-3 months on pastures or in faeces (Urquhart *et al.*, 1994). In the Trichuridae and Ascarididae, which do not have free-living infective larvae, the infective egg can survive in a warm humid environment for several years, and were shown to still be a source infection for animals (Chiejina, 1994).

Once the rainy season starts and environmental conditions become favourable for the survival of the infective larvae, the hypobiotic larvae mature and there is a continuous cycle

of infection between the host and pasture for as long as these conditions last. The number of larval peaks is a good indication of the number of generations of the nematodes, and the length of a generation interval can be estimated between 1-2 months for *Haemonchus* and *Trichostrongylus* species. It has been estimated that a minimum of 3-4 generations of *Haemonchus* and *Trichostrongylus* species can develop in small ruminants during the 6-7 month rainy season in the Nigerian sub-humid zone, while approximately 1-3 generations of the same species develop in goats in the humid zone of Malaysia. These contrast with a maximum of 2 generations of ovine trichostrongylids in the temperate conditions of north-east England (Boag and Thomas, 1971; Eysker and Ogunusi, 1980; Fakae and Chiejina, 1988; Chiejina, 1994). Vlassoff and Bisset (1991) stated that there are basically only two generations of parasites annually. The first one is derived from over-wintered larvae and those that have developed from the post-parturient rise in the ewe, while the second is derived from larvae that develop from the lambs' own contamination over the summer/autumn period. In a study conducted in Spain, Uriarte, Llorente, and Valderrabano (2003) reported that three generations of parasites occur. According to their report, the generation derived from eggs deposited the previous autumn gave rise to the first infection of the animals in January and May with *T. circumcincta* and *H. contortus* being the predominant species. The second generation occurred between June and July and the third generation in October and November.

### **2.3. LIVESTOCK PRODUCTION SYSTEM AND HUSBANDRY PRACTICES**

Livestock husbandry systems and managerial practices have a major influence on the transmissibility of infection to a susceptible host population. In most traditional systems, where animals are kept extensively, faecal contaminations and infective stages are thinly spread over a large territory, and heavy infections rarely occur.

In a study of traditionally managed small ruminants confined in sheds and zero-grazed throughout the rainy season, but allowed to roam free during the dry season, Fakae (1990) observed escalating worm burdens and faecal egg counts during the wet periods, and the opposite occurring during the latter seasons. Similarly, nomadic, and to a lesser extent livestock that moves seasonally to another region, herds and flocks usually harbour low levels of infection. However, if such animals remain in one locality for an extended period, they are liable to create a significant source of infection, particularly in areas where they are confined, and at watering places, especially during droughts. Confinement of large numbers of young animals in unhygienic and wet environments also predisposes to heavy infections,

mainly with nematodes that infect hosts percutaneously, such as *Bunostomum* spp. (Reinecke, 1983; Troncy, 1989).

According to Reinecke (1983) and Urquhart *et al.* (1994), *B. trigonocephalum*, *G. pachyscelis* and *S. papillosus* infect sheep and goats percutaneously and display similar clinical signs such as itching, weight loss, emaciation, anaemia, submandibular oedema and diarrhoea. *Bunostomum* infects percutaneously and per os, *Strongyloides* percutaneously and transmammary, but *Gaigeria* infects only percutaneously. Infection with 200-300 *Bunostomum*, 100 *Gaigeria* and 4 000 *Strongyloides* are usually fatal to small stock (Reinecke, 1983).

#### **2.4. HOST AGE, ACQUIRED RESISTANCE AND GENOTYPE**

Age influences the susceptibility to and the pathogenicity of helminth infections. Neonates are generally incapable of responding immunologically to nematode parasites. The ability of sheep to respond to *T. colubriformis* and *H. contortus* infections is not fully developed until at least 3-6 months of age (Reinecke, 1983; Hansen and Perry, 1994).

Traditionally managed flocks or herds contain a disproportionate number of old, largely female stock, which are in constant contact with their young from birth till the next pregnancy or sometimes parturition, a situation which assists the maintenance and transmission of infection (Allonby and Urquhart, 1975).

A characteristic periparturient rise in faecal strongyle egg output in ewes and does is due to a temporary relaxation or suppression of host immunity (Soulsby, 1982). This allows maturation of hypobiotic larvae, increases the fecundity of female worms and enhances establishment of new infections. *Haemonchus* and *Trichostrongylus* species have been reported to cause such a rise in sheep in the tropics (Van Geldorp and Schillhorn Van Veen, 1976).

The main factors, which are known to induce or influence larval hypobiosis are environmental factors.

#### **2.5. CONCURRENT INFECTIONS**

Concurrent infections in indigenous and exotic breeds of goats have been shown to suppress host immune response in trypanosome endemic areas where concurrent trypanosome and worm infections are common, and are frequently associated with

nutritional and climatic stresses, which are known to influence host resistance to infection (MacKenzie, Boyt, Emslie, Lander and Swanepoel, 1975). Borgsteede and Dereckson (1996) reported on the coccidial and helminth infections in goats kept indoors in the Netherlands. Oocysts were found in 26 out of 27 kids (96.3%), in 52 out of 55 weaners (94.5%) and in 72 out of 110 adult goats (65.5%) while nematode egg counts, and larval cultures and identification revealed infections with *H. contortus*, *Trichostrongylus* spp. and *Trichuris ovis*.

Health problems encountered in sheep and goats under the peasant management systems in both highland and semi-arid areas indicated ectoparasitic, helminth, and coccidial infections which sometimes include Footrot, dermatophilosis, mange, tick and lice infestations. Similar observation was reported by Kusiluka *et al.*, 1998 in Tanzania.

## **2.6. CONTROL**

Effective helminth control is a major element in ensuring the sustainability of animal production (Waller, 1997). The main aim of control is therefore to ensure that the biotic potential of a parasite is restrained at a level compatible with the biological requirements of economic livestock production (Gordon, 1973; Brunsdon, 1980). Since eradication of gastro-intestinal nematodes is not practical, only integrated control methods can be envisaged. Some of the basic principles include grazing management, acquisition of natural or artificially induced immunity, biological control and the judicious use of anthelmintics (Brunsdon, 1980; Probert, 1994). The main methods for control of helminth parasites are prophylactic treatment with anthelmintics and combined with grazing management.

## **2.7. USE OF ANTHELMINTICS**

Presently the control of gastro-intestinal helminths is mostly based on the regular use of anthelmintics. In the humid tropical zones of many countries, where *H. contortus* is dominant and weather conditions are favourable for the development and survival of infective larvae almost throughout the year, anthelmintic treatment is important if mortalities are to be reduced and satisfactory weight gains achieved (Allonby and Urquhart, 1975). Despite the accumulation of drugs in animal products and undesirable effects on non-target organisms in the environment, together with an increase in anthelmintic resistance, the use of anthelmintics still remains the corner-stone of helminth control (Van Wyk *et al.*, 1999; Waller, 1997). Because animals are often infected with a wide range of helminths, the need for broad-spectrum compounds active against trematodes, cestodes and nematodes, and their larval stages, is obvious (Probert, 1994).

Currently, there are a number of strategies that are being followed in the control of nematodes of ruminants when using anthelmintics. These are suppressive treatment, non-suppressive treatment and selective treatment. With suppressive treatment, the aim is to treat frequently to prevent the worms from becoming patent, thereby preventing transmission and reducing mortality and morbidity. The non-suppressive treatment involves the strategic use of drugs, and selective treatment aims to reduce production losses and to treat animals that show clinical symptoms of parasitism. Both field and mathematical models have indicated that treatment under conditions of low refugia, to prevent a build-up of parasites on pasture (for example, at the beginning of the worm season, or immediately before long dry and hot summers) can be highly effective for worm management. However, the greater the success of this strategy, the greater the degree of selection for anthelmintic resistance is likely to be (Waller *et al.*, 1995; Waller, 1997).

Most modern drugs have high margins of safety with therapeutic indices greater than three and therefore, the chances of overdosing are minor. Anthelmintics are administered parenterally, orally, topically or intra-uminally. Oral formulations consist of tablets, gels, pastes, drenches or granules and powders for inclusion in feed or water (Bogan and Armour, 1987; Probert, 1994; Boersma, 1992). The dose rate is calculated on the basis of milligram per kilogram (mg/kg) of body weight of the animal. Therefore, the most likely problem is under-dosing, if the estimated weight of the animal is too low (Probert, 1994). Accurate dosing is best achieved by oral or parenteral administration. Administration of the drug in feed or water is not usually accurate, since some animals may take more than others. It is also important to realize that patho-physiological changes caused by nematodes can also affect the bio-availability in a negative way (Probert, 1994; Abbott, Parkins and Holmes, 1985).

Diet can influence the bio-availability of anthelmintics, for instance, faster intestinal passage in grazing animals and consequently a lower absorption rate. Trials in sheep and cattle have shown that the bio-availability of benzimidazole, closantel and ivermectin is less in free-ranging animals than those housed (Taylor, Mallon, Blanchflower, Kennedy and Green, 1992; Ali and Hennessy, 1993). The controlled release of daily doses of anthelmintics over several weeks or months is designed to increase the efficiency of helminth control programmes (Anderson, 1985). The impact of these technologies, however, raises concern that they could provide a strong selection pressure for the development of anthelmintic resistance (Donald and Waller, 1982; Waller, 1997).

## **2.8. DEVELOPMENT OF ANTHELMINTIC RESISTANCE**

Anthelmintic resistance is the ability of an individual to survive the lethal effect of a chemical and is the result of selection acting upon the genetic variation within the population (Martin, 1987). The single most important feature of anthelmintic resistance is that it is multi-dimensional and an inherited physiological or biochemical characteristic (Le Jambre, Royal and Martin, 1979; Le Jambre, Prichard, Hennessy and Laby, 1981). The development of resistant strains is evolutionary, which depends on ecological factors that vary with species, population and location (Martin, 1987).

## **2.9. USE OF QUESTIONNAIRES IN SURVEYS OF ANTHELMINTIC USAGE.**

Questionnaire surveys in various countries have been undertaken to examine helminth management practices. The objectives of these surveys were mainly to establish any shortcomings and make recommendations for improvement based on facts gathered. Such surveys have been undertaken for cattle in England and Wales (Michel, Lotham and Leech, 1981), cattle and sheep in England (Gettinby, Armour, Bairden and Penderleith, 1987), and sheep in Denmark and Kenya (Maingi *et al.*, 1996a). In a survey by Kettle, Vlassoff, Reid and Horton (1983), which covered several regions of New Zealand, widespread resistance to both benzimidazoles and levamisole was observed, and a positive correlation between frequency of dosing and the presence of resistance on the farms was established. Pearson and McKenzie (1986) reported that 58% of goat farms in Canterbury area of New Zealand did not have predetermined drenching programmes and the majority used the doses recommended for sheep, irrespective of the anthelmintic class. This was likely to select heavily for resistance as goats are reported to metabolize anthelmintics more rapidly (Charles, Pompen and Miranda, 1989). On most of these farms, sheep were grazed alongside goats, which facilitated the transmission of resistant worms between the species. Scherrer, Pomeroy and Charleston (1990) reported that visual estimation of animals' live weight, and administering drugs based on the average weights and the overuse of one class of anthelmintics, could lead to heavy selection for resistance.

## **Chapter 3.**

### **General materials and methods**

#### **3.1. STUDY AREAS**

East and North Shewa zones located in the regional States of Oromia and Amhara respectively were identified as the study areas. East Shewa is situated in the Great Rift Valley where the altitude is between 1200-1700 meters above sea level. The Great Rift Valley extends its vast escarpments, cliffs, rivers and plains from the Red Sea southward through Ethiopia, Kenya, Tanzania, and Malawi to end into the Zambezi River in Mozambique. The valley is some 50-60 Km wide. Several large lakes occur along the study areas. North Shewa is in a highland area where the altitude is more than 2000 meter above sea level (Fig. 3.1).

A systematic sampling procedure was followed to select the study sites. Firstly, a list of all accessible sub-districts of the zone was prepared. Six sub-districts were selected randomly from this list. Similarly a list of peasant farmer's villages that met certain criteria, such as accessibility by vehicle all year round, availability of veterinary clinics, animal health representatives from the Ministry of Agriculture, willingness of peasant farmers' villages representatives to participate in the study, and the population and availability of sheep and goats in the study sites, was prepared. A total of ten peasant farmers villages were randomly selected, at least two in each sub-district of East Shewa (Fig. 3.2).

Two surveys were conducted in small ruminants in the semi-arid and sub-humid areas of East Shewa zone. The first survey on the prevalence and intensity of nematode infection was carried out in Metehara, Dugdabura and Ziwai sub-districts. The second survey that included questionnaire and anthelmintic resistance surveys as well as studies on the prevalence and intensity of helminth infections and an experimental evaluation of selective anthelmintic treatment was carried out in Modjo, Meki, Dugdabura, Ziwai and Shashemene sub-districts. The study area or nearest reference points to the areas are indicated in Fig. 3.2. In the highland areas, Sululta, Sheno, Debre Birhan, Muketuri and Selale subdistricts were selected using similar procedures and ten peasant farmers villages were selected as the study sites. The two surveys that were carried out in North Shewa were the questionnaires and surveys on anthelmintic resistance.

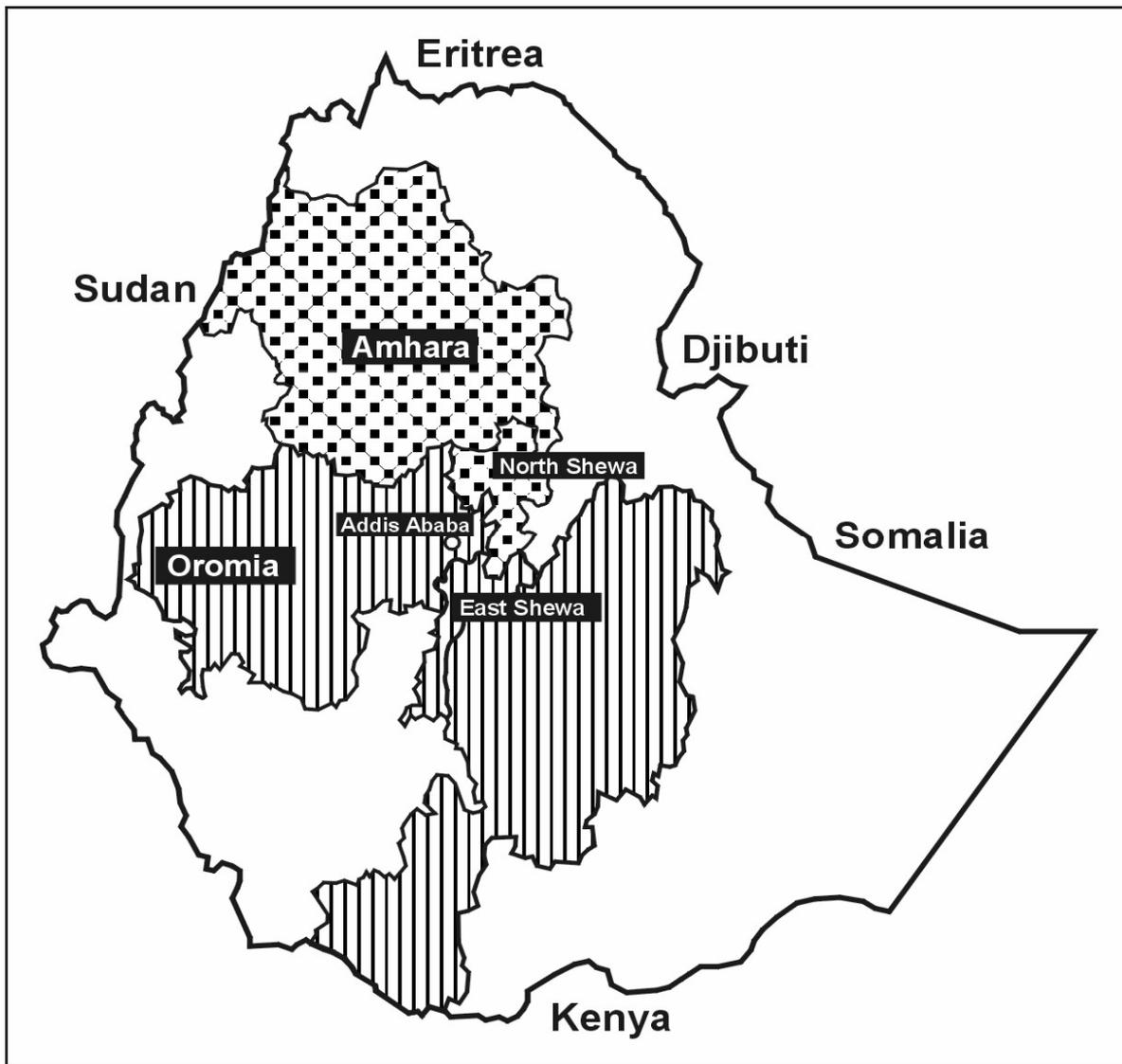


Fig. 3.1. Map of the Federal Republic of Ethiopia showing Amhara and Oromia Regional States, and North Shewa and East Shewa zones.

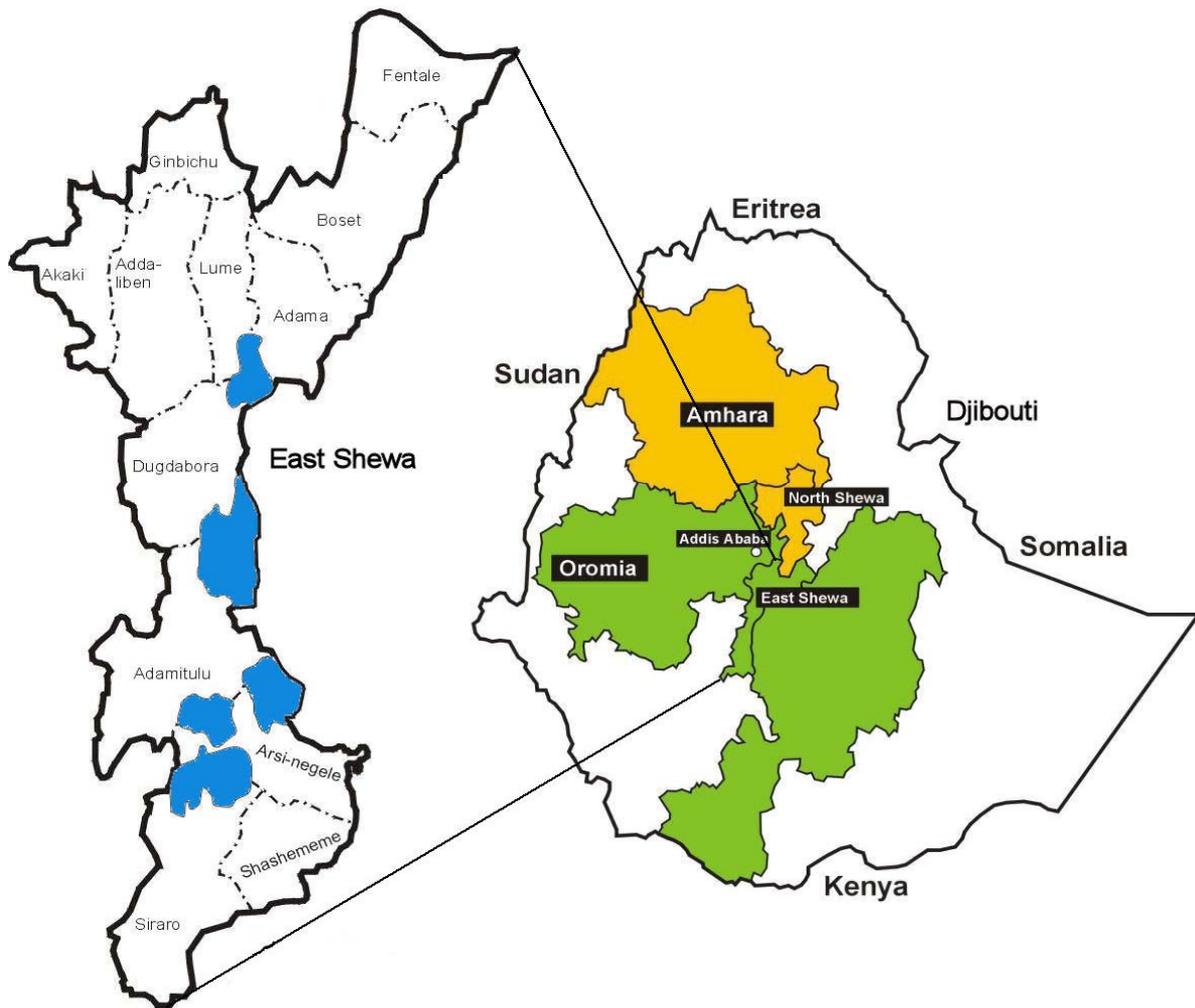
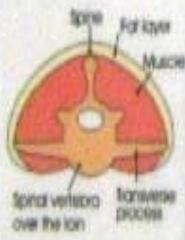
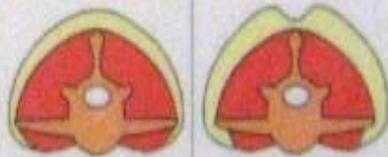


Fig. 3.2. Map of East Shewa showing sub-districts where the study sites located.

Condition Scoring in Sheep					
Spines	Individually clearly felt, sharp, obvious	Form a smooth line with deep undulations	Only slightly detectable undulations	Only detectable with firm pressure	Not detectable
Transverse processes	Fingers easily pass underneath	Smooth round edges	Well covered. Have to push firmly to get fingers underneath	Cannot be felt at all	
Muscle	Very little. Concave	Concave	Not concave. Not convex	Maximally developed. Convex	
Fat layer	No	Very thin	Moderate	Thick	Very thick to form a dip along top midline
					
Condition score	1	2	3	4	5

**Description:**

- The condition scoring is performed over the lower back area.
- Cases which do not fit these categories properly i.e. fall between whole numbers, can be assigned half scores eg. 1.5, 2.5 etc.
- This scheme may be used in goats, but half a score is added to the score, since goats preferentially store fat intra-abdominally and not over the lower back.


Fig. 3.3. Scheme for body condition scoring used in sheep and goats in the FAMACHA trial during July 2002-September 2003.



**Fig. 3.4.** Shelter for the experimental sheep and goats for the FAMACHA<sup>®</sup> trial at Abernosa viewed from outside (top) and inside (bottom).



**Fig. 3.5.** A group of experimental sheep grazing at Abernosa in the Rift Valley area of East Shewa during the dry season (top) and at the start of the rains (bottom).

### **3.2. CLIMATE**

In the semi-arid area of East Shewa the climate is hot and dry with unpredictable rains that vary from year to year. The annual rainfall averages 600 mm and the mean annual temperature is about 26°C. Relative humidity is between 50-80%. Daily minimum and maximum atmospheric temperatures, relative humidity and rainfall data were collected at Metehara Sugar Factory Research Center, Adamitulu Agricultural Research Center and the National Meteorological Services Agency.

The climate in North Shewa is characterized by a long, cool rainy season (June-September) that accounts for 75% of the annual rainfall, a short, rainy warm season (February-May) and a dry, cold season (October-January). The annual rainfall averages 960 mm and the mean maximum temperature ranges from 14-23°C and the mean minimum from 7-18°C.

### **3.3. STUDY ANIMALS**

Indigenous sheep and Rift Valley goat breeds of Ethiopia used in this study were mostly of the East African type (Galal, 1983 cited by Gatenby, 1986). (Fig. 3.4 and 3.5).

Lambs and kids used as tracers were purchased in the rural area where anthelmintics were seldom used. The tracer animals were maintained under worm-free conditions and were subsequently used to establish the seasonal incidence, for worm recovery and identification. Prior to being released onto the pastures, each animal was treated with albendazole at 7.5 mg/kg body weight. The tracers were introduced onto pasture monthly on the day the previous group was removed. The removed tracers were slaughtered after being kept indoors for 21 days at the National Animal Health Research Centre to allow larval stages that might be present to mature.

A total of 248 tracer animals were slaughtered during these studies. Experimental sheep and goats utilized in the FAMACHA<sup>®</sup> trial were kept in a shelter without a roof, but with a concrete floor to reduce contamination and provide for ventilation (Fig. 3.4 and 3.5). All other animals used in the surveys belonged to the participating farmers.

### **3.4. FAECAL WORM EGG COUNT**

Faeces were collected from the rectum of each animal, often early in the morning. The faeces were placed into specimen bottles, which were filled to the top to exclude air so that the development of the worm eggs could be delayed. Faeces were usually processed the

same day but those that could not be processed the same day were preserved with 10% formalin.

Faecal worm egg counts were done using the modified McMaster technique described by Hansen and Perry (1994) with slight modifications of our own to the procedures.

- Four grams of faeces were placed into a white mortar of medium size.
- Flotation fluid, in this case saturated salt solution (56 ml), was added to the mortar containing the faeces.
- The faeces were broken into pieces in the mortar using the pestle and then mixed by stirring with a wooden spatula.
- The mixed faecal material was sieved using a tea strainer into container 2.
- A subsample was taken from container 2 with a wide-mouthed pipette while stirring.
- A McMaster counting chamber was filled with the subsample and then allowed to stand for 5 minutes, after which it was examined under a microscope at 100x magnification
- All nematode eggs and coccidian oocysts in two chambers were counted separately.
- The number of eggs per gramme of faeces was calculated using the equation:  
Number of nematode eggs per gramme of faeces (epg) = number of eggs counted / number of chambers counted x100.
- Whenever the result of the McMaster count was negative, another subsample of the remaining suspension was examined before the result was recorded as negative.

### **3.5. FAECAL LARVAL CULTURE**

The eggs of the common gastro-intestinal roundworms differ so little from each other in appearance that they cannot be differentiated microscopically. Consequently, with a few exceptions, the worm species that are present could not be determined and faecal cultures were therefore used to identify helminths to the genus level. To determine the monthly larval helminth composition, pooled fresh faecal samples from each of the treatment groups and

thus from each of the species of animals were cultured for 7-12 days at room temperature, following the method described by Reinecke (1983) with a slight modification of the procedures.

- The pooled sheep or goat faeces were broken into fine pieces using a mortar and pestle. The faeces were mixed with an equal amount of vermiculite.
- The tip of a dowel rod was held in the middle of the bottom of a wide-mouthed fruit jar of one litre capacity, while the faeces-vermiculite mixture was placed little by little in the bottle and tamped down around the central dowel using another dowel.
- The inside of the jar was wiped with tissue paper to clean it from excess faeces. This was done to reduce contamination of the larval suspension with faeces and pieces of vermiculite during harvesting of the larvae.
- The inside of the fruit jar was rinsed down to the surface of the compacted faeces using a wash bottle. The contents was moistened and adjusted until it was damp but not soft.
- The lid was screwed on lightly and the culture left in the laboratory to incubate for 7-12 days at room temperature.
- The larvae produced migrated up the sides of the bottle. These larvae were collected by holding the flask upside down and by flushing the larvae off the sides and allowing them to run into a 100 ml measuring cylinder.
- The larvae so collected were allowed to settle down and were then examined and identified according to the procedures and techniques described by Van Wyk *et al.* (1997b, 2004).

### **3.6. BODY CONDITION SCORE**

The technique of body condition scoring in sheep and goats is an assessment of the degree of fat deposition or muscle development on different parts on body of the animal. Stockmen in several countries usually appraise the condition of their animals in verbal terms such as “fair, bad, good, lean, fat” which are often ambiguous descriptions. Over years, several attempts have been made to formalize body condition scores using numerical values. A

system based on six points scale was described by Boden (1961). Using this system as basis, Russell, Doney, and Gunn (1969) showed that subjectively assessed body condition score was closely related to the amount of chemically determined fat in sheep and that it could provide an acceptable and useful means of estimating the proportion of fat in the animal's body. For sheep this system became a useful tool in certain areas of research (Boden, 1991). In recent years, the system has been improved with better guidelines. The guideline used in the present study was developed in South Africa by the Agricultural Research Council and the University of Pretoria for use mainly to measure body condition scores in sheep (Figure 3.3) The body condition score was assessed by palpation of the sheep in the lumbar region, on and around the backbone in the loin area immediately behind the last rib, and above the kidneys as suggested in Boden (1991).

- An assessment is made of the prominence (the degree of sharpness or roundness) of the spinous process of the lumbar vertebrae.
- The prominence of and the degree of fat cover of the transverse process of the vertebrae assessed.
- The extent of the muscular and fatty tissues below the transverse process is judged by the ease with which the fingers pass under the ends of these bones.
- The fullness of the muscle area and its degree of fat cover in the angle between the spinous and transverse process estimated.
- The fat deposition area in goats is different from that of sheep, therefore, a half-score was added to the body condition scores in goats as an adjustment. Body condition scores were carried out combining the assessment points described by Boden (1991), and the illustrated guidelines (Fig. 3.3).

### **3.7. LIVE WEIGHT MEASUREMENT**

Live weight increase in livestock is the gross expression of the combined changes in carcass tissue, organs, viscera and gut fill (Orr, 1982). Similarly, Bathaei and Leroy (1996) stated that animal's growth is expressed as the positive change in body weight per unit of time or by plotting body weight against age. However, weight is strongly influenced by several factors. The adverse effects on productivity are manifested in a variety of ways with changes in body weight, which vary with level of infection, the species of parasites involved, the age,

breed (genotype), season of birth, nutritional and immunological status of the host (Gatenby, 1986; Holmes, 1987; Githigia, Thamsborg, Munyua and Maingi, 2001). Information on the correlation of helminth parasites on the live weight of small ruminants that have been managed under selective anthelmintic treatment under semi-arid conditions was not available. Therefore, live weight measurement in this study was aimed at assessing the effect of selective anthelmintic treatment on body weight gain of sheep and goats. Sheep and goats were weighed monthly over a period of 15 months using fixed spring balance (50 kg, Salter, UK), accurate to the nearest 500 g. An attempt was made to weigh the animals in the morning before they were released to graze in order to minimize fluctuation in weight that might arise due to feeding or drinking.

The monthly live weight gains were computed as: 
$$G_p = \frac{(W_t - W_{t-1})}{15}$$

where  $G_p$  is the growth at period  $p$ ,  $W_t$  the weight at age  $t$ , and  $W_{t-1}$  the weight at age  $t-1$ .

Data were analyzed by repeated measures of analysis of variance (ANOVA) of the GLM procedures of SAS (2003). In the analysis, the egg count and month (season) were considered as subject effects. The following linear models were used to analyze the data.

$$Y_1 - Y_{15} = \mu + T_{ik} + Y_{ik} + (TY)_{ijk} + \xi_{ijkl}$$

Where  $Y$  = weight, and  $Y = \log(\text{epg}+1)$  and  $i$  refers to the  $i^{\text{th}}$  treatment,  $j$  refers to the  $j^{\text{th}}$  treatment,  $k$  refers to the  $k^{\text{th}}$  month,  $l$  refers to the  $l^{\text{th}}$  individual animal and  $\mu$  is the overall means.

### 3.8. COLLECTION AND PROCESSING OF BLOOD SAMPLES

Blood samples were collected monthly from the ear veins of the experimental animals using heparinized micro-haematocrit tubes. Packed cell volume (haematocrit level) and presence of blood parasites determined from this blood. Haematocrit values were expressed as a percentage, using a haematocrit reader.

Thick and thin blood smears were made on clean glass slides at the same time that blood was withdrawn from the ear vein of each animal.

- The thin smears were fixed for 3 minutes with absolute methyl alcohol the same day they were prepared. Thick blood films were prepared by putting a drop of blood in the

centre of a clean slide. The drop was spread by spiral movements of the corner of another slide over a circular area 1.5 cm diameter. The prepared slide was left flat to dry for several hours, away from dust and insects.

- All smears, thick and thin, were stained with a 10% Giemsa solution at pH 7.2 for 30-40 minutes in the laboratory. Excessive stain was washed from each slide with tap water and slides were then allowed to drip-dry.

Smears were examined under a standard microscope under 40x and 100x oil immersion objectives. More than 100 fields were scrutinized before a slide was considered negative for haemo-parasites.

### **3.9. COLLECTION AND PROCESSING OF HERBAGE SAMPLES**

Herbage samples were collected during the short and long rainy seasons only when grass was available, usually in the morning before the pasture became drier, as the larvae could migrate to the bottom of the grass leaves and tufts (Hansen and Perry, 1994). The sampling was carried out using the methods described by Taylor (1939) and Hansen and Perry (1994).

The area of pasture to be sampled is traversed in a zigzag manner, halts about 1m apart being made at about one hundred different places and samples of herbage plucked from different points at each halt. About 2-3 pinches of grass are plucked at each halt from each of four places, one immediately in front of the toe, and 3 others as far as it can conveniently be reached in front and on either side of the foot. The grass was plucked as close to the ground as possible, but without pulling up the grass roots. Grass from areas with faecal droppings in all halts was not collected. A second collector takes samples separately at the same time, beginning at another corner.

The herbage samples were processed using the bucket washing method (Hansen & Perry, 1994). The isolation of larvae was carried out according to the procedures described by Krecek *et al.* (1991) and Hansen & Perry (1994).

Prior to washing, the net wet weight of the vegetation sample was determined. Two hundred grammes of the sample were placed into a mesh bag. The mesh bags were made from a plastic material similar to mosquito netting or fly screen, and had apertures small enough to retain the plant material but big enough to allowed soil and other small particles, including worm larvae, to be washed through.

The recovery and isolation of larvae was carried out with some modification to the procedures described by Hansen and Perry (1994).

- Each sample in the mesh bag was immersed in a bucket of water, to which 1 ml of dish washing soap had been added. The bag was prevented from touching the bottom by hanging it on a rod or dowel placed horizontally on the bucket. This was important for the larvae to be washed down into the bucket and sediment for later recovery, otherwise several larvae might remain on the grass.
- The mesh bag was raised and immersed in the bucket several times within the first 1-2 hours while each time the water was allowed to drain back into the bucket. The sample was then left overnight.
- The following day the mesh bag was slowly removed from the bucket while tap water was run over the bag into the bucket to wash down larvae that may have remained at the bottom of the grass. The content of the bucket was left to settle for 1 hour, after which the supernatant was decanted leaving about 500 ml that contained the sediment.
- The contents was then sieved onto a 25  $\mu\text{m}$  sieve through a tea strainer or bigger sieve to remove bigger particles and then re-suspend into 1 000 ml water
- The 1 000 ml suspension was poured through a Baermann apparatus and left to stand for 1-2 hours. About 30 ml of the trapped suspension was collected from the rubber tube of the Baermann apparatus in a 50 ml tube and left to cool at 4°C for 1 hour.
- The supernatant was decanted until about 10 ml remained.
- After thorough mixing two aliquots of 1 ml each were taken randomly while stirring the 10 ml sample.
- Micropipettes of 100  $\mu\text{l}$  or 200  $\mu\text{l}$  were used to pick the larvae from the randomly taken 1 ml sample. Larvae were then placed on a glass slide, stained with iodine, and identified and counted under a compound microscope.

- The washed grass samples were air dried for 30 days at room temperature and weighed.

The number of larvae recovered from each of the 200 g samples was determined by multiplying the total number counted in the two aliquots of the last 10 ml samples by 5. The total volume of larval suspension obtained from each of the samples was 10 ml.

The number of larvae recovered from 1 kg of dry grass was calculated using the following formula:

$$L_1 = L_2/V_2 \times 100,$$

Where L1 is the calculated number of L<sub>3</sub> recovered from 1 kg grass, L2 is the number of third stage larvae counted on 200 g grass and V<sub>2</sub> is the dry mass of 200g grass.

### 3.10. COLLECTION AND PROCESSING OF PARASITES

Processing and collection of the helminth parasites of sheep and goats were based on the methods described by Boomker *et al.* (1989):

- The entire gastro-intestinal tract together with the heart, lungs and liver was removed. The various organs were separated from each other and from suspensory ligaments, and were placed individually in shallow plastic trays.
- The heart was opened and examined for macroscopically visible parasites. It was then cut into slices approximately 10 mm thick and these were placed in a plastic jar with normal saline.
- The bile ducts of the liver were opened and visible parasites removed and placed in 70% alcohol. Five strips, each approximately 10 mm thick were removed from 5 places over the entire width of the liver, and placed in a plastic jar with normal saline. The strips representing 1/5<sup>th</sup>, ± 50 g of the mass of the liver and thus represented a 1/5<sup>th</sup> aliquot.
- Only the right lung, together with the trachea, was processed for parasite collection. The trachea and bronchi were opened, scrutinized for visible parasites and rinsed in running water over a sieve with 90 µm apertures. The entire lung was washed and then cut into 10 mm cubes and placed in a plastic jar with normal saline.

- The washings from various organs, together with the saline in which each organ had been incubated, were sieved over a sieve with 25 µm apertures. The residues in the sieve were collected and separately preserved in 10% formalin. The washings of the trachea and the bronchi were included with those of the lung.
- The digestive tract was divided into rumen and reticulum, omasum, abomasum, small intestine and large intestine.
- The rumen and reticulum were opened and their contents carefully removed. Visible amphistomes were collected in 10% alcohol.
- The abomasa, the small and the large intestines were opened. Each organ was rinsed twice in a small quantity of water, which was added to the respective ingesta. The washed organs were retained for further processing. The ingesta from each part of the gastro-intestinal tract was thoroughly mixed separately, put in a plastic jar with 1ℓ capacity and preserved in 10% formalin for further processing in the laboratory.
- The mucosae of the abomasa, small and large intestine were removed by scraping with glass slide and were placed in separate plastic jars of 1ℓ capacity. Digesting fluid, consisting of 10 g of pepsin powder and 35 ml technical hydrochloric acid per litre of normal saline was added to the mucosae in the ratio of four parts digesting fluid to one part mucosa. The jars were placed in the sun for incubation, and were shaken every twenty minutes until the digestion was complete. Then each digest was sieved over a sieve 25 µm apertures and the residue preserved separately in 10% formalin for further processing in the laboratory.

### 3.11. IDENTIFICATION AND COUNTING OF HELMINTHS

In the laboratory, the ingesta of the abomasa, small and large intestines were put into separate plastic containers of two litres capacity and each was made up to 1000 ml with water. Using a glass pipette the content was thoroughly mixed and 1/10<sup>th</sup> aliquot (100 ml) was taken. The digests of the abomasa and small intestines were sieved and washed over a sieve with 25 µm apertures and those of the large intestine over a sieve with 90 µm apertures. The contents of the jars containing the heart, liver, lung and entire digests were also washed separately over sieves with 25 µm apertures. The residue on the sieve was carefully washed back into the correct marked plastic bottle.

