

Helminth Parasites of Sheep and Goats in Eastern Ethiopia

**Epidemiology, and Anthelmintic Resistance and its
Management**

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Abstract

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A two-year epidemiology study of helminths of small ruminants involved the collection of viscera from 655 sheep and 632 goats from 4 abattoirs in eastern Ethiopia. A further more detailed epidemiology study of gastro-intestinal nematode infections used the Haramaya University (HU) flock of 60 Black Head Ogaden sheep. The parasitological data included numbers of nematode eggs per gram of faeces (EPG), faecal culture L3 larvae, packed red cell volume (PCV), adult worm and early L4 counts, and FAMACHA eye-colour score estimates, along with animal performance (body weight change).

There were 13 species of nematodes and 4 species of flukes present in the sheep and goats, with *Haemonchus contortus* being the most prevalent (65–80%), followed by *Trichostrongylus* spp. The nematode infection levels of both sheep and goats followed the bi-modal annual rainfall pattern, with the highest worm burdens occurring during the two rain seasons (peaks in May and September). There were significant differences in worm burdens between the 4 geographic locations for both sheep and goats. Similar seasonal but not geographical variations occurred in the prevalence of flukes. There were significant correlations between EPG and PCV, EPG and FAMACHA scores, and PCV and FAMACHA scores. Moreover, *H. contortus* showed an increased propensity for arrested development during the dry seasons.

Faecal egg count reduction tests (FECRT) conducted on the HU flocks, and flocks in surrounding small-holder communities, evaluated the efficacy of commonly used anthelmintics, including albendazole (ABZ), tetramisole (TET), a combination (ABZ + TET) and ivermectin (IVM). Initially, high levels of resistance to all of the anthelmintics were found in the HU goat flock but not in the sheep. In an attempt to restore the anthelmintic efficacy a new management system was applied to the HU goat flock, including: eliminating the existing parasite infections in the goats, exclusion from the traditional goat pastures, and initiation of communal grazing of the goats with the HU sheep and animals of the local small-holder farmers. Subsequent FECRTs revealed high levels of efficacy of all three drugs in the goat and sheep flocks, demonstrating that anthelmintic efficacy can be restored by exploiting refugia.

Individual FECRTs were also conducted on 8 sheep and goat flocks owned by neighbouring small-holder farmers, who received breeding stock from the HU. In each FECRT, 50 local breed sheep and goats, 6–9 months old, were divided into 5 treatment groups: ABZ, TET, ABZ + TET, IVM and untreated control. There was no evidence of anthelmintic resistance in the nematodes, indicating that dilution of resistant parasites, which are likely to be imported with introduced breeding goats, and the low selection pressure imposed by the small-holder farmers, had prevented anthelmintic resistance from emerging.

Keywords: Africa, Ethiopia, tropical, semi-arid, goat, sheep, small ruminant, small-holder, parasite, helminth, nematode, trematode, fluke, epidemiology, prevalence, anthelmintic resistance, refugia, FAMACHA, *Fasciola*, *Haemonchus*, *Paramphistomum*, *Trichostrongylus*.

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To

My parents

My wife, Fentanesh Emiru Zerihun

My daughters, Bethelhem and Haregewoin

My son, Yohannes

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This thesis is based on the following papers, which will be referred to by their Roman numerals.

- I. Sissay, M. M., Uggla, A. & Waller, P. J., 2006. Epidemiology and seasonal dynamics of gastro-intestinal nematode infections of sheep in a semi-arid region of eastern Ethiopia. *Veterinary Parasitology*, 143, 311-321.
- II. Sissay, M. M., Uggla, A. & Waller, P. J., 2007. Prevalence and seasonal incidence of helminth parasite infections of sheep and goats in eastern Ethiopia: I. Nematodes and trematodes. *Tropical Animal Health and Production* (accepted for publication).
- III. Sissay, M. M., Asefa, A., Uggla, A. & Waller, P. J. 2006. Anthelmintic resistance of nematode parasites of small ruminants in eastern Ethiopia: Exploitation of refugia to restore anthelmintic efficacy. *Veterinary Parasitology*, 135, 337-346.
- IV. Sissay, M. M., Asefa, A., Uggla, A. & Waller, P. J., 2006. Assessment of anthelmintic resistance in nematode parasites of sheep and goats owned by smallholder farmers in eastern Ethiopia. *Tropical Animal Health and Production*, 38, 215-222.