The various aliquots of the ingesta and the entire digests, as well as the washings of the heart, lung and liver were examined in a Perspex counting chamber using a stereoscopic microscope. All the helminths were removed, identified using the descriptions of Dunn (1978), Levine (1978), Gibbons and Khalil, 1982), and counted. Nematodes were classified according to their developmental stages, and, where possible identified to the species level. Trematodes and cestodes were identified to the genus level only.

### **3.12. USE OF ABATTOIRS IN STUDYING THE PREVALENCE OF GASTRO-INTESTINAL PARASITES**

During the dry seasons of 2002 and short and long rainy seasons of 2003, faecal and total worm counts were made from 180 gastro-intestinal tracts of goats and sheep slaughtered at Mojo export abattoir. The numbers of sheep that were processed in this study were small, because the abattoir is mainly used for slaughter of goats. The procedures described by Boomker *et al.* (1989) and Hansen and Perry (1994) for examination of gastro-intestinal tracts for adult worms and juvenile larvae were used.

### **3.13. DETERMINATION OF ANTHELMINTIC RESISTANCE**

In the study, herds of sheep and goats from two institutional farms and 22 smallholders' farms were selected from respondents to the questionnaire surveys (Chapter 7). Farmers who had at least 15 lambs or goats (<12 months old) were identified to participate in this study. The faecal egg count reduction test was carried out according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations for the detection of anthelmintic resistance (Coles *et al.*, 1992).

- The procedures for the faecal egg count reduction tests were carried out on the samples from each sheep flocks and goat herds found to have high strongyle egg counts per gramme of faeces.
- The sheep and goats had not been dewormed during the preceding 10-12 weeks.
- An untreated control group was included to monitor any changes that might occur in faecal egg counts.
- Accurate dosage of anthelmintics at the manufacturer's recommended dose rates were administered to each sheep or goats. A dose of 5 mg kg<sup>-1</sup> of albendazole and 7.5 mg kg<sup>-1</sup> of levamisole were given. The anthelmintics used in this study were suspensions for oral administration and administered to the animals with 10 or 20 ml plastic syringes.

- Faecal samples were collected pre-treatment and post treatment after 10-14 days directly from the rectum of each animal and deposited in clean specimen bottles in cool box and transported to the laboratory the same day to be examined.
- Nematode egg counts were determined using the McMaster method. Animals that did not have at least 100 nematode eggs in the pre-treatment samples were excluded from the analysis. The percentage reduction was then calculated according to the following formulas.
- $FECRT = 100 \times (1 - [T2/C2])$  Where T2 and C2 designate the counts after treatment, using the arithmetic means (Coles *et al.*, 1992).
- $FECRT = 100 \times \{1 - (T2/T1 \times C1/C2)\}$  Where T and C are the means for the treated and control groups and subscripts 1 and 2 designate the counts before and after treatment, respectively, using the geometric means (Presidente, 1985).

### 3.14. THE FAMACHA<sup>®</sup> CHART

In the search for a solution for the wide-spread anthelmintic resistance and for sustainable use of anthelmintics, Bath, Malan and Van Wyk (1996) developed a chart with illustrations that can be used as a method for selective anthelmintic treatment in small ruminants. Bath *et al.* (1996) called the concept the FAMACHA system, after its initiator, Dr. Francois (Faffa) Malan (“FA-MA-CHA”). The FAMACHA<sup>®</sup> system is a colour chart (Annexure 3) that depicts 5 categories within a range of ovine haematocrit values from healthy red (1) to severely anaemic (5). The chart is compared to the ocular mucosa of sheep and goats to assess the degree of anaemia, possibly caused by helminths.

The sensitivity and specificity of the FAMACHA<sup>®</sup> system in evaluating clinical anaemia was tested according to the method described by Vatta, Letty, Van der Linde, Van Wyk, Hansen and Krecek (2001). Briefly, two-way frequency tables of haematocrit by FAMACHA<sup>®</sup> were drawn up (haematocrit was used as the gold standard by which anaemia was measured) and the cut-off values were less than 19% (Table 3.1). In establishing the properties of a test, cut-off values are assigned to define the level of a test result that is needed to accept or reject a diagnosis (Smith, 1995).

Sensitivity is the proportion of infected or diseased individuals with a positive FAMACHA<sup>®</sup> scores. It may also be defined as the proportion of anaemic animals correctly identified as anaemic. Thus, sensitivity is the true positive rate using the formula: Sensitivity =  $(TP / (TP + FN)) \times 100$ . where TP = true positive, and FN = false negative.

Specificity is defined as the proportion of disease-free animals in a population that have negative FAMACHA<sup>®</sup> scores, or the proportion of non-anaemic animals. Thus, specificity is the true negative rate using the formula:  $\text{Specificity} = (\text{TN}/(\text{FP}+\text{TN})) \times 100$ . where TN = true negative, and FP = false positive.

The predictive value of a negative is the probability that an animal is not anaemic when the test result is negative for anaemia and vice versa for the predictive value of a positive. Using the formulas: predictive value of a negative =  $\text{TN}/(\text{FN}+\text{TN}) \times 100$ , and predictive value of a positive =  $\text{TP}/(\text{TP}+\text{FP}) \times 100$ .

**Table 3.1. Two way frequency table of haematocrit by FAMACHA<sup>®</sup> with haematocrit cut-off of <19% and FAMACHA<sup>®</sup> scores 4 and 5, or 3, 4 and 5 considered positive test results.**

FAMACHA <sup>®</sup> scores	Ht<19%	Ht>19%
Positive 4, 5 (3, 4, 5)	True positive (TP)	False positive (FP)
Negative 1, 2 (1, 2, 3)	False negative (FN)	True negative (TN)

The FAMACHA<sup>®</sup> categories of four and five or three, four and five were considered positive for anaemic animals, and categories one and two or one, two and three were considered negative. On the FAMACHA<sup>®</sup> chart, category three is on the borderline for dosing based on the level of anaemia. Recommendation with regard to treatment of animals with anthelmintics in this instance is to dose if uncertain of the level anaemia (Van Wyk *et al.*, 1997b). Grouping of animals according to the level of clinical anaemia with (categories three, four and five) and non-anaemic (categories one and two) maximized the sensitivity and specificity of the tests (Vatta *et al.*, 2001). Similar procedures were applied in the present study for the reason that categories three, four and five rendered a better sensitivity. A greater sensitivity is preferred as there is no wish to loose animals that are anaemic. Therefore, to compare the sensitivity and specificity of the FAMACHA<sup>®</sup> clinical assay, two-way frequency table of haematocrit by FAMACHA<sup>®</sup> was drawn as shown in Table 3.1. The sensitivity and specificity were tested statistically by means of Fisher's Exact Test for a two-by-two contingency table.

### 3.15. STATISTICAL ANALYSIS

The nematode egg and coccidian oocyst counts, the number of larvae per kilogram of dry herbage and worm burdens were logarithmically transformed ( $\log(x+10)$ ) to normalize their distribution and analyzed by the analysis of variance (ANOVA) in SAS for Windows Release 2003 (SAS Inc., 2003). Comparisons were made between age groups, sexes, seasons, and farm sites. Worm burdens of tracer animals used in the FAMACHA<sup>®</sup> trial, in studies on the seasonal fluctuation and intensities of helminth infection of sheep and goats, as well as worm burdens recorded from gastro-intestinal tracts from the abattoir were log transformed and their relationship examined by the analysis of variance and regression analysis. A value of  $P < 0.05$  was considered significant.

## **Chapter 4.**

# ***The seasonal fluctuation of gastro-intestinal nematodes***

### **4.1. INTRODUCTION**

The livestock population in the Mid-Rift Valley areas of East Shewa zone of Oromia regional State is estimated to be 2 million cattle, 653 940 sheep, 2 million goats, 271 950 equines, 87 100 camels and 853 500 poultry (Abule, Geremew & Aliye, 1998). The predominant production system is mixed crop-livestock farming with a limited pastoral production system in mostly the arid area. After cattle sheep, goats and equines are very important for they play a major role in the cultures and economies of the farmers. However, these animals are often affected by endoparasites, which results in clinical and sub-clinical infection and causes low performance due to stunted growth, unsatisfactory weight gain and mortality (Sykes, 1978; Barger, 1982; Armour and Gettinby, 1983; Boomker *et al.*, 1994).

Arid and semi-arid areas are characterized by hot and dry weather which affects, the development and survival of larvae. During these adverse conditions one should dose the animals because the worm eggs have little chance to survive. For sustainable and integrated parasite control, the knowledge of the epidemiology of gastro-intestinal parasites is required (Thamsborg, Roepstorff and Larsen, 1999). The purpose of this investigation was to determine the seasonal fluctuation of helminths parasitizing indigenous goats and sheep in selected sites in the arid and semi-arid areas of East Shewa zone.

### **4.2. MATERIALS AND METHODS**

#### **4.2.1. Study sites**

The study sites were chosen on the basis of climate. The first site was the arid area of Fentale sub-district about 170 km east of Addis Ababa (Fig. 3.1 ) which comprise the two locations, Kechachilo and Metehara, about 25 km apart. The area is located partially in the crop-livestock and partially in the pastoral production systems. The altitude is between 1 000 and 1 600 m above sea level. The climate is hot and dry and, although unpredictable, it has an average annual rainfall between 500-760 mm during both rainy seasons.

The second site was the semi-arid area located about 150-200 km south east of Addis Ababa. This site comprises Dugdabura, Meki and Ziwai, which are in the crop-livestock production system. The area has an altitude of 1 650 m. The rainfall is bimodal, but unevenly

distributed, and averages 760-900 mm. Smallholder farms were randomly selected from peasant farmers' villages found in Kechachilo, Fentale, Dugdabura, Meki and Ziwai sites to use their animals in the study.

## **4.2.2. Study animals**

### **4.2.2.1 Permanent resident sheep and goats**

A total of 300 animals from eight flocks, consisting of sheep and goats each with 15-40 animals per farm in three age groups (young, juvenile and adult) were selected and individually identified by ear tags with code numbers. Faecal samples were collected once a month from the rectum of each animal and faecal egg counts were monitored throughout the study period. The procedures have been described in General Materials and Methods (Chapter 2).

### **4.2.2.2 Tracer sheep and goats**

A total of 96 animals, 48 kids and 48 lambs, were purchased from the surrounding area and identified by ear tags with code numbers. Each animal was vaccinated against ovine pasteurellosis, sheep pox and anthrax and treated twice with albendazole at 7.5 mg/kg body weight. Faecal samples were collected after the second treatment and examined as described by Hansen & Perry (1994). No tracer animal was found passing nematode eggs. The tracers were kept in isolation until they were introduced with the resident animals into the study sites. Two tracer lambs and two kids were introduced every month into the arid and semi-arid areas. They were allowed to graze for a month, removed from the pastures and kept for three weeks under parasite-free conditions after which they were slaughtered for parasite recovery. The next batch of tracers were placed at each site on the same day the previous tracers were removed. Necropsies, parasite recovery, identification and enumeration were carried out as described in the General Materials and Methods. Pasture contamination was not assessed.

## **4.2.3. Climate**

Mean monthly rainfall, ambient temperature, and relative humidity data were obtained from the National Meteorological Services Agency. The weather data for Metehara area was obtained from the Metehara Sugar Factory Research Centre.

## **4.2.4. Statistical analysis**

Data were log transformed and analyzed to determine the variation in egg counts at different seasons i.e. long rainy season (July to September 1998), dry season (October 1998 to

February 1999), short rainy season (March to June 1999) and long rainy season (July to August 1999) at different sites. Monthly prevalence of sheep and goat nematode eggs of different age groups and the effects of age, sex and their interactions on monthly nematode egg counts were analyzed. Worm burdens were analyzed from the slaughtered tracer lambs and kids during the different seasons.

### 4.3. RESULTS

#### 4.3.1. Faecal nematode egg counts

##### 4.3.1.1. Sheep

The results of the faecal examinations for nematode eggs of resident sheep by age, sex, seasons and sites are shown in Tables 4.1-4.4. The mean egg counts at Kechachilo and Metehara were higher than the semi-arid sites of Dugdabura, Meki and Ziwai.

The egg counts from each age group of sheep (Table 4.2) show that there was no significant difference. Table 4.3 shows the mean nematode egg for males, 613.8 with a range of 0-12 400, while for females it was 483 with a range of 0-7 500 eggs per gramme of faeces. However, statistically there were no significant differences in nematode egg counts between the sexes of sheep ( $P < 0.05$ ). Strongyle egg counts peaked in the long rainy seasons in both the arid and semi-arid areas. In sheep, the seasonal mean nematode egg counts counts were 1 890 in the long rainy season, 150 in the dry season, and 1 156 in the short rainy season. The mean nematode log egg counts by seasons and age groups and sites are shown in Tables 4.5 and 4.6. The intensity was higher and significantly ( $P < 0.05$ ) different within seasons and age groups. There were significant differences ( $P < 0.05$ ) in egg counts between the different seasons and between study sites. The mean minimum and maximum ambient temperature, relative humidity (RH%) and the rainfall pattern and its correlation with the seasonal fluctuation of the mean nematode egg count are illustrated in Fig. 4.1–4.4.

**Table 4.1. Mean nematode egg counts in sheep from arid and semi-arid study sites during the long and short rains and dry seasons of 1998/1999.**

Site	Number of observations	Mean egg (SD)	Range
<b>Arid areas</b>			
Kechachilo	147	689.5 (1 723.6)	0 – 8 800
Metehara	92	626.1 (1 088.7)	0 – 5 500
<b>Semi-arid areas</b>			
Dugdabura	214	552.6 (1 345.9)	0 – 12 400
Meki	155	450.9 (932.0)	0 – 5 500
Ziwai	140	467.1 (991.3)	0 – 7 100

**Table 4.2. Mean nematode egg counts in sheep of different age groups during the long and short rains and dry seasons of 1998/1999.**

Age group	Number of observations	Mean egg (SD)	Range
Young (1-6 months)	231	560.2 (1134.8)	0 – 7 400
Juvenile (7-12 months)	31	487.9 (1 141.9)	0 – 8 800
Adult (> 12 months)	206	637.6 (1 562.4)	0 – 12 400

**Table 4.3. Mean nematode egg counts of different sexes of sheep and goats.**

Species	Sex	Number of observations	Mean egg (SD)	Range
Goat	Male	342	536.0 (1130.4)	0 – 9 300
	Female	407	5541.0 (1 182.3)	0 – 9 100
Sheep	Male	357	483.2 (1 059.0)	0 – 7 500
	Female	391	613.8 (1 433.0)	0 – 12 400

**Table 4.4. Mean seasonal egg counts of sheep from arid and semi-arid areas during the long and short rains and dry seasons of 1998/1999.**

Season	Number of observations	Mean egg (SD)	Range
Long rain (Jul-Sept 1998)	150	1 043.7 (1 581.8)	0 – 8 800
Dry	248	89.5 (151.3)	0 – 900
Short rain	100	575.0 (1 155.5)	0 – 7 500
Long rain (Jul-Sept 1999)	150	1 115.7 (1 889.7)	0 – 12 400

**Table 4.5. Least-square means of nematode log egg counts of sheep by seasons and age (\* = significantly different).**

Season	Age	Number of observations	Mean log egg $\pm$ SE
Long rain (Jul-Sept 1998)	1-6 months	44	1.38 $\pm$ 0.53 *
	7-12 months	58	1.78 $\pm$ 0.47 *
	> 12 months	48	1.09 $\pm$ 0.51 *
Dry	1-6 months	69	2.98 $\pm$ 0.68 *
	7-12 months	111	1.62 $\pm$ 0.58 *
	> 12 months	68	2.27 $\pm$ 0.66 *
Short rain	1-6 months	40	0.87 $\pm$ 0.57
	7-12 months	44	2.16 $\pm$ 0.55 *
	> 12 months	27	1.96 $\pm$ 0.69 *
Long rain (Jul-Sept 1999)	1-6 months	49	1.19 $\pm$ 0.53 *
	7-12 months	61	0.52 $\pm$ 0.45
	> 12 months	60	1.6 $\pm$ 0.58 *

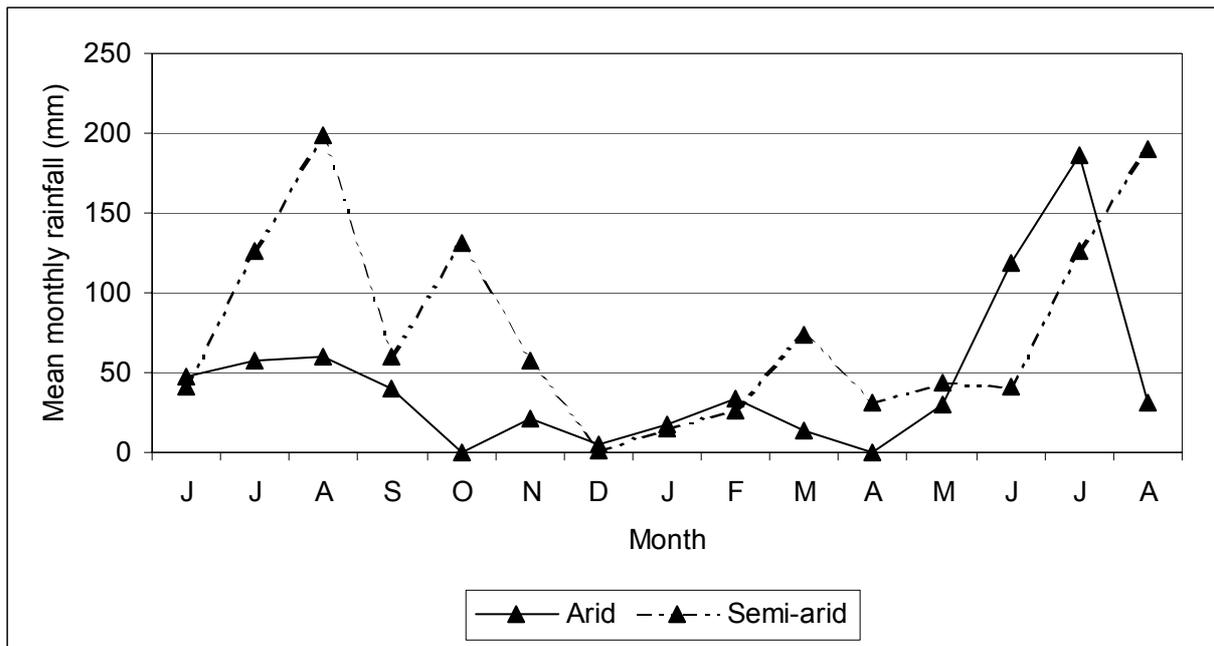


Fig. 4.1. Mean monthly rainfall at Metehara and Kechachilo (arid) and Ziwai, Meki and Dugdabura (semi-arid).

Table 4.6. Least-square means of nematode log egg counts of sheep by seasons and sites (\* = Significantly different).

Season	Site	Number of observations	Mean log egg $\pm$ SE
Long rain (Jul-Sept 1998)	Metehara	27	0.93 $\pm$ 0.68
	Kechachilo	33	1.95 $\pm$ 0.68 *
	Ziwai	35	1.42 $\pm$ 0.57 *
	Meki	35	1.87 $\pm$ 0.58 *
	Dugdabura	20	0.92 $\pm$ 0.75 *
Dry	Metehara	50	2.55 $\pm$ 0.67 *
	Kechachilo	24	2.38 $\pm$ 0.93 *
	Ziwai	74	2.08 $\pm$ 1.10
	Meki	50	2.53 $\pm$ 0.82 *
	Dugdabura	50	2.06 $\pm$ 0.80 *
Short rain	Metehara	20	0.62 $\pm$ 0.84
	Kechachilo	20	2.47 $\pm$ 1.14 *
	Ziwai	10	2.15 $\pm$ 0.64 *
	Meki	30	1.69 $\pm$ 0.77 *
	Dugdabura	20	1.22 $\pm$ 0.79 *
Long rain (Jul-Sept 1999)	Metehara	30	1.41 $\pm$ 0.63 *
	Kechachilo	15	2.63 $\pm$ 0.93 *
	Ziwai	45	0.74 $\pm$ 0.51 *
	Meki	30	1.22 $\pm$ 0.69 *
	Dugdabura	30	0.26 $\pm$ 0.67 *

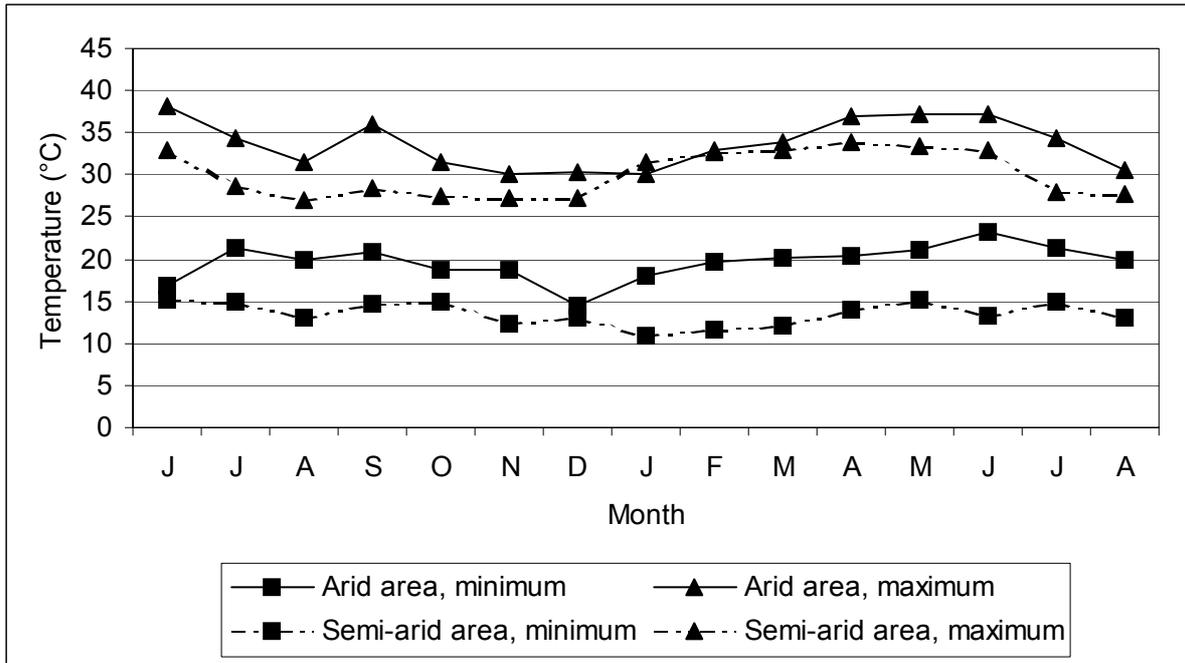


Fig. 4.2. Mean minimum and maximum ambient temperatures at Metehara and Kechachilo (arid) and Ziwai, Meki and Dugdabura (semi-arid) areas.

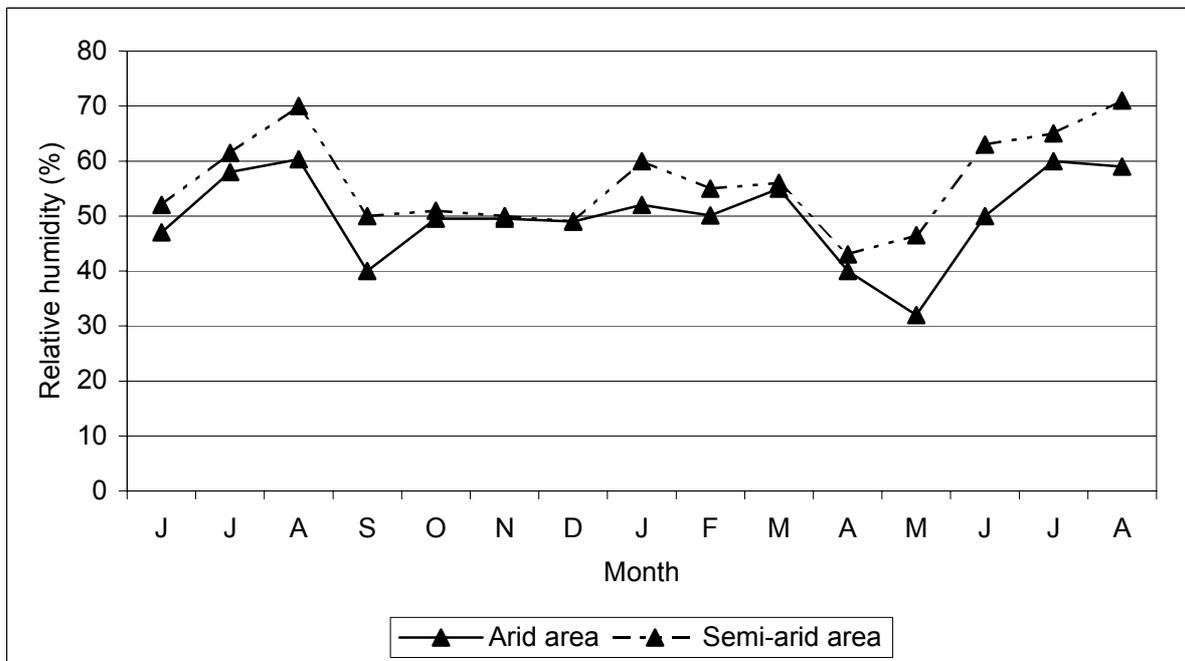


Fig. 4.3. Mean monthly relative humidity at Metehara and Kechachilo (arid) and Ziwai, Meki and Dugdabura (semi-arid) areas.

#### 4.3.1.2. Goats

The mean egg count in goats in the semi-arid area were higher than in the arid sites with the highest mean count of 654 at Dugdabura, and the lowest 440 at Ziwai (Table 4.7). The mean egg count by age groups, sex and sites are shown in Tables 4.8 and 4.9. The highest peak in egg production was in the long rainy season. The mean seasonal egg counts were 1 250 in the long rainy season, 462 in the short rainy season and 100 in the dry season. There were significant ( $P<0.05$ ) differences in nematode egg counts between seasons.

The log transformed seasonal mean egg counts by seasons, age groups and sites are shown in Tables 4.10 and 4.11. The strongyle egg counts were higher and significantly different ( $P<0.05$ ) between seasons, age groups and sites. As was the case in sheep, there were no significant differences between age groups and sexes in goats.

**Table 4.7. Mean nematode egg counts in goats from arid and semi-arid study sites during the long and short rains and dry seasons of 1998/1999.**

Site	Number of observations	Mean epg (SD)	Range
<b>Arid area</b>			
Kechachilo	155	431.6 (952.3)	0-7 100
Metehara	219	584.5 (1 212.4)	0- 9 300
<b>Semi-arid area</b>			
Dugdabura	85	654.1 (1 473)	0-9 100
Meki	150	611.1 (1 286.3)	0-7 500
Ziwai	140	440 1(884.5)	0-4 800

**Table 4.8. Mean nematode egg counts in goats of different age groups during the long and short rains and dry seasons of 1998/1999.**

Age group	Number of observations	Mean epg (SD)	Range
Young (1-6 months)	342	536.3 (1 130.44)	0-9 300
Juvenile (6-12 months)	407	554.1 (1 182.23)	0-9 100
Adult (> 12 months)	357	483.2 (1 059.01)	0-7 500

**Table 4.9. Mean seasonal egg counts of goats from arid and semi-arid areas during the long and short rains and dry seasons of 1998/1999.**

Age group	Number of observations	Mean epg (SD)	Range
Long rain (Jul-Sept 1998)	150	909.1(1 174.3)	0-5 400 *
Dry season	249	99.6 (164.5)	0-900 *
Short rain	100	462 (830.5)	0-4 800 *
Long rain (Jul-Sept 1999)	150	1 250.7 (1.922.9)	0-9 300 *

\* Significantly (P<0.05) different within seasons

**Table 4.10. Least-square means of nematode log egg counts of goats by seasons and age.**

Season	Age	Number of observations	Mean log epg ±SE
Long rain (Jul-Sept 1998)	1-6 months	41	0.98±0.54
	7-12 months	62	1.87±0.44 *
	> 12 months	47	1.57±0.48 *
Dry	1-6 months	62	0.70±0.58
	7-12 months	126	1.33±0.40 *
	> 12 months	61	2.88±0.58 *
Short rain	1-6 months	42	2.83±0.59
	7-12 months	44	0.8 ±0.56 *
	> 12 months	24	2.06±0.74 *
Long rain (Jul-Sept 1999)	1-6 months	51	1.03±0.52 *
	7-12 months	59	1.03±0.47
	> 12 months	40	1.08±0.58

\* Significantly (P<0.05) different within seasons

### 4.3.2. Worm burden

#### 4.3.2.1. Sheep

The seasonal prevalence, means and burdens of nematode parasites of tracer lambs are listed in Table 4.14 and 4.15. The most abundant and prevalent nematodes were *H. contortus* with the highest mean count of 2 980, followed by *T. colubriformis* (2 600) and *O. columbianum* (1 380) during the long rainy seasons. The worm burden increased during the long rainy seasons and the highest percentage (83.3%-100%) of infection in lambs occurred during the long rainy seasons in both arid and semi-arid areas. During the dry season, infection with *H. contortus* dropped to lower levels (16.7%) in both areas. Infection with the other nematode species dropped too (Tables 4.14 and 4.15). There were significant differences in worm burdens in lambs between the rainy and dry seasons (P<0.05).

**Table 4.11. Least-square means of nematode log egg counts of goats by seasons and sites.**

Season	Sites	Number of observations	Mean log epg $\pm$ SE
Long rain (Jul-Sept 1998)	Metehara	35	0.63 $\pm$ 0.57
	Kechachilo	39	0.79 $\pm$ 0.53
	Ziwai	25	2.39 $\pm$ 0.27 *
	Meki	31	2.48 $\pm$ 0.60 *
	Dugdabura	20	1.03 $\pm$ 0.78 *
Dry season	Metehara	50	2.30 $\pm$ 0.78 *
	Kechachilo	75	2.50 $\pm$ 0.68
	Ziwai	25	2.07 $\pm$ 1.11
	Meki	49	2.53 $\pm$ 0.82 *
	Dugdabura	50	2.06 $\pm$ 0.80 *
Short rainy season	Metehara	20	0.62 $\pm$ 0.84
	Kechachilo	30	2.53 $\pm$ 0.64
	Ziwai	10	3.41 $\pm$ 1.27
	Meki	20	2.09 $\pm$ 0.78 *
	Dugdabura	20	3.03 $\pm$ 0.78 *
Long rain (Jul-Sept 1999)	Metehara	30	2.37 $\pm$ 0.85 *
	Kechachilo	45	2.53 $\pm$ 0.64 *
	Ziwai	15	3.47 $\pm$ 1.25 *
	Meki	30	2.09 $\pm$ 0.78 *
	Dugdabura	30	3.03 $\pm$ 0.78 *

\* Significantly (P<0.05) different between seasons

#### 4.3.2.2. Goats

The seasonal prevalence, mean and range burdens of helminths in kid tracers are listed in Table 4.14 and 4.15. The same as the lamb tracers, the most abundant nematodes were *H. contortus* with the highest mean counts (1 150), followed by *T. colubriformis* (1 031) and *O. columbianum* (120). The worm burdens markedly dropped during the dry season. The prevalence of worm infection was 100% during the long rainy seasons in both the arid and semi-arid areas. During the dry period prevalences between 16.7 and 33.3 were recorded. There was a significant (P<0.05) difference in worm burdens between the dry and wet seasons.

**Table 4.12. Mean seasonal worm burdens, range and prevalence in tracer lambs during the long and short rains and dry seasons of 1998/1999.**

Season	Nematode species	n*	Mean	Range	Prevalence (%)
Long rain (July – September 1998) (n=12)	<i>H. contortus</i>	1	2 270	54-3 740	91.7
	<i>T. colubriformis</i>	11	2 315	38-2 680	91.7
	<i>O. columbianum</i>	10	1 380	125-1 670	83.3
	<i>T. ovis</i>	9	51	15-270	75
	<i>B. trigonocephalum</i>	2	34	0-68	16.7
Dry (n=12)	<i>H. contortus</i>	1	274	274	16.6
	<i>T. colubriformis</i>	4	877	10-1 700	33.3
	<i>O. columbianum</i>	3	176	12-198	25
	<i>T. ovis</i>	0	0	0	0
	<i>B. trigonocephalum</i>	0	0	0	0
Short rain (n=12)	<i>H. contortus</i>	11	288	27-557	83.3
	<i>T. colubriformis</i>	9	311	10-999	75
	<i>O. columbianum</i>	8	30	7-97	66.7
	<i>T. ovis</i>	7	10	5-30	58.3
	<i>B. trigonocephalum</i>	0	0	0	0
Long rain (July – September 1999) (n=12)	<i>H. contortus</i>	12	2 980	37-2 985	100
	<i>T. colubriformis</i>	12	2 600	120-3 450	100
	<i>O. columbianum</i>	7	1 120	16-1 480	58.3
	<i>T. ovis</i>	5	65	7-300	41.7
	<i>B. trigonocephalum</i>	0	0	0	0

n\* Number of animals positive

*Trichuris ovis* occurred more commonly in sheep than in goats, but usually the worm counts were a few in numbers. *Bunostomum trigonocephalum* was found in a few tracers in the semi-arid area only. *Moniezia* spp. was prevalent in both arid (33.3%) and semi-arid area (66.7%) during the dry and short rainy seasons. Out of 48 tracer lambs, 2 (4.2%) in the arid area had larvae of *T. hydatigena* and one (2.1%) in the semi-arid area. *Coenurus cerebralis* and *Echinococcus* spp. were found at Ziwai, Dugdabura and Meki in two (4.2%) lambs and one (2.1%) kid (Table 4.13 and 4.15). Liver fluke was found in two lambs at Metehara.

#### 4.4. Discussion

The climate at Kechachilo and Metehara (arid area) was very dry with humidity of around 50% for most of the year, except during the long and short rainy seasons. The climate in the semi-arid area of Dugdabura, Meki and Ziwai was more moderate with higher average daily

temperature and relative humidity. Between July and September, both sites had moderate rainfall (Fig. 4.1-4.3) which was reflected by the number of parasites found (Tables 4.8 and 4.15). Parasite abundance was observed to peak in the long and short rainy seasons and decline during the dry seasons. Consequently, grazing animals are constantly infected and pastures contaminated (Chiejina, 1994). Climate has direct effects on the size of parasite populations by affecting fecundity, rates of development and survival of free-living parasites (Sutherst, 1987). Variation in ambient temperatures produces obvious seasonal effects, but can also lead to large differences in the size of parasite populations between years. In dry and hot areas, except in irrigated or other permanently wet pasture, transmission is restricted to the rainy season and the only means of carry-over of infection from one rainy season to another is through animals harbouring adult worms and/or arrested (hypobiotic) larvae (Chiejina, 1994).

The seasonal worm burdens in sheep and goats followed a similar pattern. In both species, abomasal and small intestinal burdens were high during the long rainy seasons (July-September). A second peak was observed during the short rainy season (March-April) after which time, the worm numbers decreased. The worm burdens in sheep and goats showed no significant difference. The species composition of the nematodes did not vary much between sites. However, *B. trigonocephalum* was recovered from tracer lambs from the semi-arid area only. *Haemonchus contortus*, *T. colubriformis*, *O. columbianum* and *T. ovis* were the commonest nematodes encountered in both the arid and semi-arid areas. *Moniezia expansa* and larvae of *T. hydatigena*, *Echinococcus* spp., *Taenia multiceps* and *C. cerebralis* were recovered from the semi-arid areas but showed no seasonal variation.

Faecal nematode egg counts were high during the rainy seasons. The egg counts varied in different age groups, and those of necropsied tracer animals were significantly higher during the rainy seasons ( $P < 0.05$ ) than the dry season. Thus, acquisition of L<sub>3</sub> larvae began with the onset of the long rains starting about the end of June and the infection level reached peaks during August and September (Fig. 4.4). The level of faecal egg counts and worm counts in tracer animals were mostly dependent on the rainy seasons of the year and grazing period on pasture. The decline in faecal and worm counts was seen from October-March, until the start of the short rainy season. The decline in faecal egg counts may be related to the hot, dry and prolonged period to cause cessation of free-living development and survival. Maybe it is also related to the self cure phenomenon, a phenomenon also reported in East Africa by Allonby & Urquhart (1975).

Table 4.13. Seasonal prevalence of nematodes in necropsied tracer sheep from arid and semi-arid areas

Helminth species	Metehara (Arid area)								Dugdabura (Semi-arid area)							
	Long rain <sup>1</sup> n=6		Dry n=6		Short rain n=6		Long rain <sup>2</sup> n=6		Long rain <sup>1</sup> n=6		Dry n=6		Short rain n=6		Long rain <sup>2</sup> n=6	
	Pos	%	Pos	%	Pos	%	Pos	%	Pos	%	Pos	%	Pos	%	Pos	%
<i>H. contortus</i>	5	83.3	1	16.7	5	83.3	6	100	6	100	2	33	5	83	6	100
<i>T. colubriformis</i>	6	100	4	67	3	50	6	100	6	100	3	50	5	83	6	100
<i>O. columbianum</i>	5	83	4	67	0	0	3	50	6	100	3	50	4	67	6	100
<i>B. trigonocephalum</i>													3	50	3	50
<i>T. ovis</i>	3	50	0	0	4	67	3	50	5	83	2	33	3	50	3	50

<sup>1</sup> Long rain July-September 1998

<sup>2</sup> Long rain July-September 1999

Table 4.14. Mean seasonal worm burden, range and prevalence in kid tracers.

Season	Nematode species	n*	Mean	Range	Prevalence (%)
Long rain (July – September 1998) (n =12)	<i>H. contortus</i>	12	1 070	180-2100	100
	<i>T. colubriformis</i>	10	678	30-1 890	83.3
	<i>O. columbianum</i>	10	128	10-340	83.3
	<i>T. ovis</i>	1	33	0-33	8.3
	<i>B. trigonocephalum</i>	1	46	0-46	8.3
Dry (n=12)	<i>H. contortus</i>	0	0	0	0
	<i>T. colubriformis</i>	5	294	0-1 200	41.7
	<i>O. columbianum</i>	1	38	0-38	8.3
	<i>T. ovis</i>	3	10	0-65	25
	<i>B. trigonocephalum</i>	1	28	0-28	8.3
Short rain (n=12)	<i>H. contortus</i>	11	560	0-1 830	91.7
	<i>T. colubriformis</i>	10	680	0-1 220	83.3
	<i>O. columbianum</i>	11	46	0-197	83.3
	<i>T. ovis</i>	8	6	0-46	66.7
	<i>B. trigonocephalum</i>	1	11.5	0-138	16.7
Long rain (July – September 1999) (n=12)	<i>H. contortus</i>	12	1 150	25-2 220	100
	<i>T. colubriformis</i>	12	1 030	100-1 890	100
	<i>O. columbianum</i>	4	120	0-296	33.3
	<i>T. ovis</i>	6	20	0-98	50

\* Number of positive animals

Table 4.15. Seasonal prevalence of nematodes in necropsied goats from arid and semi-arid areas.

Helminth species	Metehara (Arid area)								Dugdabura (Semi-arid area)							
	Long rain <sup>1</sup> n=6		Dry n=6		Short rain n=6		Long rain <sup>2</sup> n=6		Long rain <sup>1</sup> n=6		Dry n=6		Short rain n=6		Long rain <sup>2</sup> n=6	
	Pos	%	Pos	%	Pos	%	Pos	%	Pos	%	Pos	%	Pos	%	No	%
<i>H. contortus</i>	6	100	2	33	5	83	6	100	6	100	1	17	6	100	6	100
<i>T. colubriformis</i>	5	83	4	67	5	83	5	83	5	83	0	0	6	100	5	83
<i>O. columbianum</i>	5	83	3	50	5	83	0	0	5	83	4	67	5	83	1	17
<i>T. ovis</i>	1	17	5	83	4	67	2	33	1	17	1	17	2	33		
<i>B. trigonocephalum</i>											1	17	2	33		50

<sup>1</sup> July-September 1998

<sup>2</sup> July 1998-September, 1999.

In the arid and semi-arid areas, rainfall and humidity seemed to have the most important effect on the development of eggs and free-living stages. Low recorded humidity and rainfall during October-March may have contributed to lower egg counts and lower worm burdens in the tracer lambs and kids. This suggests that the dry season is unfavourable for the pre-parasitic stages of trichostrongylids of sheep and goats in the Mid Rift valley areas of Ethiopia which is in line with what is seen in and has been published for the rest of the world. *Haemonchus contortus*, and *T. colubriformis* survive the hot and dry environment in both the arid and semi-arid areas as adult worms. The infectivity of pasture to the grazing tracer animals by the onset of the rainy seasons demonstrated the availability of infective larvae on pasture following the rains. This observation is in agreement with the findings of Fritsche, Kaufmann & Pfister (1993).

## **Chapter 5.**

# ***The prevalence and intensity of helminth and coccidial infections***

### **5.1. INTRODUCTION**

In the semi-arid areas of the Mid-Rift Valley of Ethiopia, farmers keep small ruminants mainly for consumption and trade. Sheep and goats raised in the semi-arid areas have potential for export to neighbouring countries, mainly to countries in the Middle East and for local markets. These animals therefore represent as source of meat and milk and as cash reserve in the peasant farmers' economy. Besides these, skins for local and export markets, dung for fuel and manure are very useful by-products. Sheep and goats also have some social and cultural functions and serve as a means of insurance against draught and other emergency needs. However, economic losses due to gastro-intestinal nematode parasites in both sheep and goats in the semi-arid areas of the Mid-Rift Valley are considerable. These losses seem to be intensified in the semi-arid and sub-humid area in East Shewa zone, because of favourable climatic conditions for the growth and establishment of large number of gastro-intestinal parasites population. The nematode parasites of particular concern are the gastro-intestinal nematodes *H. contortus*, *T. colubriformis* and *Oesophagostomum* spp.

Besides helminth infections, coccidiosis is a disease of sheep and goats that occurs in young animals, and in mixed infections with nematodes, is very common (Vercruysse, 1982; Smith, 1992). Infection is caused by one or more of approximately 12 different species of *Eimeria* (Smith, 1992; Kaya, 2004). Heavy infections are responsible for severe diarrhoea, which sometimes contains blood. The parasites cause lesions in various parts of intestine and, depending on the species, cause local haemorrhage, oedema and villous atrophy that results in malabsorption (Urquhart *et al.*, 1987). In Ethiopia, the role of coccidial infections in sheep and/or goats has not been clearly identified as one of the health and production constraints and published reports are scarce or unavailable.

### **5.2. MATERIALS AND METHODS**

#### **5.2.1. Study area**

The study areas are described in detail in the General Materials and Methods. Briefly, this study involved 10 small-scale farmers, with a range of 7-65 goats and 5-80 sheep for a period of 15 months, from July 2002 to September 2003. The farms were purposely selected in the five sub-districts of Lume (Mojo), Adama (Boset), Dugdabura (Meki), Adamitulu

(Ziwai), and Shashemene (Shashemene) (Fig. 3.1). The farms were selected randomly from lists of farms identified on criteria such as accessibility by vehicle all year round, willingness of farmers to voluntarily participate in the study and availability of animal health representatives from the Ministry of Agriculture in the area. The selected farms were located in the Rift Valley in different agro-ecological environments. Shashemene and Mojo are in the sub-humid area, while Meki, Dugdabura and Ziwai are in the semi-arid area.

Mean monthly rainfall, minimum and maximum temperature and relative humidity data were obtained from the National Meteorological Services Agency (NMSA, Addis Ababa, Ethiopia). Additional climatic data were obtained from the Adamitulu Agricultural Research Centre weather station in Ziwai.

## **5.2.2. Sampling procedures**

### **5.2.2.1. The study animals**

Study animals were sampled using simple random sampling techniques and sample size was determined according to the description given by Hansen and Perry (1994). Briefly, sheep or goats, at the age of 1-6 months, 7-12 months and above 12 months were categorized as age group Young, Juvenile and Adult, respectively, and identified on each farm by ear tags with code numbers. When fewer than the required number of sheep or goats on one farm were in the target age group, the nearest and the next farm household was approached and the required age group of animals were identified and included in the study. Twenty animals of each age group of each species on each farm were sampled. When animals became older than their current age group, they were moved to the next age group. New-born lambs or kids were recruited into the age group Young.

Tracer lambs and kids were used in this study to determine seasonal variability of acquired infection and to determine worm intensities. A total of 110 animals (57 lambs and 53 kids) maintained worm-free were released seasonally. From each species 2-3 lambs and/or kids were introduced on pasture to graze along with the resident sheep and goats for 21 days after which they were removed and kept for three weeks under parasite free conditions and then slaughtered for worm recovery, identification and enumeration as described in the General Materials and Methods.

#### **5.2.2.2. Sampling of animals for faecal helminth egg counts**

Three to five grammes of faeces were collected at monthly intervals directly from the rectum of the experimental animals usually in the morning, and were placed into labelled specimen bottles. Faeces that could be processed within 48 hours were stored at 4 °C whereas, those samples that could only be processed a day later were fixed in 10% formalin. The number of eggs per gramme faeces was determined by a modified McMaster technique (Hansen and Perry, 1994), using saturated salt solution, with a lower limit of detection of 100 eggs. For the detection of liver fluke eggs the sedimentation technique as described by Hansen and Perry (1994) was used.

#### **5.2.2.3. Herbage sampling**

Herbage samples were collected on each farm and the nematode larvae recovered and counted during the rainy seasons in the months of March–April and July–September, as fully described in the General Materials and Methods.

#### **5.2.2.4. Coccidia**

The faecal samples collected from all the study sites were examined for both helminth eggs and oocysts during all the seasons. The number of oocysts per gramme of faeces and the species were determined using a modified McMaster technique (MAF, 1986). Oocysts of pooled samples of either sheep or goats were allowed to sporulate in 2.5% potassium dichromate at room temperature for a week (MAF, 1986). Oocysts were then concentrated by centrifugal flotation using saturated sodium chloride solution (Hansen and Perry, 1994). Oocysts were identified using a calibrated ocular micrometer under a 40x objective, using the descriptions of Levine (1985), McKenna (1972) and Norton (1986).

#### **5.2.2.5 Sampling of gastro-intestinal tracts in the abattoir.**

During the dry and wet seasons of 2003, total worm and faecal nematode egg counts were performed on 180 gastro-intestinal tracts of sheep and goats slaughtered at Mojo Export Abattoir. The procedures described by Boomker *et al.* (1989) for examination of gastro-intestinal tracts for adult worms and juvenile larvae were used.

### **5.2.3. Statistical analysis**

Strongyle egg counts, coccidial oocyst counts, the number of L<sub>3</sub> per kg of dry herbage and the worm burdens of sheep and goats were logarithmically transformed to normalize their distribution and analyzed by ANOVA in SAS. Comparisons were made between seasons, farms, hosts, sex and age groups. The relationship between nematode burdens and the egg

counts, examined by regression analysis, was determined. A value of  $P < 0.05$  was considered significant and the prevalence and intensity of infection calculated, and the proportion of infected animals were statistically compared using the ANOVA test. The general linear model of SAS was used for statistical comparisons of the strongyle egg and coccidian oocyst counts of sheep and goats according to age, sex, site and seasons. The significance of differences between means was determined by the Student t-test.

### **5.3. RESULTS**

#### **5.3.1. Faecal egg counts**

##### **5.3.1.1. Sheep**

The results of the faecal examinations are shown in Table 5.1-5.4. The rainfall pattern, and the mean minimum and maximum ambient temperature and relative humidity are illustrated in Fig. 5.1-5.5. The seasonal fluctuation of the egg counts and its correlation with mean monthly rainfall is illustrated in Fig. 5.6.

The mean faecal egg count for young, juvenile and adults showed a similar pattern over the study period (July 2002-September 2003). There were no significant differences in the egg counts of sheep of all age groups during the dry season (October 2002 to February 2003). For most of these months, the mean faecal egg counts recorded were below 1 000 epg. The highest mean egg count in sheep was detected during July 2002 to September 2002 and July 2003 to September 2003, which corresponded the long rainy seasons. The mean, standard deviation, minimum and maximum epg counts in sheep at the different study sites are presented in Table 5.1-5.2.

The age and sex (physiological), site and seasonal (environmental) effects on the nematode egg counts were estimated by the least-square means of each category (Tables 5.3 and 5.4) for the entire sampling period (July 2002 to September 2003).

The overall results indicate that there was no site effect on the egg count in sheep i.e. there were no significant differences in egg counts between sites ( $P = 0.345 - 0.393$ ). However, with the age factor, there was significant difference between the count of young and the juvenile and adult age groups ( $P < 0.001$ ). The seasons effects were clearly seen as the egg counts were higher during the rainy season ( $2.33 \pm 0.199$ ) than the dry season ( $0.73 \pm 0.255$ ) showing a highly significant difference ( $P < 0.0001$ ).

**Table 5.1. Mean faecal nematode egg counts in sheep from different study sites for the seasons July 2002-September 2003.**

Site	Number of observations	Mean epg (SD)	Range
Meki	170	886 (1.784.4)	0-12 800
Ziwai	169	1 037 (1 596)	100-660
Boset	170	1 045 (1 596)	0-11 100
Mojo	170	1 161 (1 860.3)	0-7 500
Shashemene	168	1 061	100-7 400

**Table 5.2. Mean nematode egg counts from young (1-6 months), juvenile (7-12 months) and adult (>12 months) sheep in East Shewa from July 2002 to September 2003.**

Season	Age group	Number of observations	Mean epg (SD)	Range
Long rainy season 2002	1-6 months	80	1 655 (2 059.8)	1-11 200 *
	7-12 months	90	1 619 (1 813)	0-6 700 *
	>12 months	80	1 840 (2 346.6)	0-12 700 *
Long dry	1-6 months	85	74 (151.3)	0-900
	7-12 months	97	48 (102.2)	0-800
	>12 months	86	42 (96.4)	0-700
Short rain	1-6 months	27	541(1 420.4)	0-7 500 *
	7-12 months	29	163 (182.3)	0-700 *
	>12 months	26	258 (407.1)	0-1 800 *
Short dry	1-6 months	32	578 (1 116)	0-6 100 *
	7-12 months	36	492 (1 048.1)	0-4 800 *
	>12 months	32	331 (428.4)	0-1 700 *
Long rainy season 2003	1-6 months	48	2 119 (2 197.2)	1-11 000 *
	7-12 months	54	2 550 (1 891.1)	1-6 800 *
	>12 months	48	2 706 (2 590.2)	0-12 800 *

\* Significantly different (P&lt;0.05)

**Table 5.3. Mean nematode egg count from young (1–6 month), juvenile (7–12 months) and adult (>12 months) sheep according to age, sex, site and season for the period July 2002 to February 2003.**

Variable factor	Class	Log nematode epg	
		Mean ± SE	P - value
Age	Young (1-6 months)	1.53±0.350	<0.0001
	Juvenile (7-12 months)	0.231±0.350	0.0458
	Adult (> 12 months)	0.622±0.350	0.6440
Sex	Males	0.99±0.350	0.2522
	Females	0.60±0.312	0.0001
Site	Meki	0.72±0.360	<0.05
	Ziwai	0.99±0.437	<0.05
	Boset	0.46±0.396	0.888
	Mojo	0.29±0.434	0.1531
	Shashemene	1.49±0.424	<0.005
Season	Jul-Sept <sup>1</sup>	2.33±0.199	<0.0001
	Oct-Feb <sup>2</sup>	0.73±0.252	<0.0001
Interaction between	Age and site	1.35±0.526	0.4488
	Sex and site	1.03±0.444	0.0155
	Site and season	2.74±0.437	<0.0001
	Season and age	2.75±0.395	<0.0001
	Season and sex	2.38±0.297	<0.0001
	Sex and age	1.27±0.523	0.1591

<sup>1</sup> Long rainy season 2002      <sup>2</sup> Dry season

No interaction between age and site, age and sex or site were observed. Highly significant interaction effects were observed between season and age and season and sex ( $P < 0.0001$ ).

Besides eggs of nematode parasites, there was a 38% prevalence of tapeworm eggs in sheep during the whole study period. *Moniezia expansa* eggs were detected more often in the young and juvenile age groups than in the adult groups. A significant difference was not seen between sheep and goats with tapeworm infection in this study. Liver flukes were not detected in sheep in any of the study sites either by faecal or post-mortem examinations. Liver fluke infection is not a problem of the arid and semi-arid areas of the Rift Valley areas in East Shewa. However, infections in sheep were present in Metehara sub-district during the first phase of this study.

**Table 5.4. Least-square means and standard errors (SE) of nematode egg counts of sheep according to age, sex, site and season from October 2002 to September 2003.**

Variable factor	Class	Log nematode epg	
		Mean $\pm$ SE	P - value
Age	Young (1-6 months)	1.94 $\pm$ 0.25	0.0380
	Juvenile (7-12 months)	1.85 $\pm$ 0.281	0.0264
	Adult (> 12 months)	1.91 $\pm$ 0.236	0.4235
Sex	Males	1.924 $\pm$ 0.189	0.5730
	Females	1.88 $\pm$ 0.230	0.3996
Site	Meki	1.99 $\pm$ 0.280	0.245
	Shashemene	1.58 $\pm$ 0.315	0.027
	Boset	2.02 $\pm$ 0.287	0.004
	Mojo	1.85 $\pm$ 0.316	0.33
	Ziwai	2.05 $\pm$ 0.319	0.135
Season	Oct-Feb <sup>1</sup>	1.96 $\pm$ 0.223	<0.01
	Mar-Apr <sup>2</sup>	2.79 $\pm$ 0.269	<0.0001
	May-Jun <sup>3</sup>	0.36 $\pm$ 0.237	0.499
	Jul-Sep <sup>4</sup>	3.22 $\pm$ 0.195	<0.0001
Interaction between:	Age and site	1.20 $\pm$ 0.746	0.8022
	Sex and site	1.57 $\pm$ 0.565	0.4755
	Site and season	1.79 $\pm$ 0.384	0.522
	Season and age	3.23 $\pm$ 0.369	<0.0001
	Season and sex	3.34 $\pm$ 0.279	<0.0001

<sup>1</sup> Long dry season 2002      <sup>2</sup> Short rainy season  
<sup>3</sup> Short dry season      <sup>4</sup> Long rainy season 2003

The effect of the long dry season of 2002 (October - December 2002) which extended upto the start of February 2003, was analyzed statistically along with the effect of the short rainy season (March - April 2003 and the brief dry period of May-June and the long rainy season (July - September 2003) which demonstrated a pattern of nematode egg output. After the long rainy season of 2002, the egg count declined to low levels (Table 5.3). In the short rainy season that followed (March - April 2003) the egg count increased showing a significant difference in the epg between seasons ( $P < 0.05$ ). In the brief dry period the egg count decreased. However, during the preceeding long rainy season, (July - September 2003) the egg count increased and remained high. Statistical analysis indicated a highly significantly difference ( $P < 0.0001$ ) in egg counts between seasons (Table 5.4).

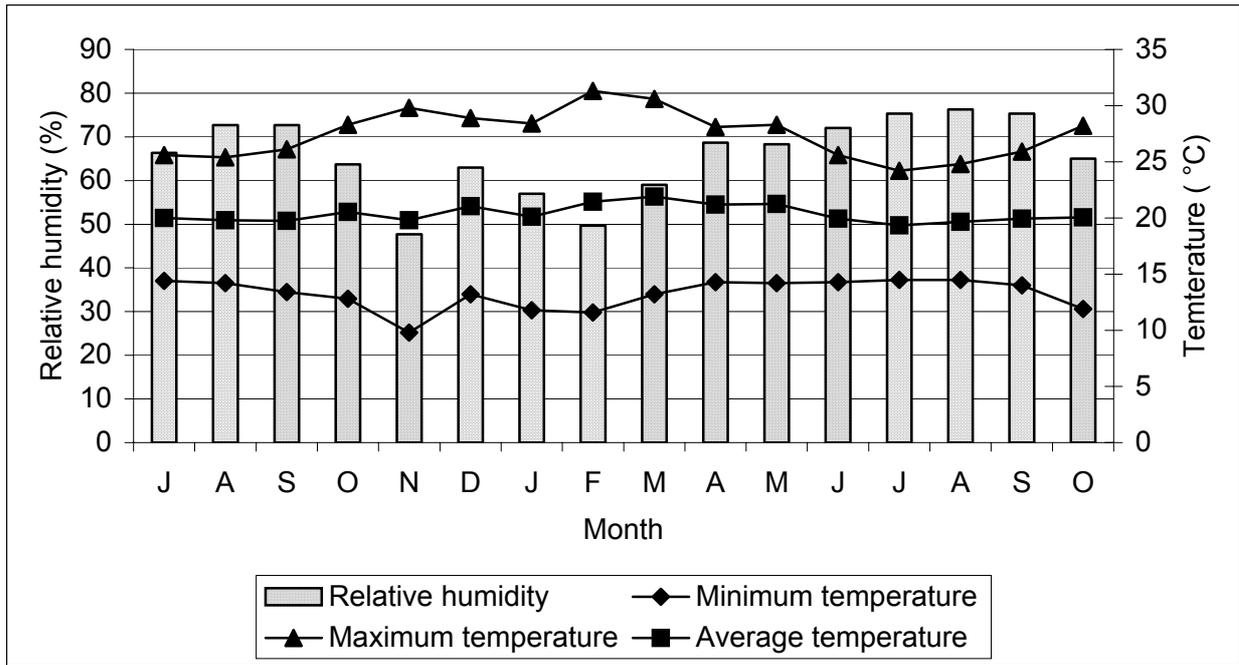


Fig. 5.1. Mean monthly rainfall data at Mojo, Ziwai, Meki, Shashemene and Boset sub-districts in East Shewa zone from July 2002 to September 2003.

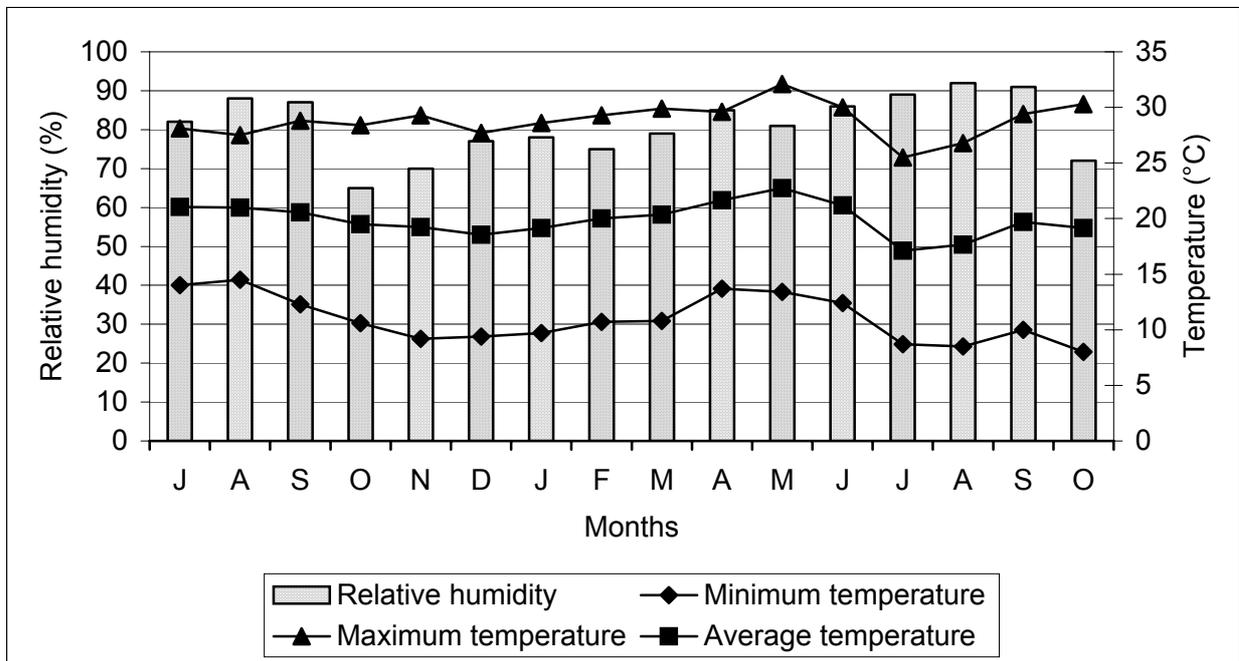


Fig. 5.2. Mean maximum and minimum ambient temperature and relative humidity at Mojo during July 2002 to September 2003.

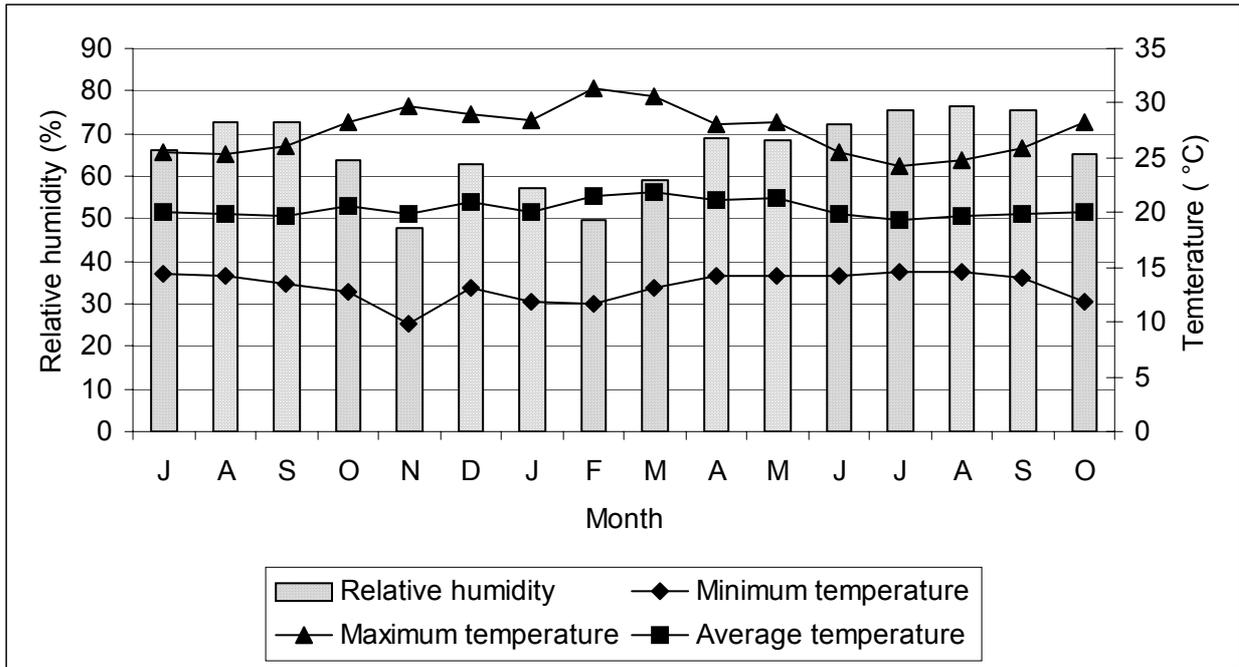


Fig. 5.3. Mean maximum and minimum ambient temperature and relative humidity at Ziwai during July 2002 to September 2003.

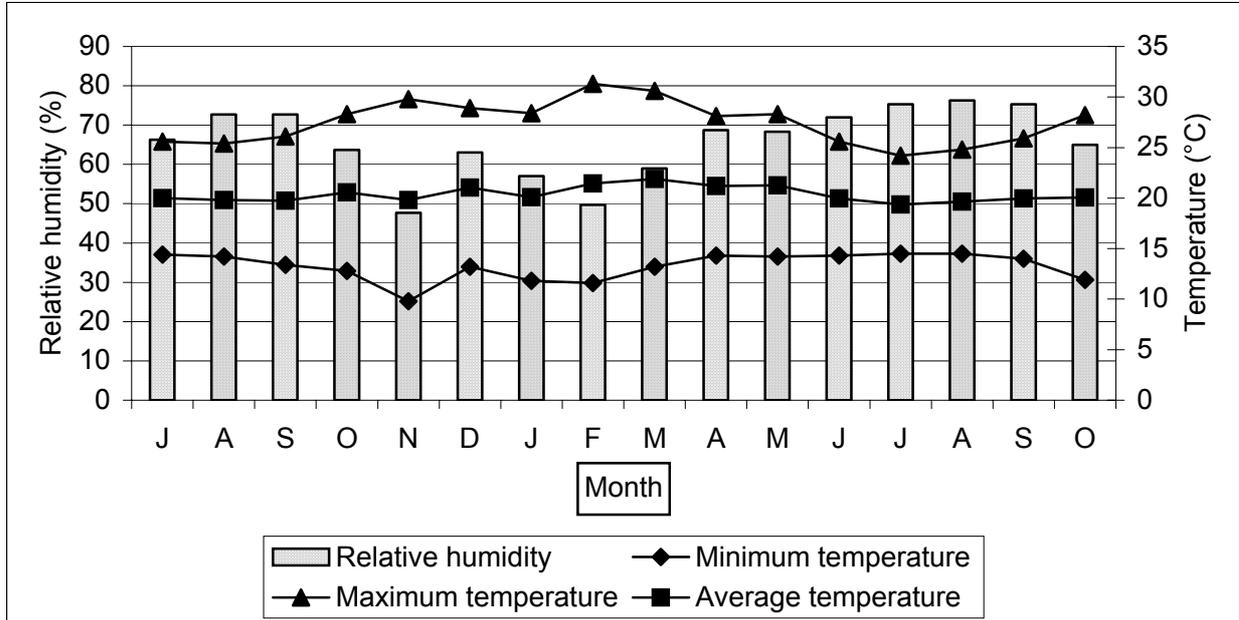


Fig. 5.4. Mean maximum and minimum ambient temperature and relative humidity at Shashemene from July 2002 to September 2003.

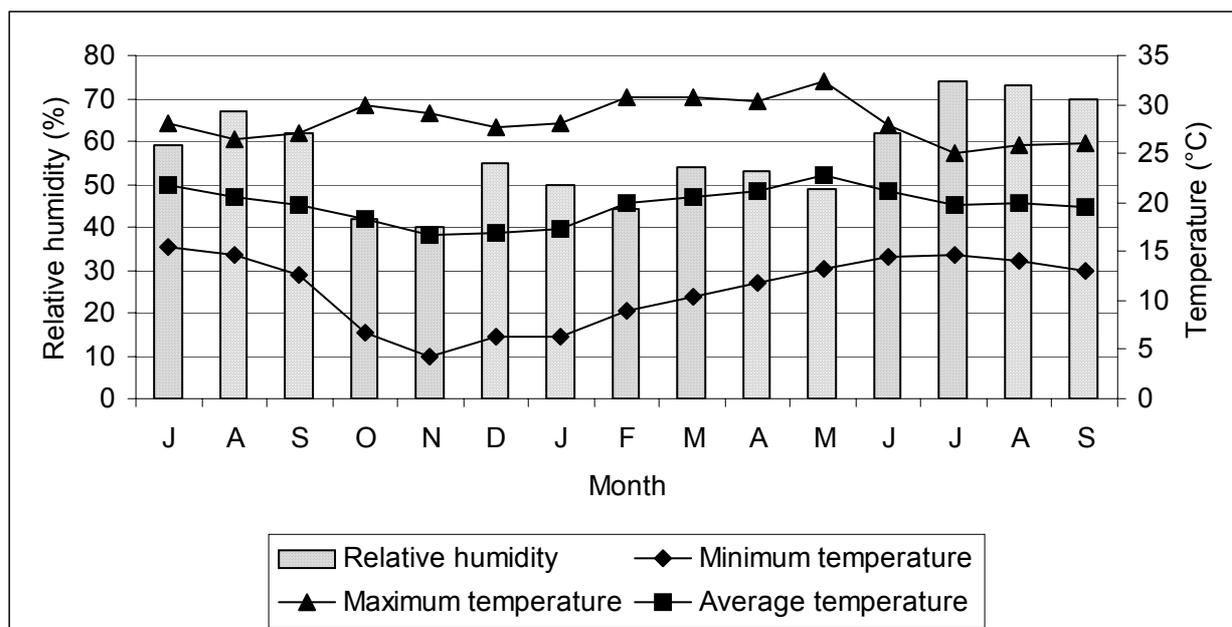


Fig. 5.5. Mean maximum and minimum ambient temperature and relative humidity at Dugdabura from July 2002 to September 2003.

### 5.3.1.2. Goats

Table 5.5 shows the mean, standard deviation, and the maximum and minimum nematode egg counts of the different age groups of goats. The counts in goats ranged from 0-4 900 in July 2002 to 0-7 400 in September 2003. The mean egg count was higher during the rainy seasons in 2002 and 2003. The infection pattern in goats tended to be similar to that of sheep, but the goats had slightly higher mean egg counts. The egg count decreased gradually between October 2002 and February 2003, but increased during the short rain that started in March 2003 and reached a peak value in April 2003. The prevalence of strongyle eggs in the young and juvenile age groups was higher than in the adult age group ( $P < 0.05$ ) during the rainy season in all the study sites. Table 5.6 shows the mean egg counts during the study period in goats at the different study sites.

In Table 5.7 the least-square means and standard errors of the egg counts in goats for the period July 2002 to September 2003 are indicated. There was no significant difference between goats from different sites, but a highly significant difference in the seasonal egg counts between the age groups. The nematode egg count also was higher in all age groups during the wet season ( $2.01 \pm 0.198$ ) than the dry season ( $-2.4 \pm 0.52$ ).

**Table 5.5. Mean nematode egg counts from young (1-6months), juvenile (7-12 months) and adult (>12 months) goats in East Shewa zone from July 2002 to September 2003.**

Season	Age group	Number of observations	Mean egg (SD)	Range
Long rain 2002	1-6 m	86	1 678 (1 511.2)	0-5 000 *
	6-12 m	80	1 563 (1 322.2)	0-4 900 *
	>12 m	83	1 833 (1 884.7)	0-9000 *
Dry season, 2003	1-6 m	91	774 (1 650.9)	0-9 800 *
	6-12 m	85	768 (1 480.8)	0-8 600 *
	>12 m	90	540 (907)	0-5 300 *
Short rain, 2003	1-6 m	28	2 114 (2 363.1)	0-9 800 *
	6-12 m	27	2 641 (2 708)	0-11 000 *
	>12 m	29	1 676 (1 368.4)	100-5 700 *
Dry season, 2003	1-6 m	34	303 (421.7)	14 000 *
	6-12 m	32	316 (580.4)	0-3 000 *
	>12 m	34	568 (1357.1)	0-7 100 *
Long rain, 2003	1-6 m	51	2 322 (1 513.7)	100-6 200
	6-12 m	48	2 810 (1 957)	7 400
	>12 m	51	2 553 (1 698.6)	7 400

\* Significant ( $P < 0.05$ )      n Number of observation

**Table 5.6. Mean faecal nematode egg counts in goats from different study sites for the seasons July 2002-September 2003. There was no significant difference in egg between sites ( $P = 0.623$ )**

Site	Number of observations	Mean egg (SD)	Range
Meki	169	1 307 (1 496.5)	0-8 600
Dugdabura	170	1 312 (1 658.5)	0-9 000
Shashemene	170	1 532 (1 894)	0-9 800
Boset	170	1 563 (1 935)	0-11 000
Mojo	170	1 400 (1 973)	0-8 500

In goats the egg counts varied between season and age ( $P < 0.001$ ), season and sex ( $P < 0.001$ ) and age and site ( $P < 0.001$ ) (Table 5.8), but not between sexes. The prevalence of *M. expansa*, *Avitellina* species, and *T. hydatigena* in goats were recorded during the post-mortem examination during all seasons. The prevalence of tapeworm eggs was higher in the young and juvenile age groups than adults. In none of the goats examined in all the study sites for the entire study period were *Fasciola* eggs found in the faeces.

### 5.3.2. Total worm counts

Of the total 110 tracer animals (57 lambs and 53 kids), 83.8% were infected with either nematodes or cestodes or both. The following species of helminths were identified from both host species: *H. contortus*, *T. colubriformis*, *O. columbianum*, *T. ovis*, *M. expansa*, *Avitellina* spp. and *C. tenuicollis*. The variation in worm burdens, the prevalence, average numbers and the statistical significance for seasonal infection in sheep and goats are summarized below.

#### 5.3.2.1. Sheep

*Haemonchus contortus* was the most predominant nematode recovered from the tracer lambs. This was followed by *T. colubriformis*, *O. columbianum* and *T. ovis*. The months with the highest mean worm counts (more than 2 000) were July, August and September 2002 and 2003. The distribution of the different nematodes species their intensities and the statistical significance for seasonal infection in the tracer lambs are shown in Table 5.9

The tracer lambs acquired low burdens of nematode parasites during the dry season that extended from October 2002 to February 2003. This was supposed to be followed by the short rain, but coincided with a severe drought in the area.

The effects of season on worm burden and worm species at necropsy were statistically examined. Sheep harboured more nematode worms than goats across all seasons. A positive correlation was determined between epg and worm burdens using the Spearman's rank correlation during the rainy ( $r=0.62$ ) and dry season ( $r=0.71$ ). A prevalence of 38% of *M. expansa* detection in sheep was observed throughout the study period.

#### 5.3.2.2. Goats

Table 5.10 shows the prevalence, mean, standard deviation and range of worm intensities and comparisons of infection of the tracer goats during the 2002 and 2003 rainy and dry seasons, respectively. *Haemonchus contortus* was the predominant nematode followed by *T. colubriformis*. Higher burdens of *H. contortus*, *T. colubriformis*, *O. columbianum* and *T. ovis* were recorded during the long rains of 2002 and 2003 than the dry season from October 2002 to February 2003. A positive correlation between epg and worm burden during the dry and wet season was observed as in sheep.

**Table 5.7. Least-square means and standard errors (SE) of nematode egg counts of goats according to age, sex, site and season for the period July 2002 to February 2003.**

Variable factor	Class	Log nematode epg	
		Mean $\pm$ SE	P - value
Age	Young (1-6 months)	0.02 $\pm$ 0.282	0.2493
	Juvenile (6-12 months)	0.150 $\pm$ 0.316	0.3011
	Adult (>12 months)	0.420 $\pm$ 0.329	0.9117
Sex	Males	0.09 $\pm$ 0.290	0.9421
	Females	0.30 $\pm$ 0.252	0.6702
Site	Meki	-0.13 $\pm$ 0.347	0.5592
	Shashemene	-0.008 $\pm$ 0.362	0.6069
	Boset	-0.46 $\pm$ 0.361	0.9373
	Mojo	-0.58 $\pm$ 0.393	0.4677
	Ziwai	-0.346 $\pm$ 0.362	0.1350
Season	Jul-Sept <sup>1</sup>	2.01 $\pm$ 0.198	P<0.0001
	Oct-Feb <sup>2</sup>	-2.41 $\pm$ 0.252	P<0.0001
Interaction between	Age and site	0.88 $\pm$ 0.520	0.8340
	Sex and site	-0.35 $\pm$	0.1243
	Site and season	2.35 $\pm$	0.0331
	Season and age	2.03 $\pm$	P<0.0001
	Season and sex	2.05 $\pm$	P<0.0001
	Sex and age	-0.44 $\pm$	0.4200

<sup>1</sup> Long rainy season 2002

<sup>2</sup> Dry season

### 5.3.3. Prevalence of coccidia

The species *Eimeria bakuensis*, *Eimeria ovinoidalis*, *Eimeria ahsata*, *Eimeria intricata*, *Eimeria granulosa*, *Eimeria faurei*, *Eimeria parva* and *Eimeria pallida* were identified.

#### 5.3.3.1. Sheep

The prevalence of coccidial infections in the different age groups of sheep during the different seasons of the study period is shown in Table 5.11. A total of 710 (83%) of 851 faecal samples examined contained *Eimeria* oocysts. Fig. 5.6 shows the mean monthly oocyst counts value and the mean monthly ambient temperature.

Lambs shed a significantly higher ( $P<0.0001$ ) number of oocysts than the juvenile and adult animals. The oocysts shed by sheep and goats are shown in Tables 5.12 and 5.13 as are the effect of age, sex, site, season, and number of animals.

**Table 5.8. Least-square means and standard errors (SE) of nematode egg counts of goats according to age, sex, site and season for the period October 2002 to September 2003.**

Variable factor	Class	Log nematode epg	
		Mean ± SE	P- value
Age	Young (1-6 m)	0.22±0.237	0.3364
	Juvenile (6-12 m)	0.15±0.265	0.5604
	Adult (>12 m)	0.22±0.277	0.4180
Sex	Males	0.38±0.244	0.3244
	Females	0.02±0.212	0.6702
Site	Meki	0.39±0.340	0.2450
	Shashemene	0.71±0.323	0.027
	Boset	-0.86±0.298	0.004
	Mojo	0.31±0.325	0.5275
	Ziwai	0.44±0.299	0.1802
Season	Oct-Feb <sup>1</sup>	3.21±0.258	<0.0001
	Mar-Apr <sup>2</sup>	0.21±0.316	0.05
	May-Jun <sup>3</sup>	0.62±0.274	0.02
	July-Sep <sup>4</sup>	3.19±0.224	<0.0001
Interaction between	Age and site	1.39±0.552	0.01
	Sex and site	0.36±0.414	0.1296
	Site and season	0.39±0.340	0.7289
	Season and age	3.24±0.419	<0.0001
	Season and sex	3.06±0.315	<0.0001
	Sex and age	0.41±0.358	0.2125

<sup>1</sup> Long dry season 2002<sup>2</sup> Short rain<sup>3</sup> Short dry season<sup>4</sup> Long rain 2003**Table 5.9. Mean and range of nematode intensities, and prevalence of infection of nematodes in 57 lamb tracers examined in East Shewa.**

Season	Nematode spp.	n	Mean (SD)	Range	Prevalence
Long rain, 2002	<i>H. contortus</i>	15	707 (1 193.3)	0–3 993	90
	<i>T. colubriformis</i>		297 (864.3)	0–400	74
	<i>O. columbianum</i>		27 (864.3)	0–209	66.6
Dry season, 2002	<i>H. contortus</i>	15	284 (537.4)	0–1 800	53.3
	<i>T. colubriformis</i>		111 (154)	0–500	60
	<i>O. columbianum</i>		59 (71.6)	0–209	66.7
Short rain, 2003	<i>H. contortus</i>	11	66 (92.9)	0–295	50
	<i>T. colubriformis</i>		3 (6.5)	0–20	30
	<i>O. columbianum</i>		10 (32.8)	0–09	10
	<i>T. ovis</i>				27
Long rain, 2003	<i>H. contortus</i>	16	246 (511.8)	0–2 038	73.3
	<i>T. colubriformis</i>		334 (645)	5–2 450	89
	<i>O. columbianum</i>		218 (312.3)	0–1 300	27.7

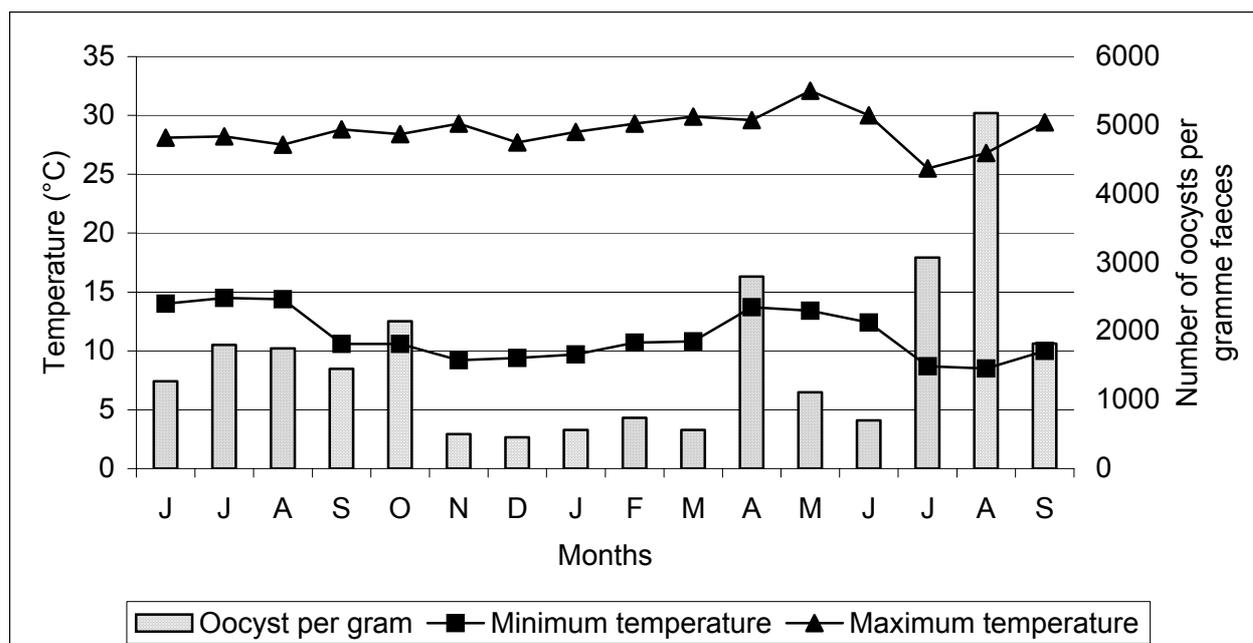


Fig. 5.6. Mean oocyst counts of sheep and monthly average ambient temperature from July 2002 to September 2003 in the semi-arid areas of East Shewa.

Table 5.10. Mean and range of nematode intensities, and prevalence of infection of nematodes in 53 tracer goat examined in East Shewa.

Season	Nematode spp.	n	Mean (SD)	Range	Prevalence
Long rain, 2002	<i>H. contortus</i>	15	1 151 (2 771)	0-1 0923	80
	<i>T. colubriformis</i>		428 (1 114.3)	0-4 300	73.3
	<i>O. columbianum</i>		51 (44.6)	0- 20	34
Dry season, 2003	<i>H. contortus</i>	15	100 (135.7)	0-27	46.7
	<i>T. colubriformis</i>		176 (151.8)	0-89	80.7
	<i>O. columbianum</i>		122 (103.2)	0-00	80
Short rain, 2003	<i>H. contortus</i>	9	51 (74.1)	0-87	50
	<i>T. colubriformis</i>		30 (70.4)	0-15	40
	<i>O. columbianum</i>		2 (5.1)	0-5	30
Long rain, 2003	<i>H. contortus</i>	14	341 (836.6)	0-030	72.9
	<i>T. colubriformis</i>		525 (966.9)	6-588	85.7
	<i>O. columbianum</i>		374 (605.6)	28-2149	20

n Number of tracer goats

**Table 5.11. The prevalence of coccidial oocysts in sheep from July 2002 to September 2003 in East Shewa.**

Season	Young			Juvenile			Adult		
	n	Infected	P	n	Infected	P	n	Infected	P
Long rain <sup>1</sup>	85	77	(90.6)	97	68	(70.1)	86	16	(18.6)*
Dry season	80	75	(93)	90	78	(86.7)	80	23	(28.8)*
Short rain	27	27	(100)	29	27	(93.1)	26	13	(46.1)*
Short dry	32	26	(81.3)	36	24	(66.7)	32	18	(56.2)*
Long rain <sup>2</sup>	48	45	(93.8)	54	26	(48.1)	48	33	(68.8)*
Total	272	225	(91.1)	306	223	(72.8)	272	103	(37.9)

\* Significantly different (P<0.05)      P    Prevalence (%)      n    Number of sheep

**Table 5.12. Least-square means and standard errors (SE) of oocyst counts in sheep compared by age, sex, site and season for the period October 2002 to September 2003.**

Variable factor	Class	Log coccidial oocyst counts		
		Mean	SE	P-values
Age	Young (1-6 months)	2.88	0.070	<0.006
	Juvenile (7-12 months)	2.76	0.077	0.0710
	Adult (>12 months)	2.64	0.064	1.200
Sex	Males	2.790	0.052	0.6700
	Females	2.74	0.063	0.450
Site	Meki	2.75	0.077	0.973
	Shashemene	2.93	0.086	<0.0001
	Boset	2.8	0.079	<0.023
	Mojo	2.81	0.087	<0.045
	Ziwai	2.52	0.087	<0.0001
Season	Dry season (Oct '02-Feb '03)	2.49	0.061	<0.0001
	Short rain (Mar '03-Apr '03)	2.87	0.074	<0.01
	Long rain (Jul-Sep) <sup>1,2</sup>	3.08	0.053	<0.0001
Interaction between	Age and site	3.12	0.205	0.210
	Sex and site	2.99	0.155	0.130
	Site and season	2.93	0.105	0.520
	Season and age	3.14	0.101	<0.001
	Season and sex	3.09	0.076	<0.0001

<sup>1</sup> Long rain, July 2002–September 2002

<sup>2</sup> Long rain, July 2003–September 2003

### 5.3.3.2. Goats

The prevalence of coccidial infections in goats was high as shown in Table 5.14. A total of 625 (74.2 %) of 831 samples were positive. Kids shed significantly higher ( $P < 0.0001$ ) number of oocysts than the juvenile and adult goats. The effect of age, sex, site and number of animals and oocysts shed by goats are shown in Table 5.13.

Highly significant differences in oocyst counts were observed between seasons and sites from goats within the sub-districts in East Shewa. The mean monthly oocysts counts and the mean monthly ambient temperature are indicated in Fig. 5.7. The effect of age, sex, site and seasons on oocysts shed by the animals are shown in Tables 5.15 and 5.16.

**Table 5.13. Least-square means and standard errors (SE) of oocyst counts in goats compared by age, sex, site and season from July 2002 to February 2003.**

Variable factor	Class	Log coccidian oocyst counts		
		Mean	SE	P-value
Age	Young (1-6 months)	2.96	0.069	<0.0001
	Juvenile (6-12 months)	2.63	0.076	<0.0001
	Adult (>12 months)	2.66	0.642	<0.0001
Sex	Males	2.72	0.051	0.561
	Females	2.77	0.061	0.621
Site	Meki	2.760	0.071	<0.0001
	Shashemene	2.924	0.084	<0.0001
	Boset	2.744	0.078	<0.0001
	Mojo	2.526	0.086	<0.0001
	Ziwai	2.797	0.087	<0.0001
Season	Jul-Sept <sup>1</sup>	3.05	0.039	<0.0001
	Oct-Feb <sup>2</sup>	2.44	0.049	<0.0001
Interaction between	Age and site	2.94	0.104	0.351
	Sex and site	2.79	0.878	0.573
	Site and season	3.07	0.086	<0.0001
	Season and age	3.30	0.078	<0.0001
	Season and sex	3.04	0.059	0.644
	Sex and age	2.83	0.103	<0.001

<sup>1</sup> Long rains, July 2002–September 2002 and July 2003–September 2003

<sup>2</sup> Dry seasons, October 2002–February 2003

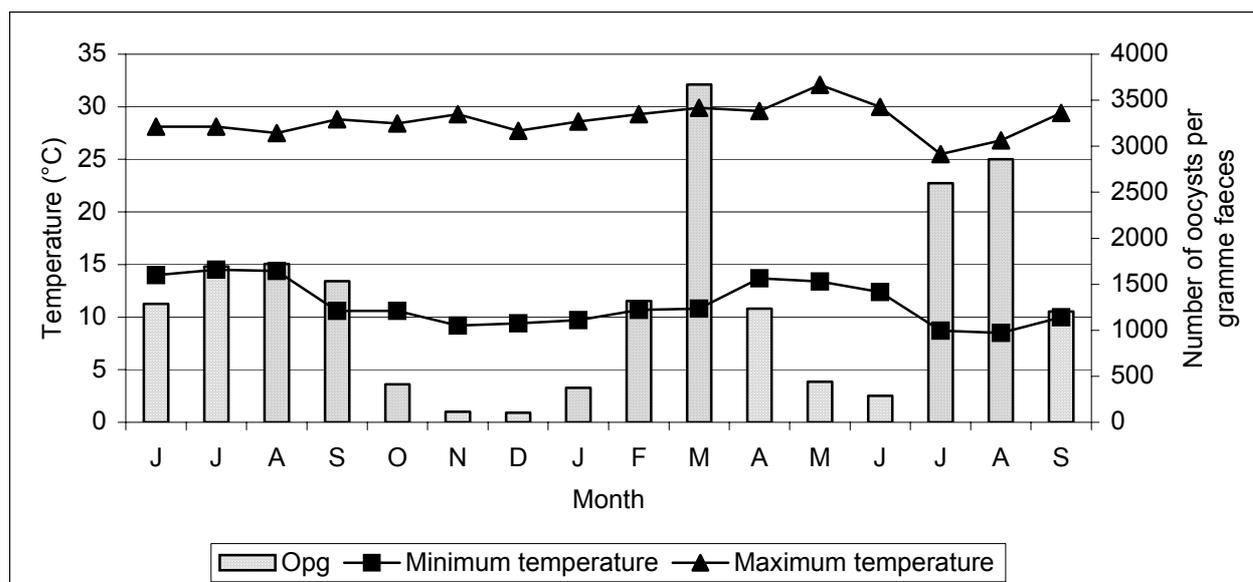


Fig. 5.7. Mean oocyst counts of goats and monthly average ambient temperature and relative humidity from July 2002 to September 2003 in the semi-arid areas.

Table 5.14. The prevalence of coccidial oocysts in goats from July 2002 to September 2003 in East Shewa zone.

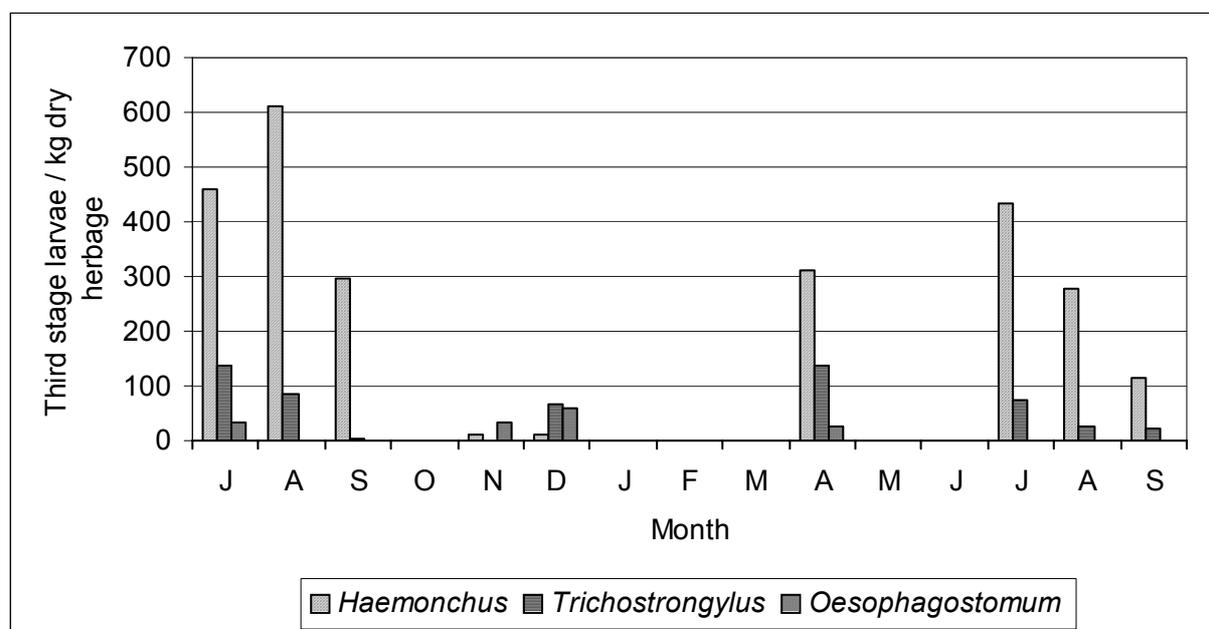
Season	Young			Juvenile			Adult		
	n	Infected	P	n	Infected	P	n	Infected	P
Dry season	86	81	94.2	80	45	56.3	93	43	46.2*
Long rain, 2002	91	84	92.3	85	35	41.2	90	40	44.4*
Short rain	28	26	92.9	27	20	74.1	29	15	51.7*
Short dry	34	30	88.2	32	28	87.5	34	12	35.3*
Long rain, 2003	51	47	92.2	48	29	60.4	51	22	43.1*
Total	290	268	92.4	272	192	70.6	263	132	50.2

\* Significant ( $P < 0.05$ ), n Number of animals P Prevalence (%)

**Table 5.15. Least-square means and standard errors (SE) of oocyst counts in goats compared by age, sex, site and season for the period July 2002 to February 2003.**

Variable factor	Class	Log coccidial oocyst counts		
		Mean	SE	P-value
Age	Young (1-6 months)	1.06	0.265	<0.0001
	Juvenile (7-12 months)	0.60	0.298	<0.05
	Adult (>12 months)	-3.68	0.311	<0.0001
Sex	Males	0.68	0.274	0.567
	Females	0.66	0.238	0.713
Site	Meki	0.29	0.366	<0.0001
	Shashemene	0.79	0.342	<0.0001
	Boset	0.62	0.341	<0.0001
	Mojo	0.72	0.371	<0.0001
	Ziwai	0.94	0.342	<0.0001
Season	Jul-Sept <sup>1</sup>	0.39	0.187	<0.0001
	Oct-Feb <sup>2</sup>	-1.74	0.238	<0.0001
Interaction between	Age and site	1.29	0.492	<0.0001
	Sex and site	-1.53	0.530	<0.004
	Site and season	1.39	0.438	<0.001
	Season and age	2.70	0.325	<0.001
	Season and sex	-1.73	0.391	<0.0001
	Sex and age	-3.897	0.536	<0.0001

<sup>1</sup> Long rain=July 2002-September 2002    <sup>2</sup> Dry season=October 2002-February 2003.



**Fig. 5.8. Herbage larval counts during the period July 2002 to September 2003.**

**Table 5.16. Least-square means and standard errors (SE) of oocyst counts in goats compared by age, sex, site and season from October 2002 to September 2003.**

Variable factor	Class	Log coccidial oocyst counts		
		Mean	SE	P-value
Age	Young (1-6 months)	1.86	0.245	<0.0001
	Juvenile (6-12 months)	1.83	0.275	<0.0001
	Adult (>12 months)	-1.40	0.287	<0.0001
Sex	Males	0.93	0.252	<0.003
	Females	0.59	0.219	<0.007
Site	Meki	0.98	0.352	<0.005
	Shashemene	0.02	0.334	0.0753
	Boset	1.27	0.308	<0.0001
	Mojo	0.85	0.337	<0.01
	Ziwai	0.68	0.309	<0.02
Season	Long rain (Jul-Sept' 03)	2.35	0.232	<0.0001
	Dry period (Oct-Feb '02)	-0.07	0.267	0.93210
Interaction between	Age by Site	0.80	0.571	<0.0001
	Sex by Site	0.80	0.429	0.061
	Site by Season	0.98	0.352	<0.005
	Season by Age	3.30	0.405	<0.0001
	Season by Sex	2.20	0.326	<0.0001
	Sex by Age	1.56	0.371	<0.0001

#### 5.3.4. Larval counts from herbage

The results of larval counts from herbage during the rainy season and two weeks after the unseasonal rains during the dry seasons are shown in Fig. 5.10. The peak pasture larval counts were determined in the long rainy season between July and August in 2002 and 2003 and in the short rainy season in April 2003. The majority of the larvae were identified as *Haemonchus* spp. ranging from 10 to 870, followed by *Trichostrongylus* spp. and *Oesophagostomum* spp.

#### 5.3.5. An abattoir survey of gastro-intestinal parasites

##### 5.3.5.1. Sheep

During the study period, only a few gastro-intestinal tracts of sheep were examined, because goats are mostly slaughtered at the abattoir at Mojo. Sheep that were slaughtered in this abattoir were purchased from areas of an extended neighbouring regions, which might have different agro-ecological environments. There is no system of tracing back information such as the name of the market, the region or place of origin of the animals that are slaughtered in the abattoir. The few sheep gastro-intestinal tract examined harboured similar parasites to

that of goats including *H. contortus*, *T. colubriformis*, *O. columbianum*, *T. ovis* and *M. expansa*.

#### 5.3.5.2. Goats

In the abattoir survey in Mojo, East Shewa the gastro-intestinal tracts of 180 goats were examined for helminths. *Haemonchus contortus*, *T. colubriformis*, *O. columbianum*, *T. ovis* and *B. trigonocephalum* were recorded as were *M. expansa*, *Avitellina* species, *C. tenuicollis* and hydatid cysts. The prevalence and mean distribution of the gastro-intestinal helminths during the rainy and dry seasons of 2002 and 2003 are shown in Tables 5.17–5.18. A significantly higher number of nematodes were found in the guts of the animals during the rainy seasons than the dry season ( $P < 0.05$ ). The most abundant and prevalent nematodes were *H. contortus* and *Trichostrongylus* spp. The remaining nematodes were present in less than 40% of the animals. The total burdens decreased with the age of the animals. The mean worm burdens during the wet seasons (370, 267, 258) indicate higher in the young age group than the juvenile and adult age groups (Table 8.17). The total worm burdens declined during the dry period (Table 5.18).

### 5.4. DISCUSSION

A nematode prevalence of 89% with an average intensity of 1 000 worms were recorded, which indicate that helminthoses are rife in small ruminants in the area. Examination of the worm population in tracer lambs at different seasons of the year confirmed the presence of a variety of helminths (Tables 5.9, 5.10 and 5.17) with *H. contortus*, *T. colubriformis* and *O. columbianum* being the predominant species. With regard to both prevalence and burden, *H. contortus* and *T. colubriformis* were the most common nematodes

Large numbers of *H. contortus* were recovered exclusively during the wet season (Tables 5.9 and 5.10). In the dry season, from October to end of February, the number of larvae on the pastures dropped markedly (Fig. 5.8) whereas few numbers of larvae were found in the abomasal mucosa of tracer sheep and goats. The number of adult parasites recovered during the dry period was lower in both host species (Tables 5.9 and 5.10). This suggests that *H. contortus* survives the dry season mainly as adult parasites and to some extent as hypobiotic larvae. Hypobiosis of both *H. contortus* and *L. elongata* (not found in this study) were observed in tracer lambs at the start of the dry season in a study conducted in the northern highland areas of North Shewa (Tembely *et al.*, 1997).

Table 5.17. Worm burden of goats from an abattoir survey in East Shewa during the wet seasons between June and September.

Helminth species	Young (6 months) (18)				Juvenile (7-12 months) (52)				Adult (> 12 months) (26)			
	Number of worms			p	Number of worms			p	Number of worms			p
	Larvae	Adult	Total		Larvae	Adult	Total		Larvae	Adult	Total	
<b>Nematodes</b>												
<i>Haemonchus contortus</i>	27	3 183	3 210	94	224	7 925	8 700	90.4	134	3 350	3 484	88
<i>Trichostrongylus colubriformis</i>	0	1 450	1 450	77	0	2 967	3 007	76.9	0	1 710	1 710	65
<i>Oesophagostomum columbianum</i>	16	1 498	1 512	66	11	1 131	1 490	65.4	33	1 200	1 233	42
<i>Bunostomum trigonocephalum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichuris ovis</i>	0	476	476	67	0	670	670	67.5	0	270	270	34
<b>Cestodes</b>												
<i>Moniezia expansa</i>	-	5	5	27.7	-	9	9	17.3	-	4	4	15
<i>Echinococcus spp.</i>	0	-	0	0	1	0	1	1.9	1	0	3.8	12
<i>Avitellina spp.</i>	-	5	5	11	-	5	5	10	-	0	0	0
<i>Coenurus cerebralis</i>	0	-	0	0	0	-	0	0	1	-	1	7.7
<i>Taenia hydatigena</i>	0	-	0	0	4	-	4	22	3	-	3	33
Total nematode burden	37	6 173	6634		235	12 693	13 867		178	6 530	6 697	
Mean nematode burden	1.2	367	370		5	244	267		2	251	258	

- Not applicable

P Prevalence

Table 5.18. Worm burden of goats from an abattoir survey in East Shewa during the dry seasons between October and March.

Helminth species	Young (6 months) (10)				Juvenile (7-12 months) (32)				Adult (> 16 months) (42)			
	Number of worms			P	Number of worms			P	Number of worms			P
	Larvae	Adult	Total		Larvae	Adult	Total		Larvae	Adult	Total	
<b>Nematodes</b>												
<i>Haemonchus contortus</i>	35	637	672	40	17	710	727	34.4	40	369	409	31
<i>Trichostrongylus colubriformis</i>	0	349	349	30	3	267	270	22	16	100	116	46
<i>Oesophagostomum columbianum</i>	5	83	88	40	0	70	70	25	6	79	85	23
<i>Bunostomum trigonocephalum</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichuris ovis</i>	0	28	28	20	0	67	67	15	0	38	38	4.8
<b>Cestodes</b>												
<i>Moniezia expansa</i>	-	11	11	30	0	5	5	16	-	5	5	7
<i>Echinococcus spp.</i>	0	-	0	0	0	0	0	0	0	-	0	0
<i>Avitellina spp.</i>	-	0	0	0	-	2	2	6		2	2	4.8
<i>Coenurus cerebralis</i>	0	-	0	0	0	-	0	0	1	-	1	2.4
<i>Taenia hydatigena</i>	3	-	3	20	4	-	4	12.5	0	-	0	33
Total nematode burden	40	1 097	1 137		20	1 114	1 134		62	586	648	
Mean nematode burden	4	109	113		2	35	35		1.5	14	15	

- not applicable

P Prevalence

Hypobiosis, and the carry-over of adult worms from one season to another, may play a major role in the survival mechanism of the nematode populations when the environment is not conducive to their development (Ogunsui and Eysker, 1979). Gatongi (1995) also reported that up to 80% of the *Haemonchus* worm burden in small ruminants in the semi-arid region of Naivasha in Kenya, survived the dry season as hypobiotic larvae. Fritsche *et al.* (1993) found that for the abomasal nematodes, *Haemonchus* and *Trichostrongylus axei*, hypobiosis is an important characteristic of larval development.

Hypobiotic larvae were not observed in tracers from Mojo and Shashemene during the dry seasons. Although these localities are in the Rift Valley, their climatic conditions are characterized more as a sub-humid environment. Rainfall, warm climate and humidity are conducive for continual development of both helminths and coccidia throughout the year. However, more studies are needed to rule out the occurrence of inhibited larval development (hypobiosis) in the arid and semi-arid areas of the zone.

After *H. contortus*, *T. colubriformis* was the most common nematode and occurred in large numbers during the long rainy seasons and declined during, the short rainy season. Their numbers were lowest during the long dry season. *Oesophagostomum columbianum* with variation in ambient temperature regimes produces obvious seasonal effects, but can also lead to large differences in the size of parasite populations between years (Sutherst, 1987). The worm counts from kids and lambs were higher than those of the adults ( $P < 0.05$ ). Animals older than 1½ years had fewer worms. This is probably attributable to age resistance, where many animals become more resistant to primary infections with some parasites as they reach maturity (Urquhart *et al.*, 1987).

The level of infection and the number of strongyle eggs in faecal specimens were influenced by weather with the highest levels being observed during the long rainy seasons followed by the short rainy seasons. This is in agreement with the observation of Gatongi (1995) in small ruminants in the semi-arid area in Kenya with a similar rainfall pattern to that of Mojo and Shashemene. Clinical infections often occur during the rainy seasons with mortalities particularly in the juvenile age groups. Strongyle egg output remained at a low level during the October to February dry season (Tables 5.3-5.8). Low egg output observed in adult animals might be because of age resistance (Soulsby, 1982). Signs of helminthoses associated with low epg (0–400) occurred in sheep early in the dry season.

During the long dry seasons, the animals would carry helminth burdens that might cause subclinical disease. This has been described as the most economically important form of

parasitic enteritis, which often passes unnoticed and affects large numbers of animals, causing retarded growth and reduced productivity. Such animals are prone to infections by other pathogens, and, in addition, continually contaminate pastures (Allonby and Urquhart, 1975).

The counts of larvae on herbage indicated that rainfall was the most important factor controlling their availability. Following the onset of dry conditions few larvae recovered but the hot and humid weather in East Shewa, particularly during rainy season, provided favourable conditions for the development and survival of the larval stages of several nematodes. Gordon (1953) suggested that a total monthly rainfall of more than 50 mm and a mean monthly maximum temperature of over 18.3 °C provide optimum conditions for the development and survival of *H. contortus*. Examination of the relationship between faecal egg counts and worm burdens that was observed in 180 goats examined during the abattoir survey showed a positive correlation during both dry and wet seasons. The positive correlation, was probably related to the high egg production of *H. contortus*. This species represent the majority of the total worm population usually following the rainy season. Thus, the species of nematodes, the duration and period of grazing are most likely to determine the level of egg out put and pasture contamination.

In the abattoir survey the age groups of the animals, which ranged from 9-24 months, were not similar and reliable. A more detailed study in both species of animals would give a definite conclusion in this regard.

Cestode infections were very common in both lambs and kids and because of the low pathogenicity in adults, tapeworm infections were considered as of minor importance. It is not known whether *M. expansa* plays any role in production loss in lambs and kids during the time of drought and lack of sufficient nutrition under arid and semi-arid environment. However, in Europe, Australia and South Africa, these tapeworms are not considered pathogenic, unless present in large numbers (Reinecke, 1983; Urquhart *et al.*, 1987).

The cause of coccidiosis in sheep and goats in the present study were found to be morphologically similar *Eimeria* species. *Eimeria* species in sheep and goats are relatively host specific and are not transmitted between hosts or to other hosts (Levine, 1985). The importance of coccidiosis in lambs and kids were proved based on the presence of large numbers of oocysts accompanied by diarrhea and clinical symptoms, such as general weakness, unthriftiness and lower productivity (Blood and Radostits, 1989).

Coccidiosis in sheep occurred as mixed infection of *Eimeria* species, several of them in one sample. In this observation, coccidiosis in lambs was more important than adult sheep. The mortality of lambs from coccidiosis was rare. Pout et al., 1966 and Levine, 1985, stated that morbidity from coccidiosis could be between 10-40% while mortality rarely more than 10%. A high level of coccidial oocysts ingested mixed with feed contaminated with manure, drinking dirty water, grazing on heavily contaminated pasture with manure, are some of the factors which contribute to the spread of the disease.

In sheep, approximately 12 species of *Eimeria* are known to exist. *E. ahsata*, *E. bakuensis* (*E. ovina*), *E. crandallis*, *E. faurei*, *E. gonzalezi*, *E. granulosa*, *E. intricata*, *E. ovinoidalis*, *E. pallida*, *E. parva*, *E. punctata* and *E. weybridgensis* (Levine, 1985; Faizal and Rajapakse, 2001). In this study the species *E. ovina*, *E. ovinoidalis*, *E. ahsata*, *E. intricata*, *E. granulosa*, *E. faurei*, *E. parva* and *E. pallida* were identified. Studies on the virulence of *Eimeria* species indicate that the most pathogenic of the above species are *E. ahsata*, *E. ovina* and *E. ovinoidalis*.

Sixteen *Eimeria* species have been described from goats worldwide (Smith and Sherman, 1994). At least nine of the species occurred in goats in this study including *E. alijeae*, *E. apsheronica*, *E. arloingi*, *E. caprina*, *E. caprovina*, *E. christenseni*, *E. hirci*, *E. jolchijevi* and *E. ninakohlyakimovae*. The pathogenic species of *Eimeria* in goats (*E. alijeae*, *E. arloingi*, *E. ninakohlyakimovae* and *E. christenseni*) suggest that coccidiosis perhaps contributes to enteric disease syndromes that affects goats, particularly kids, in the herd. Studies indicate that *E. arloingi*, and *E. ninakohlyakimovae* are regarded as the most pathogenic species in goats (Levine, 1985).

The high percentage of positive specimens in goats is consistent with findings in other studies, for example 94% in Zimbabwe (Chhabra and Pandey, 1991), 85% in Tanzania (Kusiluka et al., 1996), 85% in Senegal (Vercruyssen, 1982), 97.2% in USA (Penzhorn et al., 1994), 94% in the Southeast of England (Norton, 1986) and in South Africa, at Hammanskraal upto 98.6% (Harper and Penzhorn, 1999). In Sri-Lanka, of the representative samples examined, oocysts were found 88% of kids, 91% of young goats and 83% adults (Faizal and Rajapakse). Coccidia affected goats were also reported from other parts of Africa, for example, the Sahel region of Senegal (Vercruyssen, 1982) and Kenya (Kanyari et al., 1993), but there was no report from Ethiopia.

The prevalence of coccidia in different ecological zones of the lowland area has as yet not been reported. High rates of coccidial infections in up to 87.8% in sheep and 83.8% in goats were encountered. This finding is in agreement with observations made in other countries where infection rates of 76%-92% were found (Vercruysse, 1982; Chhabra and Pandey, 1992; Jalila, Dorny, Sani, Salini and Vercruysse, 1998; Harper and Penzhorn, 1999; Atanasio, 2000; Faizal and Rajapakse, 2001). Despite the high prevalence in all the study sites, clinical coccidiosis was limited to the young kids and lambs. Oocysts are almost always present in faeces of ruminants, sometimes up to tens of thousands or more per gramme of faeces, but clinically it usually manifests in younger animals rather than the older ones (Boomker, personal communication). On several occasions in this study, weaned kids or lambs were observed to have diarrhoea, sometimes watery and rarely stained with blood and with poor appetite and general loss of body condition. Good husbandry practices should be introduced to peasant farmers to reduce the economic losses caused by subclinical or clinical coccidiosis. Otherwise young animals, which survived either the severe or acute form and the chronic disease, will allways remain unthrifty. Thomson and Hall (1933) and Ndarathi *et al.* (1989) commented that high burdens of coccidia in young sheep and goats could represent a disease problem and thus economic importance.

No significant seasonal fluctuation was found in the prevalence and intensity of infection in sheep. This lack of fluctuation was also observed in sheep in Senegal (Vercruysse, 1982) and Australia (O'Callaghan, O'Donoghue and Moore 1987). However, in this study a significant difference ( $P < 0.05$ ) in oocyst counts between seasons and between sites in goats was observed (Tables 5.11-5.16). Similar results were obtained in semi-arid area in Kenya in goats (Waruiru *et al.*, 1991). The prevalence of oocysts and the oocyst counts were higher in the young than in adult sheep ( $P < 0.05$ ), as reported by several other workers worldwide (Asharaf and Nepote, 1990; Kanyari, 1993; Faizal and Rajapakse, 2001). Concurrent infection with coccidia and gastro-intestinal nematodes was observed in all the three age groups, which may imply that the animals were exposed repeatedly to infections. This observation is in agreement with the reports of Faizal and Rajapakse (2001).

Coccidiosis is an economically important enteric disease in the intestine of sheep or goats (Foreyt, 1990). Damage to the host is primarily that of disruption of intestinal cells. Clinical coccidiosis was observed most commonly in 4 to 8 weeks old lambs and kids, but was also seen in lambs and kids 2-3 weeks after weaning or in sheep and goats of all ages. Under unsanitary and overcrowded conditions, exposure to sporulated oocysts is routine and infection occurs throughout the year. Diarrhoea is the major sign of clinical coccidiosis. Bacterial diarrhoea and bacterial septicemia often accompany coccidiosis (Coles, 1986).

Crowding or confinement of animals under the peasant management systems might have also led to a substantial build-up of coccidial parasites (Soulsby, 1982; Levin, 1985).

In the peasant farming system, the agricultural activities are often intensified during the rainy seasons. During this time, most of the animals usually kept indoors for longer hours. The floors in the animals' shelters usually are not cemented and they become muddy during the wet seasons. Unlike cattle's and or other large animals' the small ruminants' dung is not regularly disposed of. These conditions create favorable environment for coccidial infections of particularly the young animals. The observation in this study that young animals have larger coccidial burdens confirmed the findings of Ndarathi *et al.*, 1989.

The gastro-intestinal parasites isolated and the genera of helminth identified in the current study have also been reported in both highland and semi-highland areas of the country (Graber, 1975, 1978c; Bekele *et al.*, 1992; Tembely *et al.*, 1997, 1998).

The predominance of *H. contortus* among the helminths being reported agrees with other investigations carried out under similar environmental conditions in other countries (Maingi *et al.*, 1996a). In the current study, *H. contortus* has been recognized as one of the helminths of economic importance followed by *T. colubriformis* and *O. columbianum*. In general, the high nematode burden observed in this study is significant for its pathogenic potential as a cause for clinical disease in small ruminants. The occurrence of tapeworm and *Trichuris* in sheep and goats did not cause serious or important problems. However, the economic importance of coccidiosis in sheep and goats in the area should be taken into consideration, and as the high prevalence and burden suggests a considerable loss in production can be expected.

In conclusion, gastro-intestinal helminths are wide-spread in both sheep and goats throughout the Mid-Rift Valley areas of the zone, where peasant farming system and livestock production and management practices exert a considerable influence on the occurrence and distribution. Appropriate control measures are needed which should be based on cost effective studies to optimize production and establish a sustainable worm management methods.

## **Chapter 6.**

# **FAMACHA<sup>®</sup> based selective anthelmintic treatment**

### **6.1. INTRODUCTION**

Michel (1985) stated that in the absence of a well-defined worm control program, the management of worms is more costly and less effective, and may lead to selection of anthelmintic resistance. Nematode control programs based upon epidemiological principles that have been recommended over the years in other countries are strategic worm control programmes for sheep in the UK (Taylor *et al.*, 1991) and suppressive drenching (Michel, 1969, 1976; Brunson, 1980; Lloyd *et al.*, 2000), and are highly effective for worm management. However, it has been confirmed that the greater the success of these strategies, the greater the degree of selection for anthelmintics is likely to be (Waller *et al.*, 1995; Waller, 1997). This is because all the worms in all the animals are exposed to the various drugs available. Unlike the previous approaches to maximize parasite control, recommendations must now be designed to not only control parasites, but also to minimize the development of anthelmintic resistance. It is common for 20-30% of sheep and goats to harbour about 70-80% of the worms (Galvani, 2003, cited by Kaplan, 2004). In this instance a selective treatment approach that targets the portion of the herd or flock with the high worm burden could be a successful approach in the control of parasites, while reducing drug costs, delaying the development of anthelmintic resistance and improving production.

The purpose of the present study was to test the effectiveness of selective anthelmintic treatment in sheep and goats by clinically identifying individual animals using the FAMACHA<sup>®</sup> system, together with haematocrit assessment and body condition scoring.

### **6.2. MATERIALS AND METHODS**

#### **6.2.1. Study area**

The study was conducted in Ziway district of East Shewa zone, at Abernosa Cattle Breeding and Improvement Center, which is located about 170 km southeast of Addis Ababa. The annual precipitation averages 777 mm, and the annual mean temperature and relative humidity ranges between 23-27 °C and 60-80% respectively. The highest precipitation occurs in the middle of July and August and sometimes-early part of September. Topographically, the area is flat. Soils vary from sandy to silty and the vegetation consists of different *Acacia* species with scattered grassland with good grass cover.

## 6.2.2. Study animals and experimental design

### 6.2.2.1. Study animals

About 10 hectares of paddock, which had been grazed by sheep and goats infected with *H. contortus* was fenced in before the start of the long rainy season in July 2002. The pasture was then divided into four equal paddocks with even pasture cover. After fencing, each paddock was grazed for some time by sheep and goats with known *Haemonchus* infection.

Ninety-six indigenous castrated sheep and goats between 4 and 6 months old, which were never exposed to anthelmintics, were purchased from local markets. Each animal was identified by an ear tag with code numbers and vaccinated against pasteurellosis, anthrax and sheep pox. They were treated with closantel at 10 mg/kg body weight and hand-sprayed with 1% flumethrin for ticks and diazinon for lice and keds. The animals were selected randomly into four treatment groups of twenty-four animals consisting of even numbers of sheep and goats. However, two sheep and two goats were removed from Group I and II before the start of data collection because of difficult adaptation to the conditions at Abernosa. Each group was set-stocked in an individual paddock until the end of September 2003 when the experiment was terminated.

Group I was treated with 7.5 mg/kg albendazole every month and served as the suppressive treatment control group. Group II served as selective treatment group as diagnosed by the FAMACHA<sup>®</sup> system. Individual animals with anaemia scores 3, 4 and 5 were given 7.5 mg/kg albendazole. Group III was the untreated control and Group IV served as the treated control group that received a single treatment with albendazole at 7.5 mg/kg at the beginning of the trial, which coincided with the start of the long rainy season. All animals in the trial had access to water and hay, and a balanced mineral lick. Hay that was stored for more than a year was provided to all animals in order to prevent any chance of fluke infections through metacercariae that might have been present.

Six local herdsmen were recruited. Each had to ensure that no animals from the other group entered his paddock. Two men were assigned for night guard. All were given orientation and training how to observe the animals for any health problem, and how to pass on the information to the Assistant Veterinarian who resides at Abernosa Centre.

### 6.2.2.2. Sampling procedures

Faecal samples were collected *per rectum* from each animal at monthly intervals from July 2002 until September 2003. The modified McMaster technique was used to determine the

nematode egg count in each sample. The sedimentation method to assess the presence of liver fluke infections was used according to the methods described by Hansen & Perry (1994).

Grass samples were collected during the short and long rainy seasons in 2003 and larvae were recovered from pasture samples using the methods described by Hansen and Perry (1994). Larvae were counted and identified according to the methods described by Van Wyk *et al.* (1997b).

Blood samples for haematocrit determination were collected every month. Haematocrit tubes were centrifuged in a haematocrit centrifuge for half an hour. The value was read using a haematocrit reader and expressed as a percentage. At the same time smears for the detection of haemoprotozoa were made and stained with Giemsa stain.

#### **6.2.2.3. Use of tracer lambs for worm recovery**

Forty-two lambs aged between 4-6 months were kept parasite-free by regular deworming. Four lambs were released into each paddock during the long wet season from August-September 2002, during the short rainy season from March-April 2003, and during the long rainy season July-September 2003. They were allowed to graze for three weeks and kept indoors for 21 days. Then were necropsied and processed for worm recovery using the methods of Boomker *et al.* (1989). The nematodes were classified according to their developmental stages and identified to genus and/or species level using the descriptions of Dunn (1978) and Gibbons & Khalil (1982).

#### **6.2.2.4. Live weight gain**

Weighing the animals in this study was aimed at the assessment of the effect of selective anthelmintic treatment on monthly live weight. Each animal was weighed every month, in the morning, in order to minimize mass fluctuations due to eating or drinking, and before they were released to graze.

The monthly weight gains, or losses ( $w$ ), were analyzed using repeated measures of analysis of variance. Differences between consecutive monthly weight gains (between  $w_1-w_2$ ,  $w_2-w_3$ ,  $w_3-w_4$ , until the last preceding  $w_{15}$ ) were computed. The ANOVA test was then used to calculate the actual weight difference by comparing groups or between the different treatment groups. In the analysis, helminth egg counts and weight were considered as subject effects. A model, which is shown in the General Materials and Methods was used to analyze the data.

#### **6.2.2.5. Body condition score**

On each scheduled visit sheep and goats in all the experimental groups were physically examined and their body condition scores were recorded using the guidelines developed by the Agricultural Research Council in South Africa (Fig. 3.2).

#### **6.2.2.6. FAMACHA<sup>®</sup> clinical evaluation**

At the scheduled monthly visits, the conjunctiva of both eyes of each experimental animal were examined, mostly in the morning, and scoring was carried out in the shortest possible time to avoid congestion and resulting misinterpretations. These scores were captured in the data collection formats and both sheep and goats in the selective treatment group that were considered to fall in the FAMACHA<sup>®</sup> groups 3, 4 and 5 were treated. Two-way frequency table of haematocrit by FAMACHA<sup>®</sup> with haematocrit cut-off of <19% were drawn up from the scores obtained during July 2002 to September 2003 and the sensitivity and specificity of the FAMACHA<sup>®</sup> system, the predictive value of negative and positive were tested according to the methods of Vatta *et al.* (2001), which was briefly described in Chapter 3.

The monthly FAMACHA<sup>®</sup> evaluation was sufficient and all animals in every group were inspected weekly for clinical signs such as bottle jaw and diarrhoea.

Anthelmintic resistance tests were carried out according to the methods of Coles *et al.* (1992), as described in the Materials and Methods and using RESO computer techniques for their calculation (Anon., 1990).

#### **6.2.2.7. Data analysis**

The general linear model procedure of SAS was used to analyze the data. Most of the means for worm and egg counts presented in the various tables and figures are arithmetic means. The relationships between faecal egg counts, haematocrit levels, body condition scores and live body weight gains were estimated by ANOVA following log transformation of the data that were collected from July 2002 to September 2003.

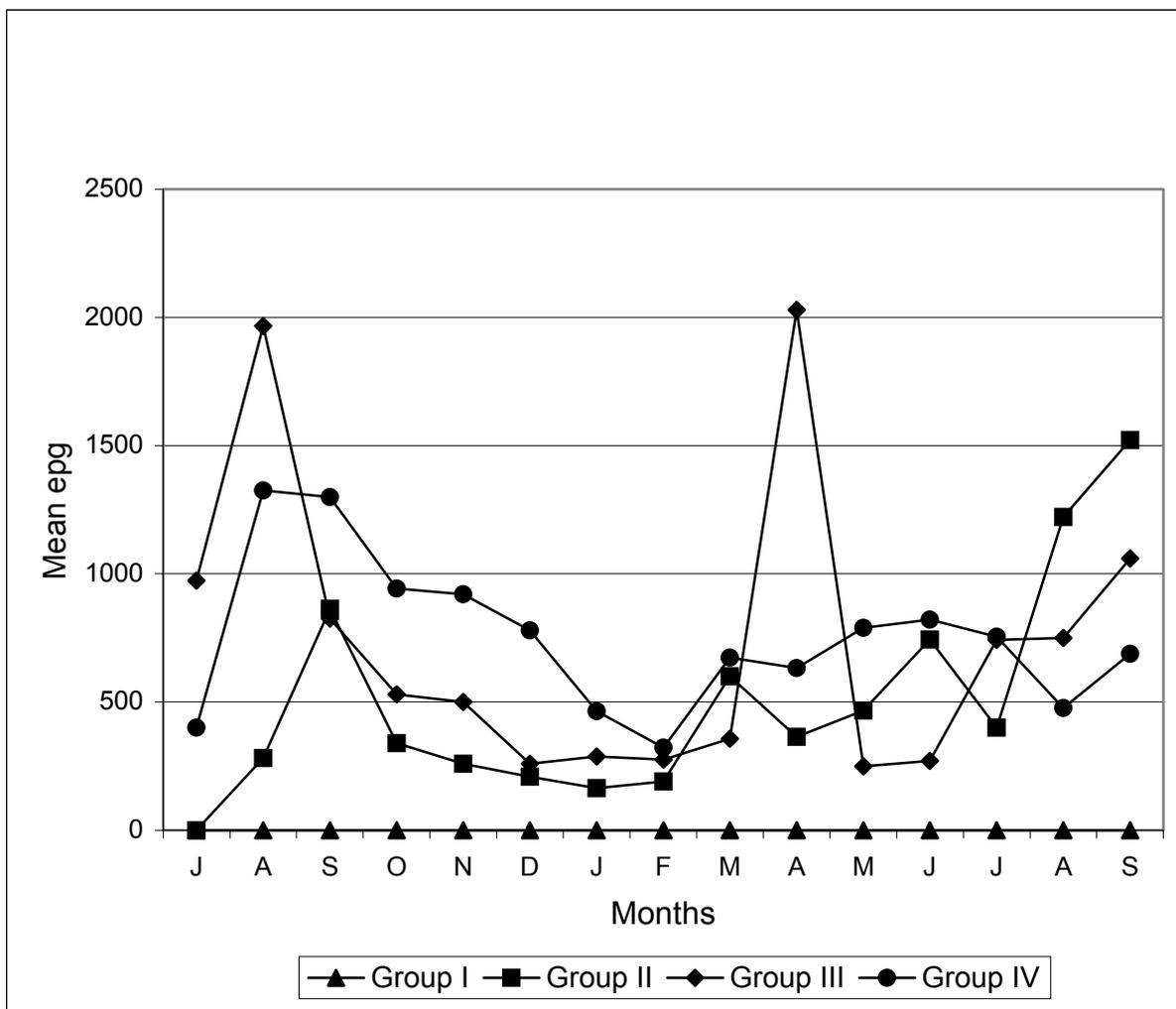
### **6.3. RESULTS**

#### **6.3.1. Egg and worm counts**

##### **6.3.1.1. Faecal egg counts**

The eggs present in faecal samples from sheep and goats in the control and selective treatment groups were predominantly those of *Haemonchus*. The mean egg count of the suppressive treatment group was very low throughout the trial period (Tables 6.1 and 6.2)

The egg count of the selective treatment group during the first long wet season showed a higher peak after the rain in August. Following the dry season in October, the egg count declined in all groups. A slight peak was observed in all except the suppressive treatment group between March and April 2003. During the second long rainy season between July and September 2003, the egg counts for the selective treatment, untreated and single treatment control groups increased (Fig. 6.1). However, the egg count between the non-treated and the selective treatment groups showed a significant difference ( $P < 0.05$ ).



**Fig. 6.1.** Mean faecal strongyle egg counts in the suppressive, selective, non-treatment and single treatment groups during July 2002-September 2003.

The statistical analysis of the faecal egg counts over the entire sampling period showed that suppressive treatment had a significant overall effect ( $P < 0.001$ ) and that the egg counts of the selective treatment group also showed a significant difference from the untreated control group ( $P < 0.001$ ).

**Table 6.1. Group mean nematode egg counts of goats in the FAMACHA<sup>®</sup> trial for the period July 2002- September 2003.**

Year	Group	n	Number of observations	Mean egg counts	Range of egg counts
2002	I	11	66	20.7	0-100
	II	11	66	629	100-12 900
	III	13	71	1 221	100-8 600
	IV	12	71	1 400	100-7 500
2003	I	11	99	60	0-500
	II	11	97	847	0-4 400
	III	10	91	832	0-6 900
	IV	10	90	860	0-5 800

n number of animals

II Group II, selective treatment,

IV Group IV, single treatment control.

I Group I, suppressive treatment,

III Group III, non-treatment control,

**Table 6.2. Group mean nematode egg counts of sheep in the FAMACHA<sup>®</sup> trial for the period July 2002- September 2003.**

Year	Group	n	Number of observations	Mean egg counts	Range of egg counts
2002	I	11	66	0	0
	II	11	66	679	100-7 400
	III	13	67	1 183	100-8 600
	IV	12	51	871	100-4 100
2003	I	11	98	14	0-700
	II	11	87	608	100-4 900
	III	10	70	1 670	100-11 100
	IV	10	85	887	0-4 900

n number of animals,

II Group II, selective treatment,

IV Group IV, single treatment control.

I Group I, suppressive treatment,

III Group III, non-treatment control,

### 6.3.1.2. Larval counts on vegetation

Larvae were counted on the vegetation during the wet seasons using samples that were often collected only during the morning from pastures in each of the paddocks. The recovered larvae were identified using the methods described by Van Wyk (1997b) and counted. In each paddock the predominant larvae were *Haemonchus*. *Trichostrongylus* larvae were present during the long rainy season. There were no other larvae (Table 6.3). The maximum number of larvae was recovered and identified from paddocks of the non-treated control group, followed by the selective treatment group. In all paddocks there were hardly any larvae during the dry periods.

**Table 6.3. Mean pasture larval counts during July 2002 to September 2003 from four paddocks where the four groups of animals were set stocked in the FAMACHA<sup>®</sup> study.**

Period	Paddock	Number of larvae (L <sub>3</sub> )	Genus of larvae	Ratio (%)
Aug-Sep (2002)	I	290	<i>Haemonchus</i> <i>Trichostrongylus</i>	69 31
	II	750	<i>Haemonchus</i> <i>Trichostrongylus</i>	66.7 33.3
	III	800	<i>Haemonchus</i> <i>Trichostrongylus</i>	75 25
	IV	650	<i>Haemonchus</i> <i>Trichostrongylus</i>	81 19
Mar-Apr (2003)	I	0	0	0
	II	70	<i>Haemonchus</i>	100
	III	200	<i>Haemonchus</i> <i>Trichostrongylus</i>	60 40
	IV	148	<i>Haemonchus</i> <i>Trichostrongylus</i>	76 24
Jul-Sep (2003)	I	170	<i>Haemonchus</i> <i>Trichostrongylus</i>	70.6 29.4
	II	640	<i>Haemonchus</i> <i>Trichostrongylus</i>	78.1 21.9
	III	1360	<i>Haemonchus</i> <i>Trichostrongylus</i>	73.5 26.5
	IV	1060	<i>Haemonchus</i> <i>Trichostrongylus</i>	79.5 19.5

I Group I, suppressive treatment,  
III Group III, non-treatment control,

II Group II, selective treatment,  
IV Group IV, single treatment control.

**Table 6.4. Mean worm counts from tracer lambs which grazed on the four separate paddocks in the FAMACHA<sup>®</sup> trial for the period July 2002-September 2003.**

Period	Group	n	epg	Mean worm counts from tracer lambs			
				<i>Haemonchus contortus</i>	<i>Trichostrongylus colubriformis</i>	<i>Trichuris ovis</i>	Total
Jul-Sep (2002)	I	2	150	20	10	10	40
	II	4	288	47	28	8	83
	III	4	2 450	541	151	18	610
	IV	2	1 100	287	43	27	357
Oct-Jan (2002)	I	2	0	0	0	0	0
	II	2	0	0	0	0	0
	III	2	0	0	0	0	0
	IV	2	200	35	0	0	35
Mar-Apr (2003)	I	2	125	75	8	8	91
	II	4	500	187	20	12	219
	III	4	1 037	309	44	21	374
	IV	4	868	500	117	23	640
Jul-Sep (2003)	I	2	125	20	10	14	44
	II	4	500	26	46	14	86
	III	4	1 037	46	179	28	608
	IV	2	940	506	174	25	705

n number of animals, I Group I, suppressive treatment,  
 II Group II, selective treatment, III Group III, non-treatment control,  
 IV Group IV, single treatment control.

### 6.3.1.3. Worm recovery and counts from tracer lambs

The results of the necropsy of the tracer animals are presented in Table 6.4. The highest worm count was recorded during the long wet seasons of both 2002 and 2003. No infection was acquired during the long dry season of 2002 and the short dry season of 2003.

*Haemonchus contortus*, *T. colubriformis*, *T. ovis* and *M. expansa* were recovered and identified from the selective treatment group and non-treated control groups in August 2002, with a peak observed towards the end of September 2002. The same parasites were recovered during the short rainy season. Out of the 42 abomasa examined in the FAMACHA<sup>®</sup> trial, 36 (75%) were *H. contortus*. More than 40% of the tracer lambs used in this trial harboured the tapeworm *M. expansa*. No flukes or lungworms were found in any of the animals examined. The overall worm burden in the tracers that grazed in the paddocks of the non-treated animals was significantly higher ( $P < 0.0001$ ) than tracers from the treated or selectively treated paddocks. No arrested larvae were recovered from any of the lambs. In all the tracers, *H. contortus* was the predominant larvae identified from faecal cultures.

#### **6.3.1.4. Faecal egg count reduction test**

Anthelmintic resistance tests were carried out according to the methods of Coles *et al.* (1992) using RESO computer techniques for their calculation (Anon., 1990). The frequency of suppressive anthelmintic treatment in Group I was high. Although prolonged use of an anthelmintic could lead to selection for resistance to that of anthelmintic (Prichard, 1990), the repeated faecal egg count reduction test for the treated animals showed that there was no anthelmintic resistance (Table 6.5-6.7).

### **6.3.2. FAMACHA<sup>®</sup> clinical evaluation**

#### **6.3.2.1. FAMACHA<sup>®</sup> evaluation in goats**

The effect of haemonchosis was demonstrated with the proportion of animals identified by the FAMACHA<sup>®</sup> categories 1 to 5. The percentages of goats identified by the categories in the different treatment groups are shown in Fig. 6.2-6.5.

Throughout the study period, the goats in Group I remained free from nematodes and most of them remained within category 1 and 2. This result was expected because of the monthly anthelmintic treatment given to each animal in the group. However, 9-18% the goats were in category 3, with the haematocrit levels within normal ranges. According to Solomons and Scott (1994) the normal haematocrit level for goats ranges from 22 to 38%. Between July and November 2002, 10-29% of the goats in Group II were identified as anaemic, and in category 3, and 9% were anaemic in category 4. From December 2002-March 2003, no anaemic goats were observed. There were 19.8% and 9.09% anaemic goats identified by category 4 and 5 during the short rain in April and in May after the rain, respectively (Fig. 6.4). In July 2003, there were no anaemic goats but in August 2003, after a month in the long rainy season, 27% of goats were anaemic in category 3, and 9% in category 5. In September 2003, 18% and 9% of goats were identified as anaemic, category three and four, respectively (Fig. 6.3 and 6.4).

**Table 6.5. Results of the faecal egg count reduction test in the experimental goats in the FAMACHA<sup>®</sup> trial.**

<b>Combined spp. (<i>H. contortus</i> &gt;79%)</b>				
<b>Drench</b>	<b>Pre-Test</b>	<b>Control</b>	<b>ALB</b>	<b>LEV</b>
Number	10	10	10	10
Arith. Mean	810	860	10	10
Var (FEC)	376556	347111	1000	1000
% Reduction			99	99
Var (Reduction)			1.05	1.05
Upper 95% CI			100	100
Lower 95% CI			90	90
Drench effectiveness			Susceptible	Susceptible

**Table 6.6. Results of the faecal egg count reduction test in the experimental sheep used in the FAMACHA<sup>®</sup> trial.**

<b>Combined spp. (<i>H. contortus</i> &gt;79%)</b>				
<b>Drench</b>	<b>Pre-Test</b>	<b>Control</b>	<b>ALB</b>	<b>LEV</b>
Number	13	13	11	10
Arith. Mean	1423	856	9	10
Var (FEC)	523590	361992	909	1000
% Reduction			99	99
Var (Reduction)			1.04	1.04
Upper 95% CI			100	100
Lower 95% CI			91	90
Drench effectiveness			Susceptible	Susceptible

**Table 6.7. Results of the fecal egg count reduction test in the experimental goats used in the FAMACHA<sup>®</sup> trial.**

<b>Combined spp. (<i>H. contortus</i> &gt;79%)</b>				
<b>Drench</b>	<b>Pre-Test</b>	<b>Control</b>	<b>ALB</b>	<b>ALB/LEV</b>
Number	10	10	10	10
Arith. Mean	800	890	10	20
Var (FEC)	206667	221000	1000	1778
% Reduction			99	98
Var (Reduction)			1.03	0.47
Upper 95% CI			100	99
Lower 95% CI			91	90
Drench effectiveness			Susceptible	Susceptible

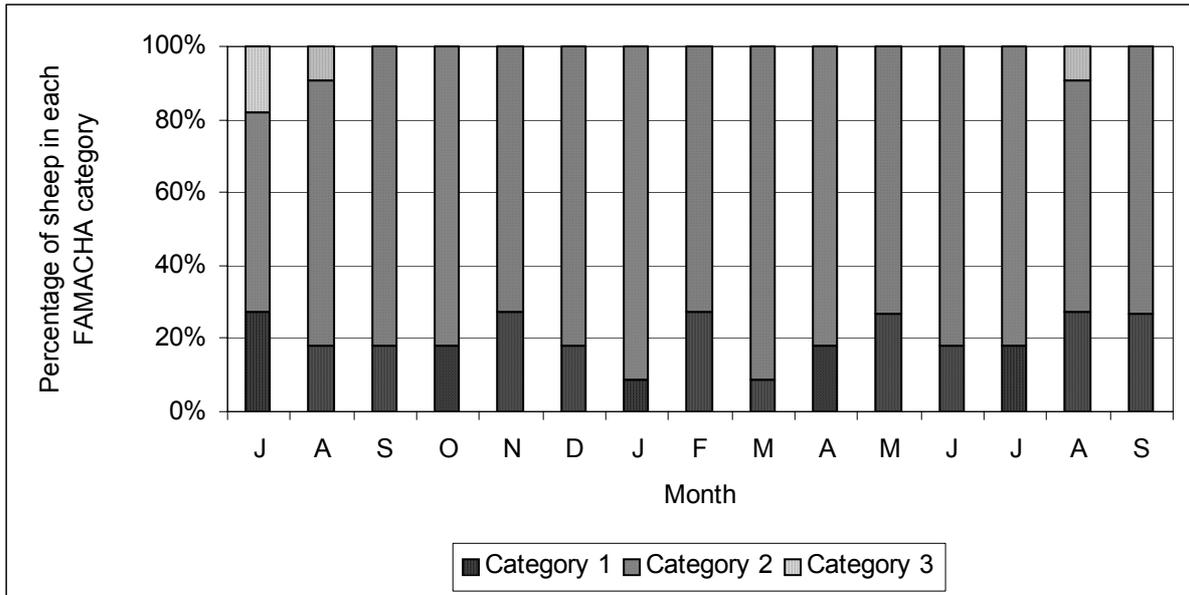


Fig. 6.2. FAMACHA® category scores of goats in the suppressive treatment control group for the seasons July 2002-September 2003.

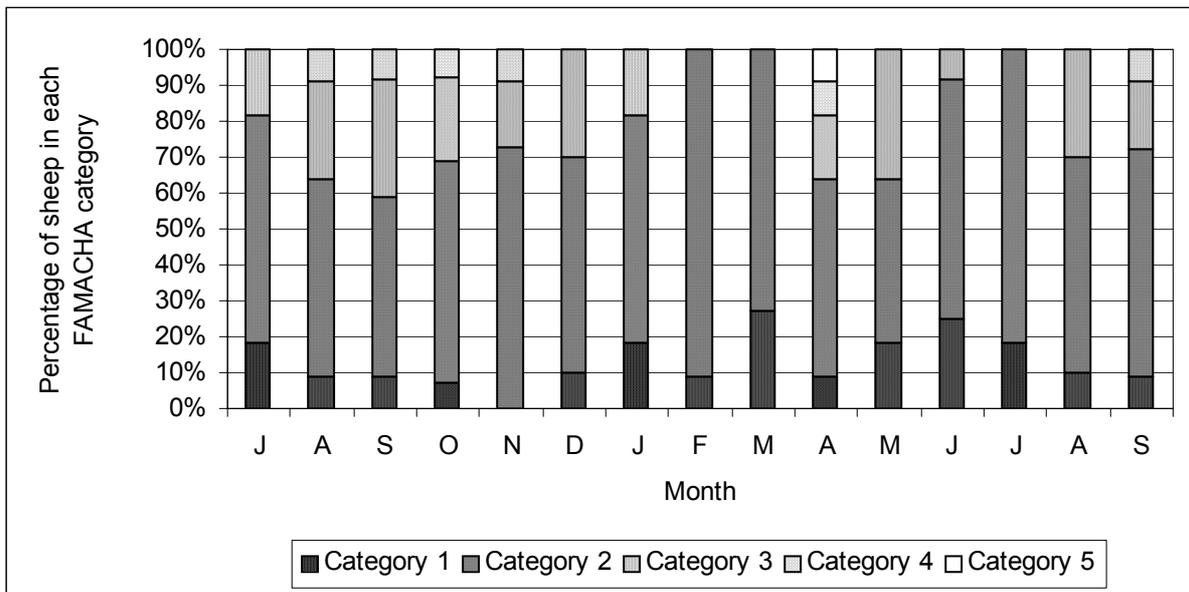


Fig. 6.3. FAMACHA® category scores of goats in the non-treatment control group for the seasons July 2002-September 2003.

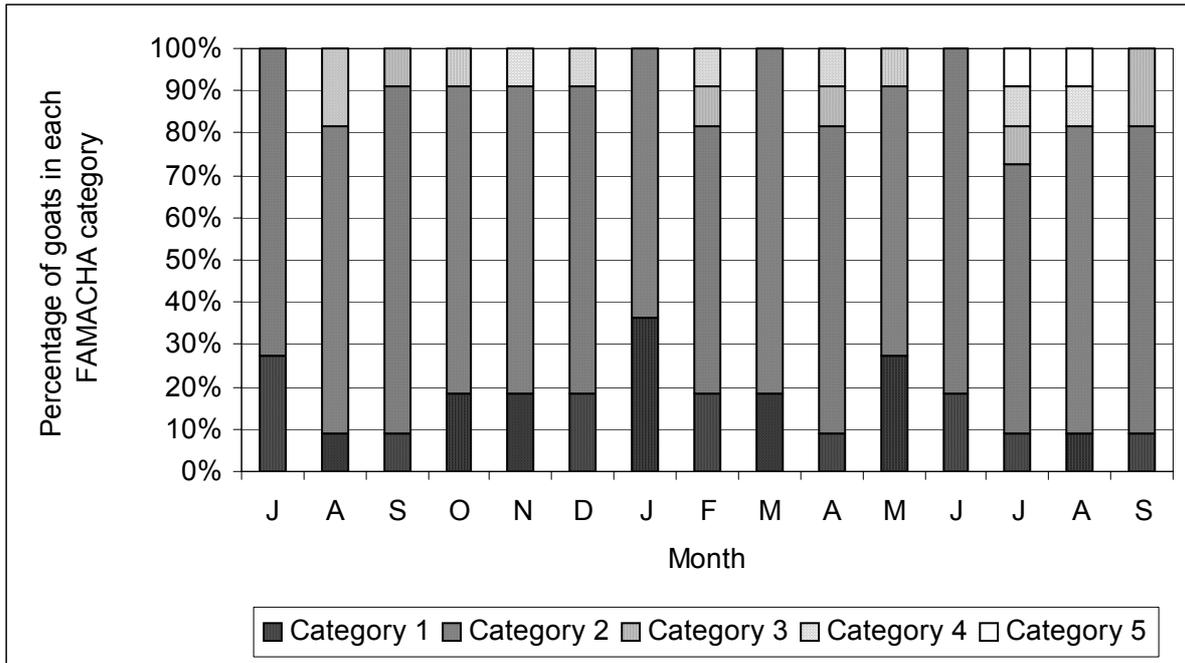


Fig. 6.4. FAMACHA<sup>®</sup> category scores of goats in selective treatment group for seasons July 2002-September 2003.

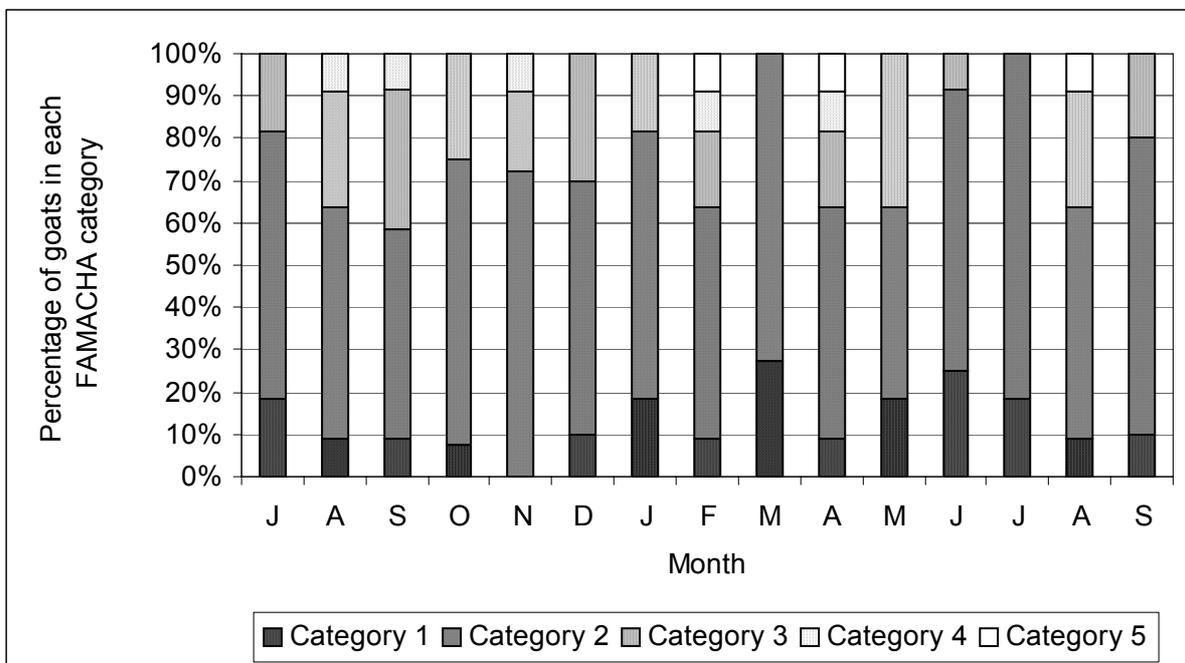


Fig. 6.5. FAMACHA<sup>®</sup> category scores of goats in the single treatment control group for the seasons July 2002-September 2003.

During the dry period in November 2003, no anaemic goats were identified by the FAMACHA<sup>®</sup> system. From July to September there were 9.09%, 9% and 18.2% of goats with FAMACHA<sup>®</sup> category three, four and five, respectively. The percentages of goats in Group 4 that showed clinical anaemia of FAMACHA category 3 was 9-18% and that of category 4 was 9-27%, both categories occurring during the rainy as well as the dry seasons (Fig. 6.5).

#### **6.3.2.2. FAMACHA<sup>®</sup> evaluation in sheep**

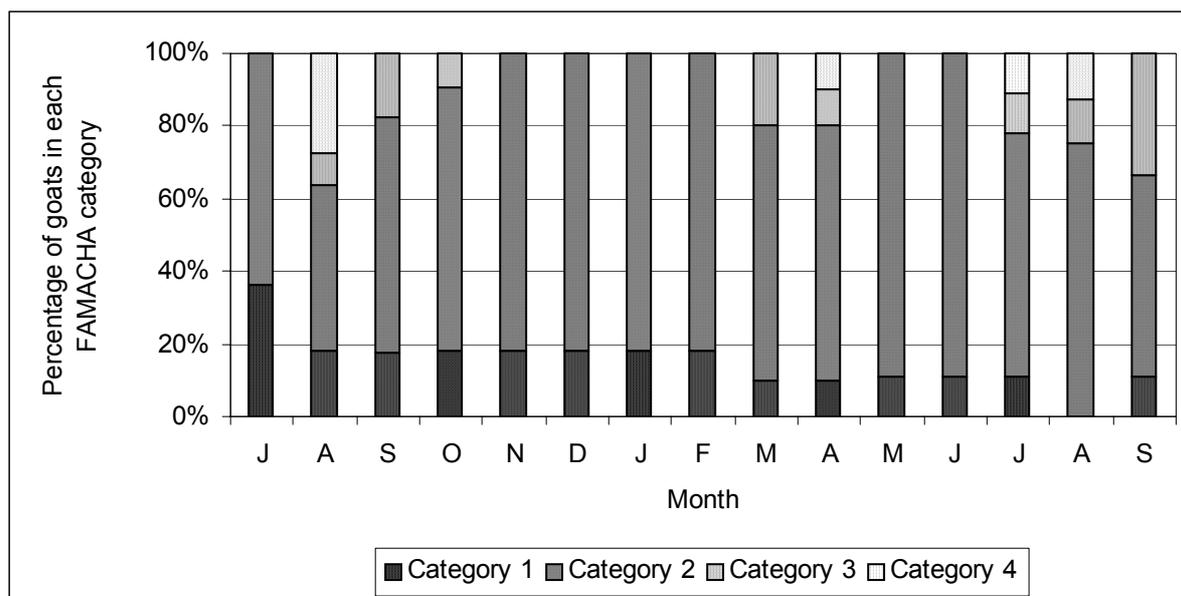
In sheep, the parasites' effect was demonstrated with variable percentages for each treatment group that was identified according to the different levels of FAMACHA<sup>®</sup> categories as shown in Fig. 6.6-6.9.

During most of the study period, the sheep in Group I remained parasite-free. The monthly evaluation indicated that the majority of the sheep were not anaemic. However, out of 11 sheep, two (18%) were identified belonging in category three (Fig. 6.6). The haematocrit of these animals was confirmed to be in the normal range. The normal haematocrit level for sheep ranges from 24 to 45% (Solomons *et al.*, 1994). This result indicates that the FAMACHA<sup>®</sup> scores 3 can not always be recorded as positive for anaemia.

There were no anaemic sheep during the long dry season, whereas during the short and long rainy seasons that followed, there were more sheep from Group II infected with nematodes. This was demonstrated when 9%, 18% and 33% of the sheep could be placed in FAMACHA<sup>®</sup> categories 5, 4 and 3 (Fig. 6.7).

The FAMACHA<sup>®</sup> evaluation of sheep in Group III is illustrated in Fig. 6.8. Thirty-three percent of the sheep in this group were identified as anaemic and in category 3 during the rainy seasons of July-September 2002. A few animals were identified that fell into categories 4 and 5 (Fig 6.7). The percentages of sheep that were identified by the FAMACHA<sup>®</sup> category three, four and five were 33%, 27% and 18%, respectively.

The FAMACHA<sup>®</sup> evaluation of sheep in Group IV is illustrated in Fig. 6.9. The percentage of those sheep which fell in the FAMACHA category three ranged between 9% and 18 %, while in category four between 9 and 27%. Clinical anaemia was recorded during the rain as well as during the dry seasons in sheep in this group (Fig. 6.9).



**Fig. 6.6. FAMACHA<sup>®</sup> category scores of sheep in the selective treatment group for seasons July 2002-September 2003**

During the rainy seasons of 2002 and 2003, the mean haematocrit values decreased, otherwise remain positive for all groups (Fig. 6.10 and Fig. 6.11). The mean faecal egg count of sheep from the suppressive treatment group remained very low throughout the study period and their haematocrit level, even during the worm seasons, remained within the normal ranges of 22-45% (Fig. 6.7). On the other hand, the mean faecal egg count of the selective treatment group remained lower during the dry periods, while it showed high peaks in the long wet period, between both July and September 2002 and 2003. The changes in haematocrit level of sheep in the selective treatment group, during all the wet seasons, were less noticeable than those of the non-treated and the single treatment control groups during the same period (Fig. 6.8-6.9 and Fig. 6.10-6.11).

Sensitivity and specificity tests were carried out to test the FAMACHA<sup>®</sup> clinical evaluations system. The data used to draw up the two-way frequency table included all values of the FAMACHA<sup>®</sup> scores and haematocrit values recorded. The results are compared with two FAMACHA<sup>®</sup> categories and cut-off points of haematocrit less than 19%. The sensitivity of the FAMACHA<sup>®</sup> test to identify animals that fell into categories three, four and five was 72.7% while the specificity 94.9% (Tables 6.12. and 6.13).

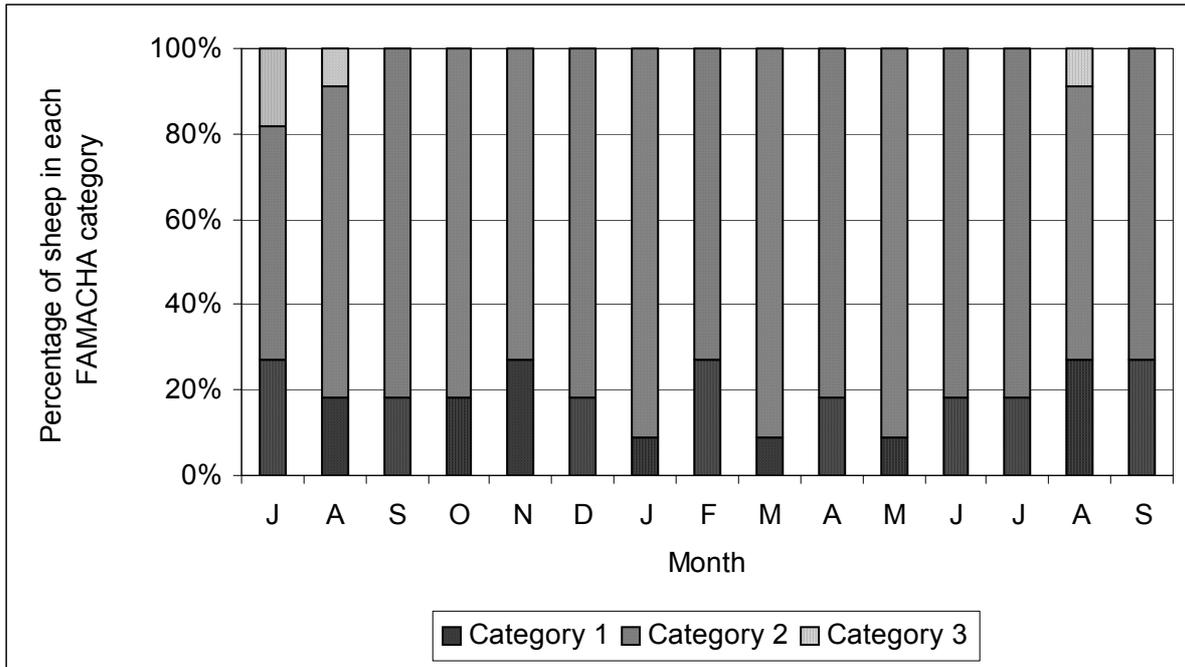


Fig 6.7. FAMACHA<sup>®</sup> category scores of sheep in suppressive treatment group for seasons July 2002-September 2003

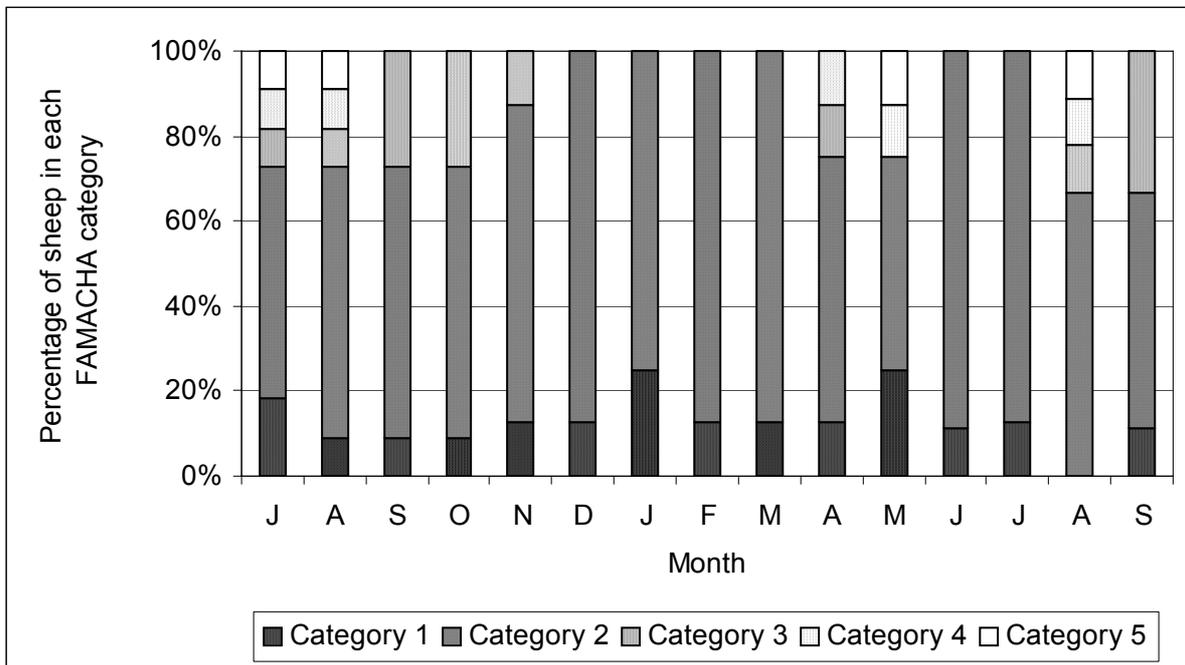


Fig. 6.8. FAMACHA<sup>®</sup> category scores of sheep in non-treatment control group for seasons July 2002-September 2003.

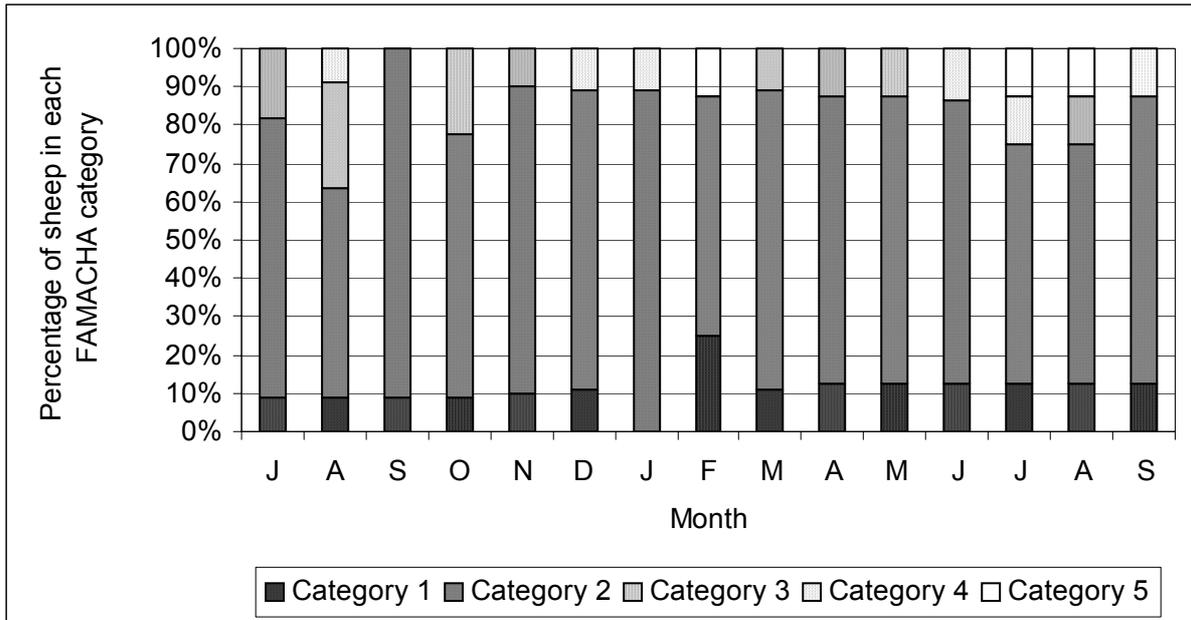


Fig. 6.9. FAMACHA<sup>®</sup> category scores of sheep in a single treatment control group for seasons July 2002-September 2003.

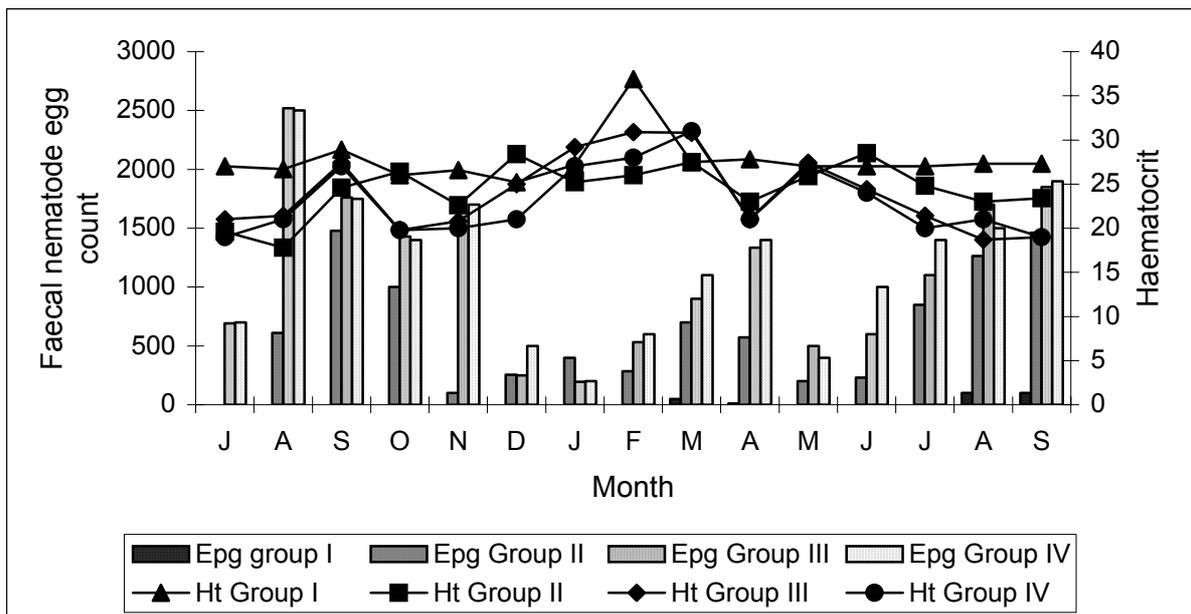
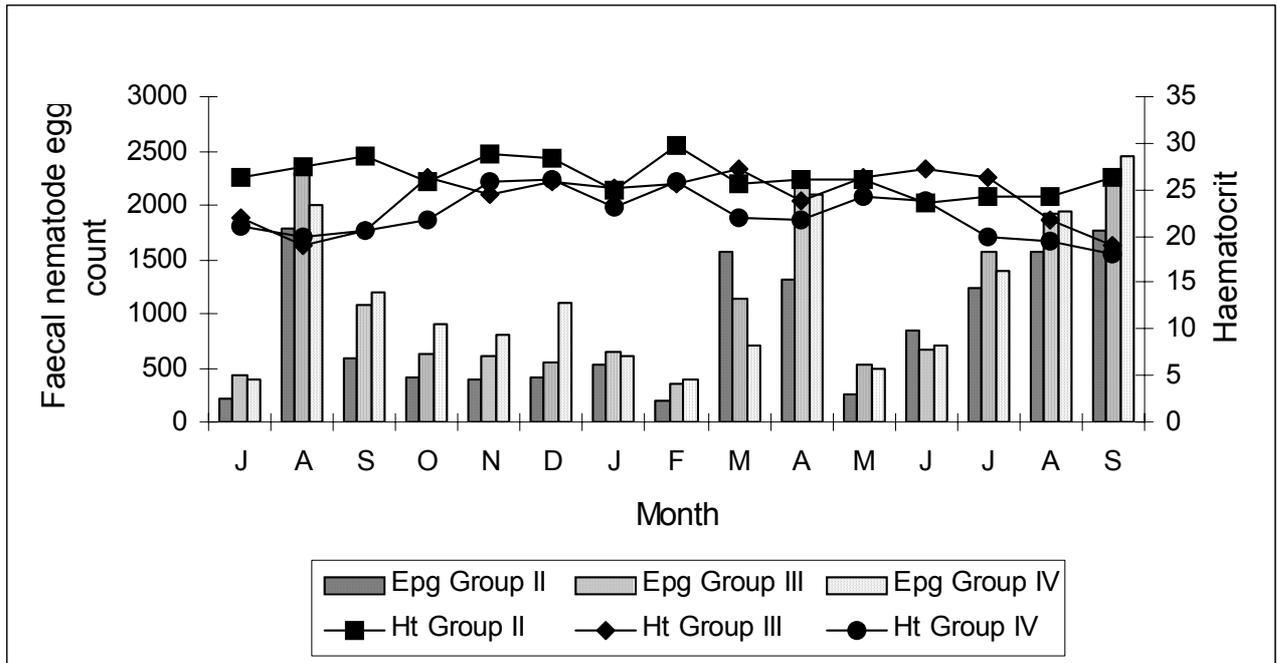


Fig. 6.10. Correlation of epg of goats with in haematocrit levels in Groups I-IV in the FAMACHA<sup>®</sup> trial for seasons from July 2002 to September 2003

**6.3.2.3. Live weight gain in sheep and goats in the FAMACHA<sup>®</sup> trial**

A significant, but variable trend was observed between months for live weight gain during the 15<sup>th</sup> month of trial period in both sheep and goats.



**Fig. 6.11.** Correlation of epg of sheep with haematocrit levels in Groups II-IV in the FAMACHA<sup>®</sup> trial for seasons from July 2002 to September 2003. The egg count for Group 1 was nil.

Comparisons of weight effect and group interaction using repeated measures of the general linear model for analysis of variance showed that the monthly weight gain in sheep between the groups was significantly different ( $P < 0.05$ ) (Tables 6.8 and 6.10). Selective anthelmintic treatment group showed a significantly higher monthly weight gain than the non-treated control group ( $P < 0.05$ ). However, Group I showed a significantly higher monthly weight gain than the other groups ( $P < 0.05$ ).

In goats, comparisons of weight effect and group interaction (Tables 6.9-6.11) showed significant differences ( $P < 0.05$ ) within groups. For most of the months there were no significant differences in weight gain in goats between the different treatment groups. The selective anthelmintic treatment group based on FAMACHA<sup>®</sup> gained more weight when compared to that of the non-treatment control and the single treatment control groups in goats. During the 15 months trial period, with the worm challenge during long rainy season in 2002 and short and long rainy seasons in 2003, there were significant differences in groups' mean live weight (Tables 6.9-6.11) (See Annexure 3).

Table 6.8. ANOVA group comparison of mean live weight gain in sheep in the FAMACHA® trial for seasons July 2002–September 2003.

Weight between months	Group comparison	Difference between means	95% confidence limit		Significance
W4-5	II and I	0.5	- 6.0	7.0	0
	II and III	6.6	0.4	12.8	P<0.005
	II and IV	0.8	- 5.5	7.1	0
	III and I	7.1	0.6	13.6	P<0.005
	III and IV	5.8	- 12.1	0.5	0
	IV and I	1.3	- 5.4	8.0	0
W5-6	II and I	1.8	0.53	3.1	P<0.005
	II and III	0.4	- 0.8	1.6	0
	II and IV	0.8	- 2.0	0.5	0
	III and I	2.2	0.9	3.5	P<0.005
	III and IV	1.2	- 2.4	0.1	0
	IV and I	1.0	- 0.3	2.3	0
W9-10	II and I	0.6	- 1.0	2.2	0
	II and III	1.3	- 0.2	2.8	0
	II and IV	0.4	- 1.2	1.9	0
	III and I	1.9	0.3	3.5	P<0.005
	III and IV	0.9	- 2.5	0.6	0
	IV and I	0.94	- 0.7	2.6	0
W12-13	II and I	0.6	- 9.1	7.9	0
	II and III	8.6	- 16.6	0.6	P<0.005
	II and IV	1.0	- 7.3	9.2	0
	III and I	9.2	- 17.7	0.7	P<0.005
	III and IV	9.6	- 1.3	17.8	P<0.005
	I and IV	0.4	- 8.4	9.1	0
W13-14	II and I	0.2	- 8.6	9.0	0
	II and III	9.9	- 1.6	18.3	P<0.005
	II and IV	0.1	- 8.5	8.6	0
	III and I	10.1	- 1.3	19.0	P<0.005
	III and IV	9.9	- 18.40	1.3	P<0.005
	I and IV	0.2	- 8.8	9.3	0

I suppressive treatment,  
 III untreated control,  
 weight gains between months.

II selective treatment,  
 IV single treatment control, W4-5 to W13-14 -  
 0 not significantly different

**Table 6.9. ANOVA for group comparison of mean live weight gain in goats in the FAMACHA® trial for seasons July 2002–September 2003.**

Weight between months	Group comparison	Difference between means	95% confidence limit		Significance
W4-5	II and I	0.1	- 0.8	1.0	0
	II and III	1.0	0.1	1.9	p<0.05
	II and IV	1.1	0.2	1.9	p<0.05
	III and I	0.9	- 1.8	0.03	p<0.05
	III and IV	0.1	- 0.8	0.9	0
	IV and I	1.0	- 0.1	1.9	p<0.05
W5-6	II and I	2.5	- 6.2	1.2	0
	II and III	1.0	- 4.6	2.6	0
	II and IV	0.9	- 2.7	4.5	0
	III and I	1.6	- 5.3	2.1	0
	III and IV	1.9	- 1.7	5.5	0
	IV and I	3.4	- 7.1	0.3	0
W9-10	II and I	0.11	- 3.5	3.3	0
	II and III	20.9	- 1.6	5.4	0
	II and IV	0.64	- 3.9	3.1	0
	III and I	21.0	- 6.5	1.4	0
	III and IV	20.3	- 1.6	5.4	0
	IV and I	0.8	- 3.7	4.2	0
W12-13	II and I	0.04	- 6.5	6.6	0
	II and III	1.5	- 7.9	4.9	0
	II and IV	3.2	- 3.1	9.6	0
	III and I	2.5	- 4.9	8.1	0
	III and IV	4.7	- 1.7	11.1	0
	IV and I	3.2	- 9.7	3.3	0
W13-14	II and I	- 0.7	- 5.7	4.3	
	II and III	2.7	- 2.1	7.6	p<0.05
	II and IV	0.05	- 4.8	4.9	
	III and I	3.4	- 8.4	1.6	p<0.05
	III and IV	2.7	- 7.5	2.3	
	IV and I	0.05	- 4.9	4.8	p<0.05

I suppressive treatment,  
 III untreated control,

II selective treatment,  
 IV - single treatment control, W4-5 to W13-14 - weight gains between months.  
 0 not significantly different

**Table 6.10. Analysis of variance of mean monthly weight gain by sheep of the four treatment groups<sup>1</sup> in the FMACHA trial during July 2002-2003**

	Group I	Group II	Group III	Group IV	Significance
Mean start weight	15.67	15.8	16.0	16.25	0
Mean end weight	30.6	26.4	19.0	18.34	P<0.05
Weight gained	14.93	10.6	3.0	2.09	P<0.05
Months on pasture	15	15	15	15	0
Average monthly gain	0.995	0.706	0.2	0.14	P<0.05

0 not significantly different

**Table 6.11. Analysis of variance of mean monthly weight gain by goats of the four treatment groups in the FMACHA trial during July 2002-2003**

	Group I	Group II	Group III	Group IV	Significance
Mean start weight	15.40	15.80	16.00	16.25	0
Mean end weight	31.98	26.12	22.31	18.7	P<0.05
Weight gain monthly	16.58	10.32	2.31	1.75	P<0.05
Months on pasture	15	15	15	15	0
Average monthly gain	1.105	0.689	0.154	0.117	P<0.05

0 not significantly different

In both sheep and goats, a steady but fluctuating positive increase in body weight was observed in the suppressive treatment group with no mortality, while a positive body weight increase was observed in the selective treatment group with two losses by death. At the beginning and in the middle of the study, two sheep and two goats died from Group III and a total of five animals died from Group IV. During necropsy worms were collected and identified from four sheep and five goats. The mean nematode worm burden that was observed from both the dead sheep was *H. contortus* (38), *T. colubriformis* (110), *T. ovis* (15) and one animal from each species, i.e two out of nine animals were found with *M. expansa*. No specific cause was found for their death during post-mortem and laboratory examinations.

**Table 6.12. Comparison of sensitivity, specificity and predictive values for positive and negative tests of sheep using FAMACHA<sup>®</sup> scores and haematocrit cut-off for positive test results and anaemia (See Annexure 4).**

FAMACHA <sup>®</sup> categories	Ht<19% n	Ht>19% n	Sensitivity	Specificity	PV* (-ve)	PV# (+ve)
3,4,5	30	74	90.9	86.6	99.3	28.9
1,2	3	476				
Total	33	550				
4,5	24	44	72.7	92.2	98.3	35.3
1,2,3	9	522				
Total	33	550				

n number of observations, Ht<19% anaemia present,  
 Ht>19% anaemia absent \* Predictive value positive,  
 # Predictive value negative.

**Table 6.13. Comparison of sensitivity, specificity and predictive values for positive and negative tests of goats using FAMACHA<sup>®</sup> scores and haematocrit cut-off for positive test results and anaemia (See Annexure 4).**

FAMACHA <sup>®</sup> categories	Ht<19% n	Ht>19% n	Sensitivity	Specificity	PV* (-ve)	PV# (+ve)
3,4,5	43	114	93.5	81.9	94.4	27.4
1,2	3	514				
Total	46	628				
4,5	35	44	76.1	92.9	98.2	44.3
1,2,3	11	584				
Total	46	628				

N number of observations, Ht<19% anaemia present,  
 Ht>19% anaemia absent, \* Predictive value positive,  
 # Predictive value negative.

#### 6.3.2.4. Body Condition

Body condition was not significantly different between both sheep and goats within each group. However, there was a significant difference in body condition between the treated and non-treated control groups ( $P < 0.001$ ). Marked difference in body condition was observed during the dry period when poor nutritional status due to scarcity of green pasture and leaves exacerbated the situation.

## 6.4. DISCUSSION

The mean nematode egg counts from sheep and goats in the non-treatment control (Group III) and treated control with single treatment (Group IV) fluctuate as seasons change. The nematode egg counts in both sheep and goats from the selective treatment groups also showed similar trends. The high egg counts in both sheep and goats in the control groups

demonstrate the increase of egg counts in the absence of anthelmintic treatments. Whereas, the egg counts in the selective treatment and suppressive treatment groups remained low throughout the study period. The high number of adult worms recovered from tracer animals which grazed along Group III and Group IV animals for the period July-September 2002 and 2003 and Mar-April 2003 was due to the increase of third stage infective larvae ( $L_3$ ) on pasture as a result of the onset of the rains and the release of larvae from eggs deposited by non-treated sheep and goats.

The worm counts from individual tracer lambs which grazed on the four separate paddocks ranging from 0-90 in Group I, 0-219 in Group II, 0-608 in Group III and 35-705 in Group IV (Table 6.4) during the entire study period demonstrates the clinical significance of the worm burdens in each the FAMACHA<sup>®</sup> trial group. The number of each species recovered from season to season also varied, but *H. contortus* dominated.

The high faecal egg counts during the worm seasons and *H. contortus* worm burdens found in the tracer lambs accounted for the decline in the haematocrit levels in the non-treatment and single treatment control groups both in sheep and goats (Fig. 6.4, 6.5, 6.8 and 6.9). The overall mean haematocrit values of both sheep and goats in the selective and a single treatment control groups remain within the normal ranges except during the rainy seasons (Fig. 6.10 and 6.11). In order to rule out the impact of other possible causes of anaemia, such as the impact of tick infestation, tick borne diseases, trypanosomosis, viral and bacterial infections, measures were taken including regular application of acaricide, vaccination against sheep pox, anthrax and ovine pasteurellosis were given. No vector-borne haemoparasite was found from the blood smears that were examined several times during the study period. There was no malnutrition problem as the animals were supplemented with hay, vitamins and mineral licks containing magnesium and copper sulphates, molasses and salt. Therefore, in this study, the most important possible cause of anaemia in the experimental sheep and goats was *H. contortus*. The lower Ht was reflected by the clinical anaemia, which was observed in sheep and goats that were identified with FAMACHA<sup>®</sup> categories 3, 4, and 5. The record of more anaemic animals was evident during the wet seasons than the dry seasons. Similar results were obtained in a study conducted in goats (Vatta *et al.*, 2001).

Variation between right and left eyes and variation between congestion and anaemia were noticed during the time of FAMACHA<sup>®</sup> clinical evaluation. To minimize differences the principal investigator always scored each animal for level of clinical anaemia applying the

categories on the FAMACHA<sup>®</sup> card strictly. Doubtful cases were usually re-examined and the final score was recorded.

The sensitivity and specificity of the FAMACHA<sup>®</sup> method was tested. The cut off points for the range of haematocrit levels for sheep and goats were determined to be <19% for the presence of anaemia and >19% for the absence of anaemia. The predetermined haematocrit values for sheep and goats in the FAMACHA<sup>®</sup> categories 3, 4 and 5 were the haematocrit values less than 18% for sheep and less than or equal to 18% for goats.

The sensitivity of the FAMACHA<sup>®</sup> system to identify sheep that were anaemic (with haematocrit value less than 19%) falling into the FAMACHA<sup>®</sup> categories at 4, 5 was good showing 72.7%. The specificity of the test was 94.9% (Tables 6.12 and 6.13). The sensitivity of the test rather increased from 72.7 to 90.9% when categories 3, 4, 5 were considered as anaemic, although the specificity showed a slight decrease from 94.9 to 86.6 %. Similarly, using the cut-off point of 19% in goats, where categories 4 and 5 considered anaemic, the sensitivity and specificity for the test was 76.1 and 92.9% respectively. These values changed to 93.5% and 81.9% when the FAMACHA<sup>®</sup> categories 3, 4, and 5 were considered positives. To adopt a selective anthelmintic treatment regimen of worm control, a higher sensitivity would be much preferable for it will leave no chance of missing anaemic plus potentially anaemic animals.

The results obtained in this study indicate that the FAMACHA<sup>®</sup> system could correctly identify more than 72% of sheep and goats in *H. contortus* problem areas that actually need treatment during the rainy seasons. The percentage of sheep scored for clinical anaemia decreased in each of the FAMACHA<sup>®</sup> categories 3, 4, 5, with the corresponding scores of haematocrit values (<18%) immediately or after the worm season.

The percentages of goats that needed anthelmintic treatment in the selective anthelmintic treatment group was 9% in the long dry period, and 10% and 27% during the short and long rainy seasons, respectively. An even higher percentage of anaemic goats in the control group (20-45%) indicated that anthelmintic treatment was necessary (Fig. 6.2 and 6.3).

The FAMACHA<sup>®</sup> score of 2 was the most frequent category observed in sheep and goats. These results were similar to those reported by Van Wyk *et al.* (2001) and Sotomaior *et al.* (2003) who found more animals in FAMACHA<sup>®</sup> score 2 than other categories. The proportion of sheep in the selective treatment group that needed anthelmintic treatment was between 9% and 33% during the rainy seasons, which was higher than in the goats. A larger

percentage of sheep from the non-treatment control group fell in the FAMACHA<sup>®</sup> category 4 and 5 and anthelmintic treatment was indicated (Fig. 6.5).

The monthly FAMACHA<sup>®</sup> evaluation was sufficient and safe, since all animals in every group were followed up and were inspected weekly for clinical signs such as bottle jaw and diarrhoea. However, in the wet seasons when the *Haemonchus* challenge often was expected to be high, more FAMACHA<sup>®</sup> evaluation was necessary. This was important to detect clinical anaemia that might have occurred due to acute haemonchiasis and to carry out intervention with anthelmintic treatments. Since the number of sheep and goats at a peasant farmer's household ranges between 5-80, it may be possible for a trained farmer to carry out the FAMACHA<sup>®</sup> about two or three times monthly.

In the present study, significant differences in live weight gain were observed in the treated and non-treated control groups. The selective treatment group, during the short and long rainy seasons of 2002 and 2003, showed a significant increase in live weight, when compared to the non-treatment control and single treatment control groups. The performance of the non-treatment control group was most affected by infections during the wet seasons and the continuation of infection into the dry months might have contributed to the loss of live weight and mortality of animals that probably resulted from the chronic effect of blood sucking parasites. There were 4 (16.5%) deaths in the non-treatment control group while 5 (25%) died in the single treatment control group. The worm burden that involved *H. contortus* and *T. colubriformis* was not sufficiently large to cause direct death as in the case of acute or sub-acute haemonchosis. *Haemonchus contortus* continuously sucks the hosts' blood and through time the gross effect contribute to their death. Reinecke (1983) states that only a few *Haemonchus* adults (100-1000) cause seepage of blood into the abomasum, possibly as little as 5-50 ml per day, but the process continues for months (40-100 days). Compensatory erythropoiesis takes place, but this depletes serum iron to levels at which it is impossible to maintain erythropoiesis, i.e. to less than 40 µg%. Iron and protein for erythropoiesis are finally exhausted and a marked anaemia precedes death (Reinecke, 1983). Although there is little information available comparing average monthly weight gain of sheep and goats under selective treatment in semi-arid environments, the results in this study indicate that selective anthelmintic treatment of sheep and goats could decrease the heavy parasite burden to some extent during the worm season as seen in the FAMACHA<sup>®</sup> scores in this study and studies conducted in South Africa and USA (Van Wyk *et al.*, 1999; Vatta *et al.*, 2001, 2002; Kaplan *et al.*, 2004).

In Ethiopia, about 14-16% of the sheep and 11-13% of the goat populations out of the national resource have been exposed to many important small ruminant diseases including helminthoses (Anon, 1999). Farmers who have access to veterinary clinics get treatment for their animals with anthelmintics after laboratory examination. The majority however, treat their animals with available drugs during numerous occasions.

Anthelmintic resistance develops as a result of several factors such as under dosing, over dosing, high drenching frequency, drench and move strategies. Resistance to any anthelmintic eventually occur as long as the anthelmintic continues to be applied on parasite population. Van Wyk (2001) urges that refugia to be considered above all when worm management in domestic animals is planned. Van Wyk (2001), suggested that refugia plays a much more important role in the selection of anthelmintic resistance than other phenomena such as reduced drenching frequency and avoiding under dosing. The term refugia itself, refers to the proportion of the parasite population that is not exposed to a given control measure, thus escaping from resistance.

Michel (1985) stated that selection pressure may be measured as a proportion of each generation that is the progeny of worms that have survived exposure to anthelmintic resistance. The main challenge here is to develop ways for overcoming the practical problems concerned with excessive build-up of worms on pasture, when the above mentioned techniques can not be resorted to without losing sustainability. In other words, while parasite burdens in animals treated with effective drug are reduced to practically nil, the untreated sheep and goats continue to propagate the progeny of parasites that do not come into contact with the drug and thus they are in refugia and unselected. In this way the balance is continually tipped in favour of the susceptible, compared to the resistant parasite (Van Wyk, 2001). Because of these advantages, selective anthelmintic treatment of infected individual animals using the FAMACHA<sup>®</sup> system may have the possibility to offer a sustainable approach of worm and anthelmintic resistance control simultaneously, particularly in regions like Ethiopia, where resistance has not yet reached many areas or not escalating and the farming system is small scale farms with available labour. Therefore, FAMACHA<sup>®</sup> as a tool for selective anthelmintic treatment against blood sucking helminth parasites is likely to be acceptable by peasant farmers as far as farmers animal health representatives get proper training on its use. Using the FAMACHA<sup>®</sup> method, would insure more animals receive at least 2 or 3 treatments annually that reduce morbidity and mortality and thereby maintain animal production. In the questionnaire survey, it was found that more than 65% of the respondents drench their animals with anthelmintics once or twice per year. Most of them drench their animals by selecting those animals showing clinical signs. This

may not be possible in commercial sheep farms where some animals required up to 8 salvage treatments (Sotomaior *et al.*, 2003). For example, the maximum experienced in South Africa, when a highly effective drug employed under similar conditions of severe *Haemonchus* challenge was four treatments (Malan, Van Wyk and Wessels, 2001).

When the selective treatment group was compared with the non-treatment control group in the present study, there was a significant difference in body weight gain ( $P < 0.05$ ). Peasant farmers and pastoralists could use the FAMACHA<sup>®</sup> system more successfully to treat animals that are unable to cope with worm challenges, and for keeping animals from severe production loss and mortality.

Selective anthelmintic treatment requires reliable means to differentiate between those animals, which, if left untreated, would be at risk, or develop severe helminthosis and possibly die and those, which would be in no immediate danger. There are several ways that are generally acceptable for this purpose. However, the most commonly known is the haematocrit measurement which provides the most accurate indication of the severity of anaemia. Although this is accurate and reliable it are also very expensive, time consuming and requires high or medium level trained personnel and specialized equipment. FAMACHA<sup>®</sup> has been validated to be a quick, simple and cheap alternative to the earlier methods. FAMACHA<sup>®</sup> has become attractive as a guide on which to base selective anthelmintic treatment for sheep and goats. From this experimental study, recommendation could be made to further study the FAMACHA<sup>®</sup> application for use on peasant sheep and goat farms. If potential improvements on the productivity of small ruminants could be achieved, the question which often arises whether the application of the FAMACHA<sup>®</sup> system brings about a better methods to control nematodes, particularly *Haemonchus* will get a better reply.

When the selective treatment group was compared with the non-treatment control group in the present study, there was a significant difference in body weight gain ( $P < 0.05$ ). Peasant farmers and pastoralists could use the FAMACHA<sup>®</sup> system more successfully to treat or cull animals that are unable to cope with worm challenges or animals which become low responders.

The heritability of FAMACHA<sup>®</sup> scores during severe *Haemonchus* challenge was relatively good in South African trials. Continued use of the FAMACHA<sup>®</sup> system will improve the resilience/resistance of the flock to worm infection progressively leading to a reduced risk of deaths and production losses. Particularly testing, selecting and culling the replacement

animals should lead to less of a worm challenge. In general, the FAMACHA<sup>®</sup> method can be an important tool in integrated helminths control, since it is useful in the reduction of the number of anthelmintic treatments, it decreases selection pressure of resistance strains and the maintenance of the refugia population with susceptible strains.

## **Chapter 7.**

# **Worm control practices and anthelmintic resistance**

### **7.1. INTRODUCTION**

The control of gastro-intestinal parasites of small ruminants is essential to improve their productivity. Effective treatment programmes are those that are based on the epidemiology of the parasites. Improper timing of anthelmintic treatment and the wrong choice of anthelmintics are not only ineffective in the control of the parasites, but also costly and potentially harmful in selecting for anthelmintic resistance (Michel *et al.*, 1981; Maingi *et al.*, 1996a; Waller, 2004).

Questionnaire surveys to determine the control practices used to reduce gastro-intestinal parasites in sheep and goats in various countries have been undertaken, with the aim to improve control programmes. At the present time worm control strategies involving anthelmintics are under serious threat because of the development of anthelmintic resistance. Thus, anthelmintic resistance has become a global problem.

The association between anthelmintic resistance and the risk factors in the management which potentially contribute to its development has been investigated on sheep farms in several countries, including New Zealand (Kettle *et al.*, 1981, 1982) and Australia (Riffkin *et al.*, 1984; Edwards *et al.*, 1986) and in goats in New Zealand (Kettle *et al.*, 1983), and Kenya (Maingi *et al.*, 1996b).

In Ethiopia, there is no officially recognized or established worm control programme. No recorded information is therefore available on the worm control practices in sheep and goats. This survey was carried out to obtain information from peasant goat and sheep farmers, and from all levels of the animal health workers at sub-district, zonal and regional veterinary clinics in North and East Shewa zones. Information from this study could indicate whether there were any worm control practices among farmers and animal health workers that could promote the development of anthelmintic resistance.

### **7.2. MATERIALS AND METHODS**

The survey information was obtained from the peasant farmers and animal health workers by means of a questionnaire, which was completed for each respondent during the course of

the interview. The peasant farmer is hereafter referred to as the farmer. The details on methods of sampling are given below.

### **7.2.1. Selection of respondents.**

#### **7.2.1.1. Selection of farmers**

The survey was undertaken in North and East Shewa zones (Fig. 3.1). The first 50 farmers were surveyed in East Shewa zone in the semi-arid area of the Great Rift Valley, where the rainfall is between 600 and 1 000 mm per annum. A further 50 farmers were surveyed in the North Shewa zone in the highland area. This zone often receives high rainfall, up to 2 000 mm and lies in the central highland area of the country. Apart from topographical differences between the two the zones the basic administrative structure of the peasant farmers sector is similar. For many years the distribution of anthelmintics and the provision of veterinary services was the responsibility of the Ministry of Agriculture.

Farmers were selected randomly from the lists of peasant farmer villages after criteria such as closeness to the main road, the presence of sheep and goats and the availability of all-weather roads, had been considered. The lists of villages were obtained from the sub-districts' Bureau of Agriculture.

If a selected smallholder owned less than 15 sheep or goats, a replacement farm was selected. The randomly selected smallholders were fully informed about the study project and its objectives. Should a farmer be unwilling to participate in the survey, a replacement farmer was selected.

Information concerning the management of helminth infections in sheep and goats, and the use of anthelmintics, was obtained during a personal interview using a set questionnaire. A total of 100 completed questionnaires were obtained from both zones. The questionnaire consisted of two main parts. The first part dealt with data concerning the farm data, which aimed at obtaining information on the husbandry practices for sheep and goats. The small holders were asked to report truthfully on the type of grazing used, the number of animals of each species, the use of pasture and feed resources.

The role of the smallholder in dealing with animal health problems, particularly worm control as well as the experiences with animal diseases, with emphasis on worm problems in the area, were required. The second part focused on the use of anthelmintics and was aimed at getting reliable information on the kind of anthelmintics used, the source of the anthelmintic,

the dosage rate and the frequency of dosing. Where farmers received diagnostic and treatment support, questions pertaining to the nature and frequencies of such activities were asked. Farmers were encouraged to provide details of any ethno-veterinary medicines or practices that were used in the area (Annexure 2).

#### **7.2.1.2. Selection of animal health workers.**

The sampling units from the animal health workers included all those serving at various levels of the government animal health stations, veterinary clinics and laboratories and private practitioners in the study areas. The “animal health workers” are defined as veterinarians, assistant veterinarians, and animal health technicians. The survey information was obtained from 64 animal health workers, from East and North Shewa zones by means of a questionnaire, which was completed individually (Annexure 2).

The questionnaires dealt with anthelmintics and were aimed at obtaining information such as the source, distribution to peasant farmers, usage, quantity of dosage, frequencies of dosage, worm control plan, and outlooks on anthelmintic resistance in the veterinary practice.

#### **7.2.1.3. Data analysis**

Data from this study was analysed using SAS (2003). Frequencies for the various responses were generated using Proc FREQ (SAS) and cross-tabulation between related responses. Differences between proportions were analysed statistically using a Chi-square test, a value of  $P < 0.05$  being considered significant.

### **7.3. RESULTS**

East and North Shewa zones are similar in many aspects as is evident from the peasant association structures, smallholders perceptions of livestock management and the distribution of anthelmintics. Information obtained from farmers and animal health workers is presented below.

#### **7.3.1. Farmers perceptions**

In Table 7.1 the number of farmers' villages visited and farmers questioned in East and North Shewa are indicated. The number of sheep and goats per farm is listed in Tables 7.2 and 7.3. Those farms with few sheep usually have more goats, therefore the average number of sheep and goats per household was 15. The majority of farms in North Shewa have a large

ratio of sheep to other species of livestock, whereas in East Shewa the majority has large ratio of goats to other species.

**Table 7.1. Randomly selected farmers' villages and number of farmers participated in for the questionnaire survey.**

Farmers villages	Number of farmers villages	Number of farmers	Percentage of farmers
<b>East Shewa</b>			
Adama	4	10	10
Shashemene	4	7	7
Arsi Negele	2	5	5
Boset	3	5	5
Dugdabura	2	5	5
Lume	2	5	5
Dukem	2	5	5
Debre Zeit	1	5	5
<b>North Shewa</b>			
Basso	6	25	25
Sululta	4	12	12
Kimbibit	4	16	16
<b>Total</b>	<b>34</b>	<b>100</b>	<b>100</b>

**Table 7.2. Distribution of the 100 farmers in the survey categorised with respect to the mean number of sheep per household.**

Category by mean number of sheep	Range	Percentage of farmers (%)
1-6	1-13	26
7-11	7-21	20
12-16	12-26	19
17-20	17-65	24
20+	20-87	11
		<b>Total</b> 100

**Table 7.3. Distribution of the 100 farmers in the survey categorised with respect to mean number of goats per household.**

Category by mean number of goats	Range	Percentage of farmers (%)
1-6	1-60	20
7-11	1-40	26
12-20+	1-87	19
17-19	17-10	25
20+	20-35	10
		<b>Total</b> 100

The majority of the farmers (96%) use permanent communal pasture. About 12% of the farmers let sheep and goats graze in lowland (moorland) which is also communal. Ninety percent of the farmers let their sheep and goats graze together with cattle, horses and donkeys. The condition of the communal pasture is rated as poor by 89% of the farmers. Hay (64%), forage as “cut and carry” feeding (25%) and crop residues (11%) are given as supplementary feed to the sheep and goats in the survey areas. Only 2% of the farmers indicated that they keep sheep and goats in the back-yard or that they do not let sheep and goats onto the communal grazing area. Thirty-five percent of the farmers indicated that the communal pasture area is situated at approximately 2-3 km from the residential area. Sixteen percent could not exactly tell how far the communal pasture area is from their houses. Eight percent said the communal grazing area is about 5 km from the residential area. The majority of respondents, however, indicated that sometimes during the dry seasons sheep and goat were driven more than 5-10 km usually to, what they refer to as, lowland or Mooreland grazing areas. In general, the majority of farms use ponds and rivers for their livestock’s water requirements.

Table 7.4 illustrates the proportion of farmers encountered with animal health problems. The majority (81%) acknowledge that worms are important animal health constraints in small ruminants followed by infectious diseases caused by viruses and bacteria (19%). When asked which specific worms they know to cause more problems, 52% replied that roundworms of the lung and stomach are important, while 41% said that all worms are important. In North Shewa, farmers (99%) indicated that liver fluke and lungworms are important followed by *Haemonchus* and *Trichostrongylus*. In East Shewa, *Haemonchus* is very important followed by *Trichostrongylus* and *Oesophagostomum*. Only 7% indicated that the most important parasitic disease of sheep and goats, as well as cattle, is liver fluke. Questions that are asked on the kinds of symptoms that were observed, were answered by the majority (58%) that the wet season is the most important period. A high percentage of farmers (between 50-70%) recognized symptoms that may have been directly or indirectly caused by parasitic diseases such as diarrhoea, coughing, emaciation, bottle jaw and general weakness (Table 7.5).

Table 7.6 shows the frequency of anthelmintic treatments carried out by peasant farmers. Fifty three percent of the farms treat sheep and goats with anthelmintics when they observe symptoms of illness. However, 27%, 12% and 5% of the farms treat their animals 2, 4, and more than 4 times during the small and long rains and during the dry periods respectively. Three percent of the respondents indicated that they do not treat their animals at all (Table 7.6). The proportion of farms where sheep and goats were treated once a year based on

symptoms of illness were significantly higher than single, double or quarterly treatments given ( $P < 0.05$ ).

**Table 7.4. The percentage of farmers encountered with animal health problems**

Source of problems	Percentage (n = 100)
<b>Infectious diseases</b>	19
Worm problems	81
<b>Known worm problems</b>	
Liver fluke	7
Roundworms	52
Roundworms, liverflukes and tapeworms	41

**Table 7.5. Percentage of farmers with regard to their response to the animal health conditions during different seasons and type of symptoms recognized.**

Criteria	Percentage of farmers (n = 100)		
	Often	Sometimes	Rarely
<b>Different seasons</b>			
Wet season	58	39	3
Dry season	18	30	52
All seasons	33	30	37
<b>Symptoms of illness</b>			
Diarrhoea	63	33	4
Emaciation	58	31	11
Bottle jaw	71	26	3
General weakness	64	14	22

**Table 7.6. The percentage of farmers where sheep and goats were treated with anthelmintics on various occasions.**

Treatment (drenching) practice	Percentage of farmers (n = 100)	
	Most commonly	Commonly
Treat once during wet season	18	11
Treat twice during wet season	5	7
Treat quarterly	5	0
Treat only when sick	29	20
No treatment at all	0	5

About 50% of the respondents replied “Yes” when asked if they knew how to correctly give the right anthelmintic dose to a sheep or goat. The weight of the animal is visually estimated

and the volume of anthelmintic to be given is based on this estimate. Fifty-eight percent of the respondents also indicated that they were able to tell when there were problems with the efficacy of anthelmintics and more than 90% of farms stated that there have been problems. Forty-five percent of respondents knew the practice of herbal medicine for both humans and animals in their areas.

The proportion of farmers using the various sources of anthelmintics is listed in Table 7.7. The majority of farmers (67%) purchase anthelmintics from the open market and consider it as a very important source. Because of a lack of information, 61% of the farmers also indicated that the purchase of anthelmintics from other farmers (drug vendors) was their next important source. Although 28% of the farmers replied that the veterinary clinics of the Ministry of Agriculture were their source of drugs, they commented that drugs from the Government's veterinary services were rarely available. Most respondents (88%) purchased anthelmintics, either because they know it or have heard about it from other farmers. However, 90% of the farmers relied on drugs which were recommended by veterinarians and other animal health workers (Table 7.7).

Farmers were asked to name the anthelmintics they currently use or the ones used for the last four or more years and Nilzan (oxyclozanide and levamisole), Wormex (albendazole), Pamizol super (tetramisole and oxyclozanide), Valbazen (albendazole), Panacur (fenbendazole) and Flukiver (closantel) were the most popular.

**Table 7.7. Major sources and criteria on selecting anthelmintics.**

Drug supply	Percentage of farmers (n=100)		
	Very important	Important	Less important
<b>Source</b>			
MoA <sup>1</sup> veterinary clinics	28	7	65
Open markets (gulit)	67	0	33
Private veterinary clinics	29	22	49
From other farmers	61	4	35
<b>Criteria for selection</b>			
Colour of drugs	6	25	69
History of drug efficacy	88	8	4
Prompt by other farmers	71	25	4
Prescription by veterinarian	90	10	0

n number of respondents

1

Ministry of Agriculture

Table 7.8 Indicates the percentage of farmers whose source of information about worms and worm control practices are ranked 1 (highest) to 5 (lowest) in terms of their importance.

Information from the zonal Bureau of Agriculture was ranked as the most important source of information (65%) followed by a number of other sources. The general comments by the peasant farmers indicate a big concern over the poor efficacy of drugs.

**Table 7.8. Sources of knowledge on anthelmintics and worm control practices.**

Source of knowledge	Percentage of farmers (n=100)		
	Very important (Rank 1 and 2)	Important (Rank 3 and 4)	Less important (Rank 5)
Farmers association	12	56	32
Radio and TV	20	45	35
MOA <sup>1</sup> extension	7	12	81
Zonal veterinary clinics	65	26	9
Private veterinary clinics	21	36	43
Rural drug vendors	4	15	81
Traditional healers	0	16	84

n number of respondents

<sup>1</sup>

Ministry of Agriculture

### 7.3.2. Animal health workers perceptions

Most of the 64 animal health workers that responded to the questionnaire are government employees but some are private practitioners in East and North Shewa.

Table 7.9 lists the various occasions for animal health workers to deworm peasant farmers' sheep and goats. Fifty-three percent of the animal health workers drench sheep and goats only after faecal examination for worm eggs and examine for nematodes, trematodes and cestodes. Anthelmintic treatments are often given by 75% of the animal health workers when seasons change.

Sixty-seven percent of the animal health workers replied positively to the question whether peasant farmers tend to select or show their preference for anthelmintics, and are summarized in Table 7.10.

Out of 64 animal health workers, 33 (51.6%) responded that anthelmintics are usually at highest demand or most needed during the period June to September (long rainy season) while 30 (46.7%) said during the period October to December (dry season).

Fifty-two (81.3%) animal health workers stated that peasant farmers purchase anthelmintics from government veterinary clinics to drench sheep and goats by themselves. Farmers prefer a bolus as the choice formulation (Table 7.10). As regards the possibility of farmers to underdose their animals, 57 (89%) of animal health workers said this could happen

knowingly or unknowingly. The percentage of animal health workers that weigh the animals, and the dosing regimen, is summarized in Table 7.11. The percentage of animal health workers that dose according to individual weights of the sheep or goats were 12.5%, whereas 67.7% the average weight and 27.7% dose according to the heaviest animal.

**Table 7.9. Percentage of animal health workers response for the various reasons/ occasions to treat sheep and goats with anthelmintics.**

Occasions	Percentage of animal health workers (n=64 )		
	Always	Sometimes	Never
When farmers request	75	25	0
When mortality occur	37.5	2	54.7
Based on examination result	53	47	0
At seasons change	75	25	0
During field visit & observation	21.9	78.1	0

**Table 7.10. Response of animal health workers on the prerequisites of farmers on anthelmintics choice.**

Choice of drugs	Percentage animal health workers (n=64)		
	Most important (Rank 1 & 2 )	Important (Rank 3 & 4)	Least important (Rank 5)
Attractive price	53.1	31.3	15.6
Formulation	35.9	54.7	9.4
Colour	48.4	21.9	29.6
History of efficacy	4.7	59.3	35.9
Recommend by veterinarians	95	5	0

The main supply of anthelmintics and other veterinary drugs to the government veterinary clinics in all the survey areas is the zonal Bureau of Agriculture. Although the annual drug requirements of districts or sub-districts is decided on at a lower level, the planning and purchase of all drugs is done at a higher level. Ninety-nine percent of animal health workers responded positively that the zonal Bureau of Agriculture is most important for their drug supply. Very few (10%) replied that non-governmental organizations did supply some groups of anthelmintics (Table 7.12).

The response on the use of the different classes of anthelmintics by the zonal and sub-district level animal health workers for the period 1998 to 2001 is summarized in Table 7.13 and 7.14. When the questions on anthelmintic use were analysed to determine the changes of anthelmintic classes used, 95% of the animal health workers response, that the same

class of anthelmintic was used mostly during the 1998-1999 period. In the following years (2000–2001) the percentage of animal health workers' response for using the same class of anthelmintics was 100% (Table 7.14). The results from this survey indicate that there were no changes in the use anthelmintic classes for the last 4 years.

**Table 7.11. Percentage of animal health workers with respect to their practice of weight measurements and dosing responses with anthelmintics.**

Type of measurement	Percentage (n=64)
<b>Sheep weight estimate</b>	
Weighing using kg	36.9
Eye measurement	63.1
<b>Goat weight estimate</b>	
Weighing using Kg	27.7
Eye measurement	73.3
<b>Dosing sheep based on</b>	
Average weight	67.2
Heaviest weight	20.3
Individual weight	12.5
Lightest weight	0
<b>Dosing goats based on</b>	
Average weight	71.8
Heaviest weight	17.3
Individual weight	10.9
Lightest weight	0

n number of animal health workers

**Table 7.12. Major source of anthelmintics to veterinary clinics.**

Source	Percentage of respondent AHW (n=16)		
	Most important	Important	Less important
Zonal Agricultural Bureau	100	0	0
Regional Agricultural Bureau	13.5	86.5	0
Non Gov. organization	18.8	0	78.2
Local drug stores	55	45	0

AHW animal health workers

Table 7.14 shows the responses of animal health workers on the use of the different anthelmintic classes available in government veterinary clinics and at private practitioners. A significantly higher number of veterinary clinics ( $P < 0.05$ ) had not changed anthelmintic classes from 1998 to 2001. Benzimidazole and pro-benzimidazole were exclusively (100%) used by all veterinary clinics and centers. The most commonly available and used benzimidazole was albendazole. About 5-10% respondents indicated that the use of ivermectin has dropped almost to nil since 1998.

From the general comments given by the animal health workers, it became apparent that they were confronted by and complained to by many farmers, about the failure of some anthelmintics to produce the desired results. Hundred percent of the animal health workers involved in this questionnaire survey confirmed that there was no test for anthelmintic resistance that was carried out in their localities. About 7% of the animal health workers have attempted an efficacy test when farmers returned their animals with continued diarrhoea, coughing, bottle jaw and/or lack of general improvement after treatment.

**Table 7.13. Frequency of anthelmintic groups used by animal health workers for the past four years.**

Class	1998 (n=64)	1999 (n=64)	2000 (n=64)	2001 (n=64)
<b>Class I</b>				
Albendazole	76.6	78.1	96.7	75
Fenbendazole	1.6	1.6	0	1.6
Thiabendazole	3.1	3.1	1.6	3.1
Febantel	1.6	1.6	0	0
Thiophanate	0	1.6	0	0
Oxyclozanide	1.6	0	0	1.6
Total for BZ <sup>1</sup>	84.5	86	98.3	81.3
<b>Class II</b>				
Levamisole	6.3	6.5	1.6	12.5
<b>Class III</b>				
Ivermectin	1.6	1.6	0	1.6
<b>Class IV</b>				
Closantel	3.1	0	0	4.7
Triclabendazole	4.6	1.6	0	3.1

n number of animal health workers <sup>1</sup> Benzimidazoles

**Table 7.14. Frequency of anthelmintic classes used by veterinary clinics for the past four years.**

Class	1998 (n=16)	1999 (n=16)	2000 (n=16)	2001 (n=16)
<b>Class I</b>				
Benzimidazole and Probenzimidazoles	100	100	100	100
<b>Class II</b>				
Levamisole	62.5	68.8	43.8	0
<b>Class III</b>				
Ivermectin	6.3	12.5	0	0
<b>Class IV</b>				
Closantel	50	56.3	50	43.8

n number of animal health worker respondents in veterinary clinics

#### 7.4. DISCUSSION

This study presents the assessment of a questionnaire survey carried out in East and North Shewa zones for the first time involving peasant farmers and animal health workers on worm control practices and the occurrence of anthelmintic resistance. The one hundred questionnaires were given to randomly selected peasant farmers from the list of peasant farmers' villages which represented from 2-5% of the farmers in each village, where in each village an estimated 100 households were expected to respond. The animal health workers' questionnaires were given to 64 workers selected at random for the interview but were representatives from each zone.

The average number of sheep and goats per household both in North and East Shewa was 15. Eventhough the majority (81%) of the farmers acknowledged that worms are important animal health constraints, little work has been done towards the establishment of a sustainable worm management. To date, in the study area as well as in other parts of the country, there are no recognized worm control practices. Due to lack of other options, the use of anthelmintics for the control of gastro-intestinal helminths is common for small ruminants.

Mixed grazing of sheep and goats with other species is very common and practised on the majority of the farms. Most of the farmers, however, are not aware of the advantage of the mixed grazing system. Alternate grazing of small stock with horses and donkeys during different seasons or different times of the day is likely to reduce pasture contamination due to the host specificity of the predominant parasites such as *Haemonchus* and *Trichostrongylus* (Reinecke, 1983). The majority of farms in North Shewa have a large ratio of sheep to other species, whereas in East Shewa goats are in the majority. The ratio of sheep and goats to other species was higher in all the survey areas.

Maingi *et al.*, (1996b) found that a lower ratio of sheep to other species enhanced the effectiveness of worm control. This was similar to the observation made by Coles and Roush (1992). However, in New Zealand, goat farmers were reported to graze sheep on goat farms and 41% of farmers grazed sheep and goats together (Pearson and McKenzie, 1986).

Twenty-seven percent of the farmers indicated that they treat sheep and goats twice a year while 12% treat four times. Eight percent of the respondents never treat their animals at all. Fifty-three percent of the respondents either treat once annually or treat only when necessary. Overall, it is concluded that the frequency of anthelmintic treatment that was carried out annually was too small to result in the development of anthelmintic resistance. In

spite of differences in the management type and size of flocks, there are similarities between the observations made between these and other studies. For example, the mean number of treatments that were recorded in New Zealand was 2.3 for sheep and 3 for goats (Kettle *et al.* 1981, 1982; Brunndon *et al.* (1983), in Australia (Edwards *et al.*, 1986) and 2-3 in Kenya (Maingi *et al.*, 1996b). However, up to 12 treatments per year were reported in the USA by Tritschler *et al.* (1986).

Although the risk of the development of anthelmintic resistance seemed unlikely because of the low frequency of anthelmintic use, other practices that might cause the development of anthelmintic resistance exist. In this survey, 58% of the farmers are in a position to give the correct dose to sheep and goats. This was not confirmed in a cross-checking with the animal health workers, of whom 89% stated that farmers could knowingly or unknowingly underdose their animals. On the other hand, the most likely cause of underdosing is due to estimation of weight rather than actual weighing, and, secondly, dosing according to the average mass of the animals rather than the heaviest one.

In addition, 90% of respondents complained that the anthelmintic they used was not as effective as expected. Many respondents (88%) buy anthelmintics from the open market. However, most of the drugs in the open markets did not come from any reputable company or distributors. Most of these drugs were illegally imported from unknown sources and are often unreliable. Such types of adulterated and dubious quality of drugs could contribute to the development of anthelmintic resistance (Waller, 1997).

When the questions on anthelmintic use were analysed to determine the changes of anthelmintic classes used, 95% of animal health workers responded that the same classes of anthelmintic have been used for four or more consecutive years before being changed. This was confirmed by studying the data obtained from the zonal and sub-district level veterinary clinics where the major groups or classes of anthelmintics were not changed for the last several years. This situation could select for resistance to anthelmintics.

## **Chapter 8.**

### ***A survey on the occurrence of anthelmintic resistance***

#### **8.1. INTRODUCTION**

Resistance is an inevitable consequence of the use of anthelmintics, and the history of parasite resistance to anthelmintics starts with the first report on phenothiazine resistance in sheep in the USA (Drudge, Leland and Wyant, 1957). After five decades since the first cases of anthelmintic resistance occurred, the problem, has become an important limiting factor in the control of nematode parasites of ruminants. The geography of resistance is widening and as a result there have been numerous reports of anthelmintic resistance in Africa, e.g. in Cameroon (Ndamukong and Sewell, 1992), Kenya (Maingi, 1991; Maingi *et al.*, 1996a), Tanzania (Ngomuo *et al.*, 1990), Zimbabwe (Boersema and Pandey, 1997), and Mozambique (Atanasio *et al.*, 2002). In South Africa anthelmintic resistance has become a major problem and surveys indicate that 90% of farms harbour resistant helminth strains; as much as 40% are resistant to three or more anthelmintic groups (Van Wyk *et al.*, 1997a, b, 1999).

Based on data from surveys of the Office of International Epizootics member countries, FAO questionnaires (1998) and literature searches (1999) indicate that Ethiopia is among the few countries in Africa where anthelmintic resistance has not been reported (Anon, 2004). The results obtained from this survey indicates that it is likely that anthelmintic resistance is present, due to, among other reasons, the frequent use of same class of anthelmintic and inappropriate dosage rates. However, worm control continues to rely on anthelmintics because of their high performance and the absence of other alternatives, thus they are likely to continue into the foreseeable future as the first and foremost line of defence against parasites (Martin, 1985). The emergence of resistance is therefore a practical problem.

This part of the study aims to investigate the occurrence of resistance to anthelmintics, particularly to the benzimidazole and levamisole groups, by using the faecal egg count reduction test methods in selected areas in Ethiopia.

## **8.2. MATERIALS AND METHODS**

### **8.2.1. Sampling of farms and animals**

Farms for this study were selected from the villages that participated in the questionnaire survey. The criteria for selecting the farms were: farmers' willingness to cooperate and participate in the study, the availability of more than 15 sheep or goats on the farm, a nematode egg count of more than 500 per gram of faeces, and the assurance that animals in the flock were not treated in the last 8-10 weeks. Twenty-four farms from 17 localities which complied to the stipulated criteria were chosen. A code number identified each location. Thus, a total of 24 smallholder farms in 17 localities in East and North Shewa zones and two institutional farms were involved in this study (Table 8.1).

All sheep and goats belonging to the peasant farmers in East Shewa were of the local breeds and their age classes included, where possible, weaned animals, but when few such animals were available, adult animals were used.

The different goat breeds that occurring on the farms were used in this survey. There was no specific reason for the tests to involve the different breeds, but simply to clear any doubts if there was any worm resistant breed among the groups. The goat breeds that were used in this study included local Rift Valley goats, their crosses with Toggenburg or Anglo-Nubian, and pure Toggenburgs. The animals were divided into different age groups, namely the weaned local Rift Valley goats, the Rift Valley cross-bred kids, the Rift Valley adult goats, the crossed adults and the pure-bred Toggenburgs.

Faecal egg count reduction tests were carried at Debre Birhan on flocks of exotic sheep breed and Menz, the local sheep breed in North Shewa.

Three treatment groups were constituted on every sheep or goat farm, namely benzimidazole, levamisole and non treatment control groups. Animals were randomly allocated to each of the groups. Each animal in every group was identified by ear tag with code numbers.

### **8.2.2. Anthelmintics**

The anthelmintics used in this survey are listed in Table 2. Albendazole (Albenol) and levamisole were selected to represent benzimidazoles and imidazothiazoles. The anthelmintics that had been used mostly frequently and for six consecutive years were used

in the anthelmintic resistance test. The anthelmintics were identified during the questionnaire survey as drugs that did not change for over four or more years in the study area.

Albendazole and levamisole were used in North and East Shewa because they have been used for a long time to control *F. hepatica* and nematode parasites. The recommended dose of levamisole for sheep was 7.5 mg/kg and on the goat farm at Debub University, up to double the dose for sheep was given to the goats.

On the first day, each animal was drenched with the calculated dose based on its actual weight. The anthelmintics were carefully administered using a syringe to deliver an accurate dose and to ensure that the animals swallowed the drenches properly.

### 8.2.3. Faecal egg count reduction test

Faeces were collected from the rectum of each sheep and goat and placed into clean specimen bottles. These were kept in a coolbox and transported to the laboratory at Sabata. Faecal samples were examined the same day they were collected by using a modified McMaster technique (Hansen and Perry, 1994). Faecal samples were collected 14 days after the first treatment. The reduction in faecal egg count was determined using the method of Coles *et al.* (1992), as advocated by the WAAVP. The method of Presidente (1985) was used merely to observe the distinctions of the methods. The following formulas were used to assess the reduction of faecal egg counts.

1)  $FECRT\%_1 = 100 \times (1 - [T_2 / C_2])$ , Coles *et al.* (1992), use arithmetic means, 95% confidence level is provided; Resistance is present if the percentage reduction less than 95% and the lower 95% confidence limit for the reduction is less than 90%.

2)  $FECRT\%_2 = 100 \times (1 - [T_2 / T_1] [C_1 / C_2])$ , Presidente (1985), use logarithmic transformation of egg counts to stabilize variances. Efficacy is corrected for changes that occur in the control group by the equation listed. Resistance is present if the percentage reduction is less than 90%. In this formula the T and C were the geometric means for the treated and control groups and the subscripts <sub>1</sub> and <sub>2</sub> designate the counts before and after treatment.

### 8.2.4. Larval identification and counts

Pooled samples were collected from each group on the day of the first treatment and post-treatment, 14 days after treatment. Pre-treatment samples with fewer than 100 egg were omitted from this experiment. Cultures were made as described in Chapter 3 and 100 larvae

identified according to the descriptions of Reinecke (1983) and Van Wyk *et al.* (1997b, 2004).

**Table 8.1. Zone, location and number of sheep and goats in the anthelmintic resistance survey.**

ID Number.	Survey zone	Location of* smallholders	Number of Farms	Sheep	Goats
1	East Shewa	Shashemene	1	0	35
2		Meki	2	26	26
3		Zewai	2	30	30
4		Dugdabura	1	0	23
5		Wolenchiti	2	0	26
6		Fentale	1	22	20
7		Alemgena	1	0	30
8		Arsi negele	1	25	35
9		Boset	1	0	22
10		Chabi	1	30	35
11		Mojo	2	35	35
12	North Shewa	Karafino	1	27	0
13		Yato	1	37	0
14		Chacha	1	45	0
15		Faji	1	30	0
16		Sululta	1	20	0
17		Sheno	2	45	0
18	Debre Birhan	DSBIC <sup>1</sup>	1	45	0
19	Awasa	DU <sup>2</sup>	1	0	197
TOTAL		19	24	417	514

<sup>1</sup> Debre Birhan sheep breeding centre

<sup>2</sup> Debub University, Awasa

**Table 8.2. Anthelmintics drenched orally in the anthelmintic resistance survey**

Active ingredient	Trade name	Company	Dosage (mg kg <sup>-1</sup> )
Albendazole	Albenol	Interchemia	5
Levamisole	Levamisole	Pfizer	7.5

### 8.3. RESULTS

The arithmetic and geometric means of the faecal egg counts of the experimental group were determined before and after treatment, and of the control groups. For the faecal egg count reduction percentage calculation using the arithmetic mean the WAAVP method (Coles *et al.*, 1992) was used, while for faecal egg count reduction percentage calculation using the geometric mean the method of Presidente (1985) was used.

Tables 8.3. and 8.4 show the faecal egg count reduction percentages for the 22 smallholder and the two institutional farms, which were calculated according to the methods proposed by the WAAVP (Coles *et al.*, 1992). Both the benzimidazole and levamisole anthelmintics were tested on six smallholder farms, and benzimidazole alone on 15. According to the WAAVP recommendations (Coles *et al.*, 1992), resistance to benzimidazole was present on three out of the 22 flocks belonging to smallholders and suspected on one. The faecal egg count reduction percentages according to the WAAVP method in these instances were 67%, 77%, 85%, 92% with the 95% confidence interval below 86 (Table 8.3). Levamisole resistance was present on one out of the seven smallholder farms.

**Table 8.3. Results of faecal egg count reduction percentages on smallholder farms calculated based on arithmetic means (Coles *et al.*, 1992) and geometric means (Presidente, 1985).**

ID	Number of farms	Number of animals sampled	Drug	Coles <i>et al.</i> (1992)	Presidente (1985)
1	1	35 goats	Albendazole	96 (88-99) LR	90 (91-99)
2	2	26 sheep	Albendazole	98 (93-100)	91 (90-98)
		26 goats	Levamisole	98 (90-100)	92 (91-99)
3	1	30 goats	Albendazole	95 (84-98) R	76 (75-95) R
		30 sheep	Levamisole	95 (84-98) R	72 (65-90) R
4	1	23 goats	Albendazole	99 (90-99)	92 (92-98)
5	1	26 goats	Albendazole	98 (92-99)	*
6	2	22 sheep	Albendazole	98 (91-99)	93 (91-99)
		20 goats	Levamisole	99 (95-100)	90 (92-100)
7	1	30 goats	Albendazole	98 (93-100)	90 (92-99)
8	2	25 sheep	Albendazole	98 (93-98)	93 (91-100)
		35 goats	Levamisole	98 (93-100)	91 (92-99)
9	1	22 goats	Albendazole	98 (91-100)	92 (94-100)
10	2	30 sheep	Albendazole	99 (95-100)	88 (58-98)
		35 goats	Levamisole	98 (90-99)	92 (91-99)
11	1	35 sheep	Albendazole	67 (25-86) R	65 (32-88) R
		35 goats	Levamisole	92 (78-97) R	89 (76-95) R
12	1	27 sheep	Albendazole	98 (90-100)	92 (91-99)
13	1	37 sheep	Albendazole	98 (95-100)	93 (92-99)
		25 sheep	Albendazole	77 (50-89) R	46 (25-68) R
14	2	20 sheep	Levamisole	63 (9-85) R	
		30 sheep	Albendazole	97 (90-99)	94 (93-99)
15	1	20 sheep	Albendazole	98 (90-99)	95 (92-100)
16	1	45 sheep	Albendazole	65 (15-89) R	66 (25-79) R

LR= low resistance, R=resistance, number in brackets are 95% confidence interval

\* No data

**Table 8.4. Results of faecal egg count reduction percentages on institutional farms calculated based on arithmetic means (Coles *et al.*, 1992) and geometric means (Presidente, 1985).**

ID	Locality	Spp.	Breed	n	Drug	Coles <i>et al.</i> , 1992	Presidente. 1985
18	MOA <sup>1</sup>	sheep	Menz crossbred	15	Albendazole	97 (88-99) LR	86(72-98)
			“	15	Levamisole	95 (93-98)	84 (89-98)
19.1	Debub university	goat	Rift-Valley local breed	15	Albendazole	86 (61-95) R	31(25-80) R
			“	15	Levamisole	89 (77-94) R	55 (27-72) R
19.2	Debub university	goat	Rift-valley crossbred	10	Albendazole	88 (71-95) R	24 (12-26) R
			“	10	Levamisole	93 (80-98) R	35 (2-65) R
19.3	Debub university	goat	Toggenburg ”	7	Albendazole	92 (77-98) R	50 (1-20) R
19.4	Debub university	goat	Mixed goat breed	20	Albendazole	92 (77-97) R	80 (58-90) R
			“	20	Levamisole	58 (33-87) R	71(35-92) R
19.5	Debub university	goat	Rift-Valley kids	10	Albendazole	95 (86-95) R	-26 (21-45) R
			“	10	Levamisole	85 (60-94) R	28(10-46) R
19.6	Debub university	goat	Rift-Valley crossbred	10	Albendazole	71(28-88) R	12 (1-42) R
			“	10	Levamisole	96 (82-99) LR	32 (15-68) R

n= number of animals tested for resistance, LR=low resistance, R=resistance, number in brackets are 95% confidence interval.

**Table 8.5. Results of the faecal egg percentage reduction tests (calculated using RESO) according to Coles *et al.* (1992) in goats on Debu University's goat farm**

Combined species	Control	Bz <sup>1</sup>	Lev <sup>1</sup>	Control	Bz <sup>2</sup>	Lev <sup>2</sup>	Control	Bz <sup>3</sup>	Lev <sup>3</sup>	Control	Bz <sup>4</sup>	Lev <sup>4</sup>
Drench number	10	10	10	10	10	10	15	15	15	10	10	10
Arith mean epg (pre)	1 010	1 290	2 390	720	820	720	850	1 607	1 007	2 696	1 490	1 560
Arith.mean epg (post)		800	420		90	50		120	93		790	80
Variance (FEC)	16 544	1 5111	510 667	315 111	7 667	5 000	491923	38 857	13 524	4 330 827	77 111	21 778
% Reduction		92	58		88	93		86	89		71	96
Variance (Reduction)		0.25	0.31		0.16	0.26		0.23	0.11		0.18	0.43
Upper 95% c.l.		97	87		95	98		95	94		88	99
Lower 95% c.l.		77	33		71	80		61	77		28	82
Interpretation		R	R		R	R		R	R		R	LR
<b>Pre-treatment L<sub>3</sub></b>	<i>85 H. contortus</i> , 14 <i>T. colubriformis</i> and 1 <i>O. columbianum</i> larvae were recovered from a pooled faecal sample											
<b>Post-treatment L<sub>3</sub></b>												
<i>H. contortus</i>	87	89	90	79	90	74	88	80	91	75	86	72
<i>T. colubriformis</i>	10	11	7	15	10	16	12	14	5	15	14	18
<i>O. columbianum</i>	3	0		6	0	10	0	6	4	10	0	10

The superscript 1, 2, 3 and 4 designate the albendazole and levamisole tests on the Rift Valley mixed, Rift Valley crossed, Rift Valley local and Rift Valley crossed with Toggenburg kids, respectively. R- resistance, LR-low resistance. Number in parentheses refers to levamisole tests.

**Table 8.6. Results of the faecal egg percentage reduction tests (using RESO) according to Coles *et al.* (1992) in goats and sheep on Debub University's goat farm and Debre Birhan sheep breeding centre.**

Combined species	Control	Bz <sup>5</sup>	Lev <sup>5</sup>	Control	Bz <sup>6</sup>	Lev <sup>6</sup>	Control	Bz <sup>7</sup>	Control	Bz <sup>8</sup>	Lev <sup>8</sup>
Drench number	10	10	10	7	7	7	5	7	15	15	15
Arith.mean epg (pre)	1 770	1 610	2440	1429	1 586	1 829	760	686	780	1 260	900
Arith.mean epg (post)		90	370		71	71	68 000	57		20	47
Variance (FEC)	1 646 778	14 333	27 122	1 019 048	5 714	12 381	68 000	6 190	277 429	3 143	6 952
% Reduction		95	85		95	94		92		97	95
Variance (Reduction)		0.23	0.21		0.23	0.41		0.29		0.55	0.23
Upper 95% c.l.		98	94		98	98		98		99	98
Lower 95% c.l.		86	60		86	77		77		88	86
Interpretation		R	R		LR	R		R		LR	LR
<b>Pre-treatment L<sub>3</sub></b>	80 <i>H. contortus</i> , 13 <i>T. colubriformis</i> and 7 <i>O. columbianum</i> larvae were recovered from a pooled faecal sample										
<b>Post-treatment L<sub>3</sub></b>											
<i>H. contortus</i>	70	79	80	74	90	74	85	92	88	71	70
<i>T. colubriformis</i>	22	21	15	20	10	16	15	8	12	29	26
<i>O. columbianum</i>	8	0	5	6	0	10	0	0	0	0	4

The superscript 5, 6, 7 and 8 designate the albendazole and levamisole tests on the Rift Valley kids, Rift Valley crossed kids, Toggenburg breeds and Debre Birhan Crossed sheep, respectively. R- resistance, LR-low resistance, Number in parentheses refers to levamisole tests

**Table 8.7. Results of faecal egg percentage reduction according to Coles *et al.* (1992) in sheep and goats on smallholder farms in East and North Shewa.**

Combined species	Control <sup>1</sup>	Bz <sup>1</sup>	Control <sup>2</sup>	Bz <sup>2</sup>	Control <sup>3</sup>	Bz <sup>3</sup>	Control <sup>4</sup>	Bz <sup>4</sup>	Control <sup>5</sup>	Bz <sup>5</sup>	Bz <sup>6</sup>	Control <sup>7</sup>	Bz <sup>7</sup>
Drench number	20	20	10	20	15	20	15	20	10	20	15	7	15
Arith.mean epg (pre)	1013	1135	860	805	973	1220	933	1055	855	805	640	757	1067
Arith.mean epg (post)		80		45		40		305		45	27		113
Variance (FEC)	411 238	376 079	347 100	9 974	482 095	6 737	463 810	223 658	347 111	9 974	3 524	469524	24 095
% Reduction		92		95		96		67		95	96		85
Variance (Reduction)		0.25		0.29		0.24		0.16		0.29	0.35		0.24
Upper 95% c.l.		97		98		99		86		98	99		95
Lower 95% c.l.		78		84		88		25		84	86		58
Interpretation		R		R		LR		R		R	LR		R
<b>Pre-treatment L<sub>3</sub></b>	89 <i>H. contortus</i> , 11 <i>T. colubriformis</i> and 5 <i>O. columbianum</i> larvae were recovered from a pooled faecal sample												
<b>Post-treatment L<sub>3</sub></b>													
<i>H. contortus</i>	87	89	90	80	90	74	88	80	90	80		0	91
<i>T. colubriformis</i>	10	11	7	15	10	16	12	14	7	15		0	0
<i>O. columbianum</i>	3	0	0	5	0	10	0	6	0	5		0	0

The superscript designate FECRT tests in goats and sheep, <sup>1</sup> and <sup>2</sup> at Zewai, <sup>3</sup> and <sup>4</sup> at Mojo, <sup>5</sup> and <sup>6</sup> in sheep at Karafino and Faji, and Cha-cha, respectively.

The results of pre- and post-treatment larval recovery in the anthelmintic resistance test were determined using the faecal egg count or worm count reduction test analyses (RESO) (Anonymous, 1990). In the pre-treatment larval cultures, *Haemonchus* was the predominant nematode (75-89%), while *Trichostrongylus* and *Oesophagostomum* were present in small numbers (1-15%). Post-treatment faecal cultures indicated that *Haemonchus* was resistant, and to a lesser extent *Trichostrongylus* to both a benzimidazole and an imidothiazole. Detailed results of the calculations on faecal egg count reduction percentages are presented in Tables 8.5-8.7

#### 8.4. DISCUSSION

This is the first report on the presence of anthelmintic resistance shown by helminthes of goats and sheep in Ethiopia. The results of the faecal egg count reduction percentage tests are in agreement with studies carried out in South Africa (Van Wyk *et al.*, 1997a, b; 1999), in Kenya (Mwamachi *et al.*, 1995), in Denmark (Maingi *et al.*, 1996a), in Malaysia (Pandey and Sivarja, 1994; Pandey *et al.*, 1996, cited by Atanasio, 2000) and in Peninsular Malaysia (Dorny *et al.*, 1994) with respect to the species of nematodes involved and the percentage reduction results obtained, where resistance of *Haemonchus* and *Trichostrongylus* spp. to benzimidazole and levamisole was found.

In this study, the results of the faecal egg count reduction test (Coles *et al.*, 1992) indicate that anthelmintic resistance to both benzimidazole and levamisole is present on two of the farms, and against benzimidazoles on five out of the 22 smallholder farms. On the Debu University goat farm, the three flocks, consisting of the local goat breed (Rift Valley breed), the local breed crossed with Toggenburg or Anglo-Nubian (Rift Valley cross-bred) and the pure-bred Toggenburg were sampled several times for anthelmintic resistance with an interval of more than two months. Resistance to albendazole and levamisole were confirmed to be present on the farm. Resistance to albendazole and levamisole on the farm was suspected as the percentage faecal egg count reduction calculated was lower, which partially agrees with the method of the FECRT of Presidente (1985). The level of resistance detected to benzimidazole and levamisole were high with the percentage faecal egg count reduction ranging from 45% to 65%. The pre-treatment and post-treatment larvae recovered indicated that *Haemonchus* was the predominant resistant nematode found on this farm.

The anthelmintic resistance test on a flock of sheep that was carried out at Debre Birhan government sheep farm showed a percentage reduction of 94 and 95% in the faecal egg

count. The lower 95% confidence interval was less than 90. This indicates that anthelmintic resistance to benzimidazole and levamisole could be present on this farm.

Prolonged use of the same classes of anthelmintics, and the distribution and availability of drugs of dubious quality, may have contributed to the development of resistance in some flocks of sheep and goats belonging to smallholders. Unless control measures are designed and uniformly applied, anthelmintic resistance has the potential to spread rapidly and widely. This is likely to further aggravate the “ill thrift” or nutritional inadequacies, poor helminth control and husbandry practices in small ruminant production in the area, and may further enhance selection for resistance to wide spread resistance.

The use of the same groups of anthelmintics for a number of years is very common in most of the government veterinary clinics. In the questionnaire survey (Chapter 7) the majority of animal health workers (87-90%) used the same groups of anthelmintics for four or more consecutive years before changing to a different group.

Currently the responsibility of supplying and distributing of anthelmintics and other veterinary drugs to regional, zonal or district level veterinary clinics has been taken over by the zonal or regional Bureau of Agriculture of each of the regional states in the country. This should have brought changes from using same groups of anthelmintics in most of the veterinary clinics. On the other hand, because of the Government’s free market economic policy, there are several private companies which import veterinary drugs in bulk and distribute products from various manufacturers in Africa, Europe and Asia in Ethiopia. This has contributed towards reducing the shortages of drugs, but there are certain discrepancies and questions such as the quality of the drugs, marketing the same group of anthelmintic under different trade names, formulation and presentation, all of which require serious attention from the drug regulation authority. Despite the facts mentioned above, there are often anthelmintics available in the open market, the sources of which are not known. These illegal products are seen in the open markets on a daily basis, and peasant farmers easily purchase them because of the trade names and their cheap prices. Waller (1997) indicated that adulterated and anthelmintics of dubious quality obviously lead to development of resistance.

In this study, four farms belonging to smallholders in North and East Shewa as well as two institutional farms were shown to have resistance against benzimidazole and levamisole. The arithmetic mean of the faecal egg count for faecal egg count reduction percentage calculation according to Coles *et al.* (1992) is universally accepted, because it provides a better estimate of the worm egg output and it is a more conservative measure of anthelmintic

efficacy. McKenna (1997) therefore suggested that the estimate of anthelmintic efficacy should be calculated from the arithmetic mean. Generally, the two methods of calculating the percentage faecal egg count reduction agreed in confirming resistance in flocks or herds of sheep and goats. Relatively high levels of resistance were detected.

The number of farms examined in both North and East Shewa were too few to represent the smallholder farms in the two zones and no conclusions can therefore be made on the prevalence of anthelmintic resistance among the sheep and goat flocks of the smallholders. The anthelmintic resistance tests that were carried out at Debub University using the local and cross-bred goats confirmed the existence of resistance by *Haemonchus contortus*. Because of the scope of this study, other potential areas where anthelmintic resistance possibly prevailed was not assessed. In order to reach a better understanding and positive decision on the current anthelmintic resistance situation in the country, a broader and extensive survey, involving more farms in other regions and at a national level, is crucial. However, this study gives indications that anthelmintic resistance in nematode parasites of sheep and goats is becoming an emerging problem, to which special attention should be paid.

A number of strategies have been proposed that may help to avoid or slow down the development and spread of anthelmintic resistance. They include limiting the frequency and number of anthelmintic treatments, strategic dosing at particular times of the year based on epidemiological findings and annual rotation using drugs from different chemical groups (Fairweather and Boray, 1999). To prevent the build up of resistance to anthelmintics and to minimize the passage of resistance genes in the selection process, rotation and or combination of anthelmintics have been considered as effective approaches. Resistance to any anthelmintic eventually occurs when the anthelmintic is continually used.

Van Wyk (2001) suggested that refugia should be considered an important part of a strategy when worm management in domestic animals is planned. Refugia refers to the proportion of the parasite population that is not exposed to a given control measure particularly using anthelmintics. Van Wyk (2001) stated that refugia play a crucial role in the selection of anthelmintic resistance than other applications such as reduced drenching frequency and avoiding under-dosing.

Strategies such as the drench and move system in which all the animals in a flock are drenched before they are moved to pastures containing few or no worms in refugia, the farming system would probably not be sustainable in view of the generally high levels of

resistance already present. The term “refugia” was coined to define the proportion of the parasite population that is not exposed to a particular given control measure, thus escaping selection for resistance (Van Wyk, 2001). After an effective drench, the progeny of the worms that survive the treatment develop among the free-living worms in refugia. Thus, the population in refugia has direct bearings on the degree of selection for resistance with particular anthelmintics.

## **Chapter 9.**

### **General discussion, significance of the study and concluding remarks**

#### **9.1. GENERAL DISCUSSION**

This study has described the seasonal occurrence, prevalence and intensity of gastro-intestinal parasite infections in sheep and goats, the perception of farmers and animal health workers on worm control management practices, the occurrence of anthelmintic resistance and the effects of the FAMACHA<sup>®</sup> system-based selective treatment on the control of nematodes in small ruminants in selected areas in Ethiopia.

The longitudinal studies on gastro-intestinal parasites of sheep and goats were carried out in two periods in the arid and semi-arid environments in East Shewa zone to determine the seasonal occurrence and intensity of helminths and coccidia infections in relation to sites, seasons and age of the host animals. The overall results indicate that nematode counts were higher in all sites following the rainy seasons and decline during the dry seasons. The mean egg counts during the long rainy seasons in the initial survey showed 536, 554 and 483 for young, juvenile and adult goats while for sheep for the same age group the egg counts were 560, 487 and 637 respectively.

The worms recovered during the initial survey of the 48 tracer kids in the survey showed that *H. contortus* (95-100%) and *T. colubriformis* (83-100%) were predominant followed by *O. columbianum* (58-83%) and *T. ovis* (41-74%). The prevalence of worms in the same rainy seasons from the 48 tracer lambs indicate that *H. contortus* (91-100%) and *T. colubriformis* (90-100%) were predominant followed by *O. columbianum* (33-83%) and *T. ovis* (8-33%). Significant differences in worm counts were observed between seasons ( $P < 0.05$ ), and the mean egg counts were higher than during the initial study period. Higher numbers of worms were recovered in the current study than the initial survey. The worms recovered during the long rainy seasons from 53 kid tracers in the survey showed *H. contortus* (95-100%) and *T. colubriformis* (83-100%) to be the predominant ones, followed by *O. columbianum* (58-83%) and *T. ovis* (41-74%). The same result was found in the 57 tracer lambs where *H. contortus* (91-100%) and *T. colubriformis* (90-100%) were predominant followed by *O. columbianum* (33-83%) and *T. ovis* (8-33%). Regarding the age and worm burden relationship, the worm counts for juvenile animals were higher than the adult animals.

Significant differences in worm count were observed between seasons ( $P < 0.05$ ) and sites. The mean worm burden during this study was found to be much higher than the initial period in almost all study sites during the worm seasons. The species composition of nematodes does not vary between sites. *Bunostomum trigonocephalum* was found less frequently and few were found in sheep and goats during the initial survey. This nematode was absent from almost all the sites during the later study. Other helminths such as *M. expansa*, *T. hydatigena*, *Echinococcus* spp. and *Fasciola hepatica* were found in sheep and goats in East Shewa zone. Lambs and the kids were more often parasitized by *M. expansa* than were the adult animals. This result is similar to the findings of Horak *et al.*, 1991. However, most of these worms were found a small percentage of the animals examined in the semi arid areas. According to Horak (1981) helminths such as *T. hydatigena*, and probably *Echinococcus*, can be classified as occasional parasites, while the infrequent occurrence of *F. hepatica* in the arid area makes it an accidental parasite of sheep in this locality.

The high prevalence of *Haemonchus*, *Trichostrongylus* and *Oesophagostomum* indicates that infection with these nematodes is probably the major constraint to small ruminant production in the area. The highest percentages of infected animals and pastures were recorded during the rainy seasons. Gatongi (1995) in Naivasha in a semi-arid area of Kenya with similar climatic conditions to East Shewa zone, reported similar findings. The main economic loss due to chronic sub-clinical infections (Allonby and Urquhart, 1975; Soulsby, 1982; Reniecke, 1983) occur as a result of reduced growth rates, emaciation, unthriftiness, and decreased resistance to other secondary diseases (Allonby and Urquhart, 1975; Soulsby, 1982). The correlation between the faecal nematode egg count and the actual worm burdens showed positive correlations during the rainy and dry seasons. This result is different from the findings of Gatongi (1995) who reported that positive correlation between egg counts and worm burden was observed only during the wet season.

Reports from studies that were carried out in the tropics indicate that arrested larvae would tend to accumulate within the grazing sheep and persist in this form over the long dry season, commencing maturation at the start of the rainy season (Vercruyse, 1985). In this study, nematodes were observed to have dual survival means. They survive the dry season either as adult nematodes or as inhibited larvae. The resumed development of inhibited larvae together with the increased fecundity by the existing adult worm population in the animal hosts, at the beginning of the rain appeared to be responsible for pasture contamination and the continuity of the parasites life cycles. This study therefore, demonstrated the seasonal occurrence of nematodes in both species of animals, which

implies the possibility to conduct successful strategic use of anthelmintics together with proper and appropriate management strategies.

Treatment under conditions of low refugia, to prevent a build-up of parasites on pasture (for example, at the beginning of the rainy season, or immediately before long dry period) can be highly effective for worm management. The strategic use of anthelmintics has been effective in controlling *H. contortus* and other nematode parasites in Australia (Waller, 1987; Rolfe *et al.*, 1990). Besides this, numerous field trials have confirmed that strategic drenching can be more effective for worm control than the suppressive or non-suppressive treatment that is applied at a time when the worm challenge on pasture is high (Michel, 1969; Brunsdon, 1980; Lloyd *et al.*, 2000). However, the greater the success of this strategy, the greater the degree of selection for resistance to anthelmintics is likely to be (Waller, 1997).

One of the strategies that is being encouraged to use in the control of nematodes of small ruminants and anthelmintic resistance is selective anthelmintic treatment, which aim at reducing production losses and treatment of sick animals that show clinical symptoms of parasitism and thereby slowdown the development of resistance. Correlations between haematocrit levels and FAMACHA<sup>®</sup> scores, haematocrit and faecal egg count, worm and faecal egg counts were significant for both sheep and goats in the selective treatment group ( $P < 0.001$ ). Sheep and goats receiving selective treatment had significantly higher weight gains than non-treated or animals treated on one occasion ( $P < 0.05$ ). Animals receiving monthly anthelmintic treatment as a positive control group had significantly higher weight gains than all the other groups ( $P < 0.05$ ). The sensitivity of the FAMACHA<sup>®</sup> test to identify animals that fall into categories 3, 4 and 5 was 72.7% while the specificity 94.9%. This results indicate that the FAMACHA<sup>®</sup> method can be used as a quick, simple and cheap alternative for an integrated control of nematode parasites, particularly when *H. contortus* is the primary pathogen which would result in reducing number of anthelmintic treatments, thereby increasing the proportion of worms in refugia. Michel (1985) stated that selection pressure might be measured as a proportion of each generation that is the progeny of worms that have survived exposure to anthelmintic resistance. Van Wyk (2001) also suggested that refugia play a much more important role than for instance, reduced drenching frequency and avoidance of under-dosing. The main challenge is to develop ways for overcoming the practical problems concerning the excessive build-up of worms on pastures. In other words, while parasite burdens in animals treated with effective drugs are reduced to practically nil, the untreated sheep and goats continue to propagate the progeny of parasites that do not come into contact with the drug, and thus they are in refugia and unselected for resistance to anthelmintics. In this way the balance is continually tipped in favour of the

susceptible, compared to the resistant parasite. The advantage of using FAMACHA<sup>®</sup> was clearly seen in the present study as it reduced the amount and frequency of anthelmintic treatments. Because of this advantage, selective anthelmintic treatment offer a sustainable approach to worm control and anthelmintic resistance simultaneously, particularly in countries like Ethiopia, where resistance is not yet widely distributed.

The FAMACHA<sup>®</sup> system as a tool, for selective anthelmintic treatment to control the blood sucking nematode parasites is likely to play an important role in an integrated worm control strategies. The FAMACHA<sup>®</sup> method is simple and easy to use and to integrate with clinical examination and sampling procedures such as faeces and blood collections, weight measurement, scoring of body condition, vaccination and treatment. However, to detect animals that require anthelmintic treatment using this method, require examining each and every animal's ocular mucous membrane. In this regard, in commercial sheep farms or in farms where there are large number of animals it may require a substantial length of time and labour whenever the FAMACHA<sup>®</sup> method is applied to detect clinical anaemia.

In this study, coccidia were found to be the most prevalent parasites in young lambs and were present in about 98% of both host species. Often less attention is given to coccidia as oocysts appear in thousands in almost every sheep or goat. It should however, alert the attention of animal health workers if a high prevalence is encountered, as seen in the present study, where 83% of the faecal samples examined from sheep, and a 74.2 % from goats were positive for *Eimeria* oocysts. Lambs and kids had higher oocyst counts per gramme of faeces than either juvenile or adult age groups ( $P < 0.05$ ). Several factors including microclimate, stress factors such as underfeeding or starvation (Brunsdon, 1964; Vercruysse, 1982), adequate moisture, and rainfall contribute to infection (Soulsby, 1982). The traditional management system of keeping lambs, kids, mature and juvenile and adult sheep and goats crowded under the same shelter with almost no disposal of faeces and urine is certain to contribute to high levels of infection. This study has shown that coccidial infections are widespread in lambs and kids in the Rift Valley areas, and that the parasite have pathogenic effects. Since no previous study was carried out concerning coccidia of small ruminants in the country, the predominant *Eimeria* species of sheep and goats should further be identified, and coccidiosis as a disease of young lambs and kids needs further investigation.

Information concerning worm control practices with emphasis on the use of anthelmintics and the implications for the development of resistance was obtained through a questionnaire survey that was carried out on sheep and goat farms and veterinary clinics in East and North

Shewa zones. The main factors identified in the study, which may contribute significantly to the risk of selection of worms resistant to anthelmintics according to Coles and Roush (1992) and Waller (1997) were failure to alternate classes of anthelmintics from different groups, underdosing and the use of drugs of dubious quality.

In a questionnaire survey that was carried out in Kenya and Denmark (Maingi *et al.*, 1996b) found that the majority of sheep farmers (98%) and goat farmers (85%) in Denmark, and sheep farmers (97%) in Nyandarua district in Kenya were not alternating anthelmintics annually. This was likely to enhance the development of resistance to the anthelmintics used (Maingi *et al.*, 1996b). Results of the present survey (Chapter 7) indicated that the majority of sheep and goat farmers (96% in North Shewa and 98% in East Shewa) did not alternate anthelmintics. Only 2% of 64 animal health workers interviewed in both zones alternated anthelmintics from the different classes annually. Annual alternations of anthelmintic classes were first suggested by Prichard *et al.* (1980) as a way to slow down the development of anthelmintic resistance. The main purpose of this practice was to avoid exposure of one generation of worms to more than one anthelmintic type, preventing selection for resistance and assume that some reversion to susceptibility takes place following withdrawal of an anthelmintic (Coles and Roush, 1992; Maingi *et al.*, 1996a; Van Wyk *et al.*, 1997; Chandrawathani, 2004).

Selection studies support the concept of annual alternation as a proper option (Waller *et al.*, 1985). In the survey by Maingi *et al.*, (1996b), most sheep and goat farmers (84% and 69%) in Denmark and 98% of sheep farmers in Kenya did not weigh their animals when drenching, but determined live weight by visual appraisal. Coles and Roush (1992) and Maingi *et al.* (1996b) pointed out that the most frequent cause of underdosing is probably guessing the weight of animals. Results of this study showed that more than 90% of the farmers and 78% of the animal health workers were using the average weight of the animals when drenching anthelmintics and this likely lead to under-dosing most animals that were treated. According to Coles and Roush (1992), underdosing allows optimal survival of resistance genes in a population of susceptible individuals and is heterozygotes worms. On the other hand, application of high doses, which is enough to kill heterozygotes and prevent their interbreeding to produce fully resistant individuals, would therefore delay the development of resistance (Coles and Roush, 1992).

The occurrence of anthelmintic resistance was investigated on 22 smallholder farms and two institutional farms in North and East Shewa zones and in Awasa and Debrebirhan areas respectively. These farms were selected from those participating in the questionnaire survey

on worm control practices. Results of the faecal egg count reduction tests carried out according to Coles *et al.* (1992) and Presidente (1985) indicated that resistance to the benzimidazole group was present on 4 (18.2%), and levamisole on 1 (4.5%) of the farms examined in North and East Shewa zones and on two institutional farms. Particularly at Debub University goat farm there was resistance to both albendazole and levamisole. At Debre Birhan sheep farm resistance to albendazole and levamisole is suspected. Few farms were surveyed but the results indicate that resistance is present and will soon be an emerging problem in the country.

As reviewed by Kaplan (2004) the most relevant factors that affect the rate with which resistance develops include: the biology and epidemiology of the parasite, the dynamics of the host–parasite relationship, the treatment frequency and the treatment strategies that result in various levels of refugia. There are several factors which may contribute to the development of resistance in the study areas in Ethiopia, among which, overuse of anthelmintic drugs at institutional farms, underdosing, repeated use of the same types of drugs (Waller *et al.*, 1995; Waller, 1997) both at farmers and veterinary clinics and animal health centers.

Poor drenching technique is another way to accelerate the development of resistance (Chandrawathani, 2004). In the resource poor area peasant farmers do not use drenching guns, rather they give anthelmintic boluses to their animals orally with water. The way smallholders administer drugs, does not ensure the boluses to be properly swallowed by the animals. It is important, therefore, to supply simple instruments such as drenching guns and train farmers on the use and handling of anthelmintic drugs.

Only two institutional farms were surveyed for resistance in the current study and the presence of anthelmintic resistance was confirmed. The animals were allowed to graze on pastures all year round and were treated more regularly and frequently to avoid high mortality. As a consequence resistant strains of nematodes may have spread slowly when these farms through their program of supplying breeding stock to smallholder farmers. There is a strong possibility that resistant worms could have been introduced to the smallholder farms where resistance already occurs, but this has not been assessed in the present study. However, resistance might have occurred on farms in Kenya and Mozambique through acquiring breeding stock from neighbouring commercial farms or countries (Mwamachi *et al.*, 1995; Maingi *et al.*, 1998; Atanasio, 2000).

In the record books of most of veterinary clinics and institutional farms, case numbers, species of animals treated, and addresses of owners are usually entered. However, records concerning basic details on the anthelmintics used were rare and in some instances very poorly recorded. In this study, statistical comparison can not be made on the frequency occurrence of anthelmintic resistance on sheep and goat farms, as reported in New Zealand where more wide spread resistance occurred on goat farms (Kettle *et al.*, 1981; Kettle *et al.*, 1983). No previous report or record was found on resistance nematodes in goats directly imported into the country for cross breeding purposes, and which were translocated to several regions in the country, including regions to the east and the south. In this study, it was found that resistance is confirmed in Toggenburg and the crossbred offspring of Toggenburg and Anglo-Nubian with the local Rift Valley goat breeds.

There is no policy of testing and treating of newly introduced small ruminants for worm resistance, while it is strictly applied for infectious diseases of viral and bacterial origins of any animal. In this study, the WAAVP method was found to be conservative in confirming resistance and most appropriate in surveys and monitoring of anthelmintic resistance. It is easy to use and helps to create awareness, keeps the local animal health workers alert to detect the slightest change in the efficacy of an anthelmintic. The overall results of the anthelmintic resistance survey in this study prompts for more investigation in other potential areas. Therefore, investigation concerning the status of resistance and its extent in broader areas of the country should be urgently initiated. Control strategies, which limit its further development and spread need to be addressed through a joint effort between research centers, field agricultural extension officers, and animal health workers at zonal, regional and national level.

For the local resource-poor farmers, traditional herbal remedies obtained from local plants offer an alternative to the expensive and often inaccessible commercial anthelmintics. The development of anthelmintic resistance, particularly in nematode parasites of small ruminants, and the trend towards non-chemical (ecological, organic or green) farming has provided an opportunity for research and development of alternative methods for parasite control, which include herbal de-wormers (Waller and Thamsborg, 2004). The response of farmers (17% of 100 respondents) showed that there was limited tendency for the use of herbal preparations for worm control in animals, whereas herbal medicine for the treatment of people for round- and tapeworms is a known practice in Ethiopia. As the small ruminant industry is increasingly threatened by worm infections and anthelmintic resistance, Besier and Love (2003) suggested for the need to adopt new approaches that minimise reliance on chemical control, such as the breeding of worm resistant sheep, use of specific grazing

strategies for worm control, nutritional regimens and flock treatment tactics that minimise the impact of worm infections and further resistance development. In this study, the use and practice of ethno-veterinary activities were assessed during the questionnaire survey.

## 9.2. SIGNIFICANCE OF THE STUDY AND CONCLUDING REMARKS

a) The main gastro-intestinal parasites occurring in sheep and goats in the Great Rift Valley areas of East Shewa zone and their relative distribution and the importance of seasonal weather factors, species of animals, and age of hosts in determining the level of infection was determined. *Haemonchus contortus* (95-100%) and *T. colubriformis* (83-100%) were found to be the most prevalent species of nematodes in the Rift Valley areas of East Shewa. The highest prevalence and pasture infectivity were recorded during the rainy seasons. No study previously carried out in these areas or else where in the country on coccidiosis of sheep and goats, probably because of the absence of information or under estimating of the disease particularly in kids and lambs.

Losses occur as a result of reduced growth rates, emaciation, unthriftiness, reduced resistance to helminths and other secondary diseases during both the dry and rainy seasons. Poor nutrition, sheep pox, pasteurellosis and other respiratory infections and ectoparasites are common problems which exacerbate the effect of gastro-intestinal parasitism (Kusiluka *et al.*, 1998).

The findings of this study indicate that management of worms in sheep and goats is important. Effective management of worms could be achieved through integrated control methods. However, farming systems such as communal grazing is largely practiced, and where other strategies are not available and may not be applicable, the option for the control of worms is limited.

In the short term, management of worms may continue through the regular and strategic use of anthelmintic treatments. To reduce the sheep and goats' worm infections in all the stocks that belong to the peasant farmers, strategic drenching still plays a role. However, for the strategic treatment to be effective and the efficacy of the drugs to be maintained, control programmes need to be developed considering factors that include the species of parasites present, their epidemiology and the farming systems and the worm management that may enhance selection for resistance. In the semi-arid and arid areas of East Shewa, gastro-intestinal nematodes, particularly *H. contortus* survive mainly as adults and to some extent as hypobiotic larvae during the dry season. Therefore, strategic drenching could be

applicable if the majority of farmers deworm their animals at the same time before or on the onset of the rain. Most of the farmers can afford one or two treatments per annum.

For sustainable worm management, an integrated approach should be developed. The communal pastures have to be controlled for the benefit of all peasant farmers, and a method should be designed to break the major infection cycle, by regulating the pasture to be grazed for a certain period or by rotational use of the different communal pastures, by encouraging cut-and-carry feeding of animals from areas protected from grazing (uncontaminated) by sheep and goats particularly during the rainy seasons.

b) The application of selective anthelmintic treatments based on the FAMACHA<sup>®</sup> system as a strategy in a broad base approach in the semi-arid and arid areas in East Shewa or in similar agro-ecological areas in the country may not be immediately practical for many reasons, which include the size of the flocks/herds and lack of skilled labour. However, through individual training of farmers and farmers' animal health representatives, in monitoring the health status of animals and faecal egg counts along with the use of FAMACHA<sup>®</sup> system, selective anthelmintic treatment could be feasible. Individual farmers could use the FAMACHA<sup>®</sup> system to selectively treat his or her animals by after identifying anaemic animals, and those animals that appear ill, lag behind others, and the young and old suckling ewes and does. The results of the present study on selective anthelmintic treatment on *Haemonchus* infection will form a basis for further investigations as part of an integrated worm control strategy.

c) Benzimidazole and levamisole resistance on sheep and goat nematodes are confirmed at smallholders and institutional farms. Worm control practices, which may contribute to the occurrence of anthelmintic resistance, were assessed through questionnaire surveys. This is the first report in the area and in the country. The findings will initiate to start further investigation on the development and spread of anthelmintic resistance to nematodes of small ruminants.

In conclusion, the survey data provided here on helminths and coccidia in goats and sheep demonstrates the importance of these parasites on health and productivity. The occurrence of anthelmintic resistant nematodes in sheep and goats shown in this study is an emerging problem in the control of gastro-intestinal nematodes in small ruminants in Ethiopia. In fact, gastro-intestinal parasitism emerges with the highest global index as an animal health constraint in resource poor communities (Perry *et al.*, 2002).

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# **Annexure 1**

## **QUESTIONNAIRE SURVEY ON WORM CONTROL IN SMALL RUMINANTS**

### **Section I. To be answered by the Animal Health Workers (AHW)**

Name .....

Zone.....

Address.....

To answer questions, please tick or choose number and write in the space provided.

Q I. Do you treat sheep and goats to control worms? 

Yes	No
-----	----

Q II. During which seasons do sheep and goats in your area need anthelmintics treatment most?

1. Between June and September	
2. Between October and December	
3. Between January and May	

Q III. Please specify if you drench at the following occasions

1. Drench when farmers request ?	
2. Drench in connection to mortality sheep & goats?	
3 Drench after faecal examination for worms?	
4. Drench as seasons change	

Q.. IV. How do you get your anthelmintic supply?

1 From Zonal bureau of agriculture	Yes	No
2. Regional state bureau of agriculture	Yes	No
3. Any local drug store/pharmacy	Yes	No

Q V Rank from 1 to 5 the criteria most commonly used (1) to the least commonly used (5) by farmers when selecting anthelmintic.?

1. Price of drugs	
2. The drug is of bolus & easy to take	
3. Known by colour & trusted efficacy	
4. Experience of good effect	
5. Recommendation from veterinarians	

Q VI Do farmers in your area buy anthelmintics so that they can treat sheep and goats on their own?

Yes	No
-----	----

Q VII Do you think farmers knowingly or unknowingly under-drench the recommended dosage?

1. Yes, they could without knowing	
2. No they do not	
3. Do not know	

Q VIII. Do you change the class of anthelmintics you use each year?

Yes	No
-----	----

Q IX. Do you change the class more than once per year?

Yes	No
-----	----

Q X. Have you had flocks tested for anthelmintic resistant nematode in your area?

Yes	No
-----	----

Q XI Have you had any problems with any anthelmintic?

Yes	No
-----	----

Q XII. Please tick the anthelmintics you used during the last four years (tick for every year).

	1999	2000	2001	2002
ALBENDAZOLE				
1 Fenbendazole				
2 Wormita				
3. Wormex				
4 Oxibendazole				
5 Albendazole				
6 Vetalbel				
7 Thiabendazole				
8 Triclobendazole				
9 Albenol				
LEVAMISOLE				
10 Febantel				
11 Deaxamine				
12 Pamizole sheep				
13 Tetramisole				
14 Bolumisol				
15 Fenbendazole				
AVERMECTINS				
16 Ivermectin				
17 Doramectin				
18 Avimec				
19 Ivectin				
SALICYLANILIDES				
20 Closantel				
21 Rafoxanide				
If others write & tick				

Q XIII Do you know of any farmer in your area who uses herbal preparation for the control of worms in animals?

Yes	No
-----	----

Q XIV. Animals may be treated according to their live-weight. How do you estimate the weight of sheep or goat?

Sheep 1. Visual estimate

2. After weighing

Goats 1 Visual estimate

2 After weighing

Q XV. Which body weight of sheep or goats do you use for estimating dose for drenching (please tick once at sheep and sheep) ?

Sheep 1. Weight of lightest animal

2. Average body weight

3. Weight of heaviest animal

4. Weight of individual animal

Goat 1. Weight of lightest animal

2. Average body weight

3. Weight of heaviest animal

4. Weight of individual animal

Q XVI. Do you agree to participate in similar study in the future?

Yes	No
-----	----

Thank you for your cooperation

## **Annexure 2**

### QUESTIONNAIRE SURVEY ON WORM CONTROL IN SMALL RUMINANTS

**Section II. To be answered by farmers (or his/her representative).**

Farmer's name .....

Zone .....

District .....

Peasant farmers' association (PA) .....

**A. Questions on farm data**

Q I. Is the respondent the farm owner or employee? 

Yes	No
-----	----

1. Farm owner 

Yes	No
-----	----

2. Employee 

Yes	No
-----	----

3. Relative 

Yes	No
-----	----

**Q II. Gender**

1. Female

2. Male

**Q III. What is the level of your education?**



1. Primary

2. Secondary

3. Read & write

4. Illiterate

Q IV. How many sheep do you have?

- 1. Lambs
- 2. Adult
- 3. None


Q V. How many goats do you have?

- 1. Kids
- 2. Adult
- 3. None


Q VI. Where do your sheep and goats graze?

- 1 Back yard
- 2 Communal grazing
- 3 Anywhere the animals get grass

Always (1)	Sometimes (2)	Never (3)

Q VII. Do your sheep graze together with?

1. Cattle

Yes	No
-----	----

2. Horse and donkeys

Yes	No
-----	----

3. Goats

Yes	No
-----	----

Q VIII. What is the condition of the communal grazing area?

- 1. Poor
- 2. Average
- 3. Very good

--

Q IX. How far is the communal grazing area from your house?

1. About Km
2. Between and 2 Km
3. About 3 Km
4. About 5 Km or more

Q X. Do you supply feed to your sheep and goats?

Yes	No
-----	----

Q XI. What type of supplement ?

1. Hay
2. Forage leaves
3. Concentrates

Q XII. Which health problems have encounter with your sheep and goats?

1. Infectious diseases
2. Parasitic diseases (worms)
3. If other specify

Q XIII. When do you think the animals' health deteriorates?

1. During wet season
2. During dry season
3. All year round

Always (1)	Sometimes (2)	Never (3)

Q XIV. Did you have sick animals last season?

Yes	No
-----	----

Q XV. Did your sheep and goats die?

Yes	No
-----	----

Q XVI. Which worms cause problems?

1. Flukes
2. Round worms
3. All worms

B. Questions on worm contro

Q XVII. Do you treat sheep and goats for worm control?

Yes	No
-----	----

Q XVIII. Who takes care of your animal health?

--

1. Self
2. Veterinarians from the Ministry of Agriculture
3. Private veterinary practitioners
4. Traditional healers

Q XIII. When do you think the animals' health deteriorates?

1. During wet season
2. During dry season
3. All year round

Always (1)	Sometimes (2)	Never (3)

Q XIX. How many times do you treat you sheep and goats with anthelmintics?

1. Once in a year
2. Once every six months
3. Only when illness occur
4. More than three times in a year

Commonly (1)	Sometimes (2)	Never (3)

Q XX. Do you know how to dose animals with anthelmintics?

Yes	No
-----	----

Q XXI. How do you recognize worm problems in your sheep and goats?

1. Diarrhoea and/or coughing
2. Emaciation
3. General weakness/illness sign
4. Bottle jaw

Always (1)	Sometimes (2)	Rarely (3)

Q XIV. Do you observe diarrhoea, loss of body condition or other signs in ewes or dams around the time of parturition?

Yes	No

Q XXIII. If you deworm your animals yourself, from where do you obtain the drugs?

1. Open market
2. From drug vendors
3. From other farmers

Always (1)	Sometimes (2)	Rarely (3)

Q XXIV. How important are the following criteria to select the anthelmintics ?

1. Colour of the drug
2. History of the drug efficacy
3. Recommendation by other farmers
4. Recommendation by veterinarians

Very important (1)	Important (2)	Not important (3)

Q XXV. Have you had any problems with any anthelmintics?

Yes	No

Q XXVI. Have you noticed any drug which was not effective?

Yes	No

Q XXVII. Do you know people in your area who use herbs to control worms in animals?

Yes	No

Q XXVIII. How important are the following sources for you to gain knowledge about worms and their management?

1. Farmers' Associations
2. Radio, TV and News papers
3. Extension program of the MOA
4. Government vet. Clinics
5. Private vet. Clinics
6. Rural vet. drug vendors
7. Traditional healers

Very important (1)	Important (2)	Not important (3)

Q XXIX. Was there any governmental or non-governmental program design for worm control strategy in your locality?

Yes	No
-----	----

Q XXX. Do you agree to participate in a project to study the management of worm control in sheep and goats in your area?

Yes	No
-----	----

Thank you for your cooperation

### Annexure 3

**Table A1. Manova Test criteria and exact F statistics for the hypothesis of no weight effect in sheep in the FAMAQCHA<sup>®</sup> trial.**

Statistics	Value	F value	DF	Den DF	Pr>F
Wilks' Lambda	0.0531	25.43	14	20	0.0001
Roy's Greater Root	17.801	25.43	14	20	0.0001

The dependent variables are the levels of monthly weight gain or loss (w1-w15)  
Reject the "H<sub>0</sub>" hypothesis that there is no weight effect (P<0.0001).

**Table A2. Manova Test criteria and exact F statistics for the hypothesis of no weight effect and group interaction in sheep in the FAMAQCHA<sup>®</sup> trial**

Statistics	Value	F value	DF	Den DF	Pr>F
Wilks' Lambda	0.028	3.37	42	60.095	<0.0001
Roy's Greater Root	5.082	7.99	14	22	< 0.0001

The dependent variables are the levels of monthly weight gain or loss (w1-w15)  
Reject the "H<sub>0</sub>" hypothesis that there is no weight\*group effect (P<0.0001).

**Table A3. Manova Test criteria and exact F statistics for the hypothesis of no weight effect in goats in the FAMAQCHA<sup>®</sup> trial.**

Statistics	Value	F value	DF	Den DF	Pr>F
Wilks' Lambda	0.094	17.89	14	26	<0.0001
Roy's Greater Root	9.634	17.89	14	26	< 0.0001

The dependent variables are the levels of monthly weight gain or loss (w1-w15)  
Reject the "H<sub>0</sub>" hypothesis that there is no weight effect (P<0.05).

**Table A4. Manova Test criteria and exact F statistics for the hypothesis of no weight effect and group interaction in goats in the FAMAQCHA<sup>®</sup> trial.**

Statistics	Value	F value	DF	Den DF	Pr>F
Wilks' Lambda	0.161	1.58	42	77.894	<0.004
Roy's Greater Root	9.634	3.75	14	26	< 0.001

The dependent variables are the levels of monthly weight gain or loss (w1-w15)  
Reject the "H<sub>0</sub>" hypothesis that there is no weight\*group effect (P<0.05).

## Annexure 4

Sensitivity, specificity and predictive values for positive and negative tests of sheep using FAMACHA<sup>®</sup> scores and haematocrit cut-off for positive test results and anaemia

FAMACHA <sup>®</sup> categories	Ht<19% n	Ht>19% n	Sensitivity	Specificity	PV* (-ve)	PV# (+ve)
3,4,5	30	74	90.9	86.6	99.3	28.9
1,2	3	476				
Total	33	550				
4,5	24	44	72.7	92.2	98.3	35.3
1,2,3	9	522				
Total	33	550				

n=number of observations, Ht<19% anaemia present, Ht>19% anaemia absent, \*Predictive value positive, #Predictive value negative.

When FAMACHA<sup>®</sup> values equal 4 and 5, the Chi-square = 136.1037, P <0. 0001  
Fishers Exact Test: 2-sided P <3.579E-26, Thus, P <0.001

When FAMACHA<sup>®</sup> values equal 3, 4 and 5, the Chi-square = 197.6721 P <0. 000  
Fishers Exact Test: 2-sided P <5.078E-7, Thus, P <0.0001.

Sensitivity, specificity and predictive values for positive and negative tests of goats using FAMACHA<sup>®</sup> scores and haematocrit cut-off for positive test results and anaemia

FAMACHA <sup>®</sup> categories	Ht<19% n	Ht>19% n	Sensitivity	Specificity	PV * (-ve)	PV # (+ve)
3,4,5	43	114	93.5	81.9	94.4	27.4
1,2	3	514				
Total	46	628				
4,5	35	44	76.1	92.9	98.2	44.3
1,2,3	11	584				
Total	46	628				

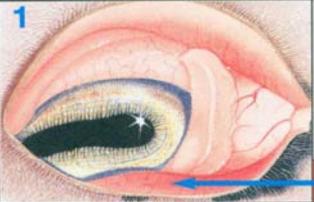
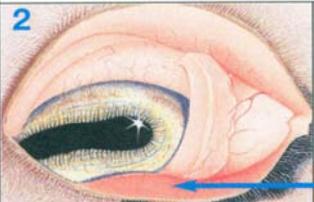
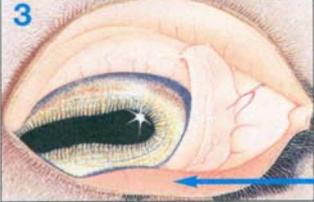
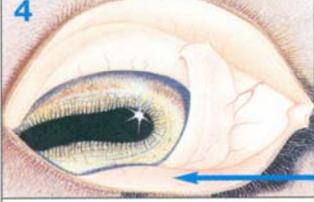
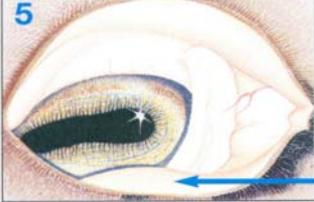
n=number of observations, Ht<19% anaemia present, Ht>19% anaemia absent, \*Predictive value positive, #Predictive value negative.

When FAMACHA<sup>®</sup> values equal 4 and 5, the Chi-square = 175.31105, P <0.001,  
Fishers Exact Test: 2-sided P <3.579E-26, Thus, P <0.001

When the FAMACHA<sup>®</sup> values equal 3, 4 and 5 the Chi-square = 127, 4296, P <0. 0001  
Fishers Exact Test: 2-sided P <3.579E-26, Thus, P <0.001.

# Annexure 5

Obverse

<b>FAMACHA<sup>®</sup></b> <b>ANAEMIA GUIDE</b>	
<b>1</b>	 <div style="display: flex; justify-content: space-between; align-items: center;"> <div style="text-align: center;"> <p><b>OPTIMAL –</b> <b>(NO DOSE)</b></p> </div> <div style="text-align: center;">  </div> </div>
<b>2</b>	 <div style="display: flex; justify-content: space-between; align-items: center;"> <div style="text-align: center;"> <p><b>ACCEPTABLE –</b> <b>(NO DOSE)</b></p> </div> <div style="text-align: center;">  </div> </div>
<b>3</b>	 <div style="display: flex; justify-content: space-between; align-items: center;"> <div style="text-align: center;"> <p><b>BORDERLINE –</b> <b>DOSE?</b></p> </div> <div style="text-align: center;">  </div> </div>
<b>4</b>	 <div style="display: flex; justify-content: space-between; align-items: center;"> <div style="text-align: center;"> <p><b>DANGEROUS –</b> <b>DOSE!</b></p> </div> <div style="text-align: center;">  </div> </div>
<b>5</b>	 <div style="display: flex; justify-content: space-between; align-items: center;"> <div style="text-align: center;"> <p><b>FATAL –</b> <b>DOSE!!!</b></p> </div> <div style="text-align: center;">  </div> </div>

DEVELOPED AND SUPPORTED BY:



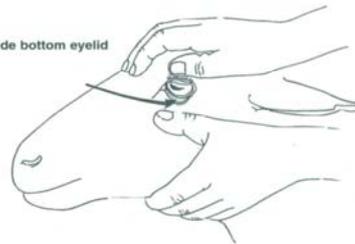
Reverse

**INSTRUCTIONS FOR USE**

**Examination**

- Examine sheep in good, natural light
- Open the eyelid as shown in the sketch
- Push the upper eyelid down with the upper thumb, while the lower thumb gently pulls the lower lid downward
- Look especially at the colour inside the lower eyelid
- Open the eyelid for a short time only, or else the mucous membrane may become redder
- Compare the colours seen to those on the reverse side of this card
- Score the sheep 1 to 5 and proceed as explained in the pamphlet
- If in doubt, score the sheep at the lower (paler) category
- Examine weekly and no less than every 2 to 3 weeks
- Contact your veterinarian if you have any questions

Look inside bottom eyelid



**Precautions**

- Only properly trained persons should use this card
- Read the full information pamphlet before using the guide and follow instructions carefully
- This guide is intended for sheep only
- If used for goats, all those in category 3 should also be treated
- This card is an aid in the control of wireworm only
- Paleness or reddening of the eyes may have other causes
- Maintain standard worm control measures
- The colours of this card will fade with time, especially if exposed to the sun
- Replace the card after 12 months use
- As the system is used in conditions outside their control, no organisation involved in its development or distribution accepts liability for losses or problems associated with its use

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