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Abbreviations

ABZ	Albendazole
BHO	Black Head Ogaden sheep
BW	Body weight
EL4	Early fourth-stage larvae (inhibited larvae)
EPG	Eggs per gram of faeces
FAMACHA	Anaemia guide chart with 5-colour scale
FECRT	Faecal egg count reduction test
GI	Gastro-intestinal
GLM	General linear model
HU	Haramaya University
IVM	Ivermectin
L1	First-stage larvae
L2	Second-stage larvae
L3	Infective third-stage larvae
L4	Fourth-stage larvae
m.a.s.l.	Metres above sea level
PA	Peasant association
PCV	Packed red cell volume
PGE	Parasitic gastroenteritis
PPR	Periparturient rise
TET	Tetramisole
TWC	Tracer worm counts

Introduction

This study was performed to identify the important nematode and fluke parasites of sheep and goats in eastern Ethiopia, and to determine factors affecting their epidemiology. An evaluation of the efficacy of the anthelmintic drugs commonly used for treatment of small ruminants in the region was also done.

Ethiopia lies within the tropical latitudes of Africa, and has an extremely diverse topography, a wide range of climatic features and a multitude of agro-ecological zones, which makes the country suitable for different agricultural production systems. This in turn has contributed to the existence of a large diversity of farm-animal genetic resources in the country (Anon., 2004). Ethiopian livestock production systems are broadly characterized as low input, mixed crop-livestock, agro-pastoral and pastoral systems; as well as medium input, peri-urban and urban enterprises (Anon., 2004). The current cattle, sheep, goat and camel populations of Ethiopia are approximately 38, 23, 18 and 1 million, respectively (Anon., 2005). These livestock are almost entirely managed by the resource-poor, small-holder farmers and pastoralists. However, they make a critical contribution to food self-sufficiency for rural households by providing milk, meat, skin, manure and traction, as well as generating direct cash income. In addition, livestock are a source of risk mitigation against crop failures. Small ruminants (sheep and goats) are particularly important resources for their owners, because they require smaller investments, have shorter production cycles, faster growth rates and greater environmental adaptability than cattle. Therefore, they form an important economic and ecological niche in all agricultural systems throughout the country.

Despite the large livestock population of Ethiopia, the economic benefits remain marginal due to prevailing diseases, poor nutrition, poor animal production systems, reproductive inefficiency, management constraints and general lack of veterinary care. The most prevalent animal diseases include trypanosomiasis, foot and mouth disease, bovine pneumonia, peste des petits ruminants, contagious caprine pleuro-pneumonia, lumpy skin diseases and helminth parasitism. These diseases have a major impact on morbidity and mortality rates, with annual losses as high as 30–50% of the total value of livestock products of Ethiopia (Anon., 1992; Mukasa-Mugerwa *et al.*, 2000; Tibbo *et al.*, 2001; Kassa, 2003; Tibbo *et al.*, 2003). It is also estimated that approximately 2 million cattle and 5–7 million sheep and goats die from these diseases and malnutrition each year in Ethiopia, accounting for an annual financial loss in excess of 90 million USD (Anon., 1992; Tilahun, 1993). Even more significant, however, are the enormous losses resulting from inferior weight gains, lower milk yields and condemnation of organs and carcasses at slaughter (Bekele *et al.*, 1992; Ngategize *et al.*, 1993; Tilahun, 1993). Endoparasites are responsible for the death of one third of calves, lambs and kids, and considerable losses of parts of carcasses condemned during meat inspection (Anon., 1997; Anon., 2000b).

It is well recognized that in resource-poor regions of the world, helminth infections of sheep and goats are major factors responsible for economic losses through reduction in productivity and increased mortality (Over *et al.*, 1992;

Anon., 1994; Gatongi *et al.*, 1997; Nari *et al.*, 1997; Perry & Randolph, 1999; Perry *et al.*, 2002; Tibbo *et al.*, 2006). Small-holders or pastoralists may not easily detect the effects of internal parasites on their animals, because of the generally sub-clinical or chronic nature of the helminth infections (Soulsby, 1982; Urquhart *et al.*, 1996). Thus, the sub-clinical parasite infections are responsible for significant economic loss, because once clinical disease is noticed in a group of animals much economic loss in terms of animal productivity has already occurred (Kaplan, 2006; Tibbo *et al.*, 2006).

Although helminth parasites of ruminant livestock are ubiquitous in all of the agro-climatic zones of Ethiopia with prevailing weather conditions that provide favourable condition for their survival and development, their presence does not mean that they cause overt diseases. Therefore, it is important to assess the type and level of parasitism in ruminant livestock, in order to be able to determine the significance of parasite infections and to recommend the most beneficial and economically acceptable control measures. Therefore, the first step in the investigation of helminth infections of ruminants is to establish what parasite species are present in an area, or region.

Although the causes of helminth parasitism in ruminant livestock are multiple and often interactive, the vast majority of cases are due to any of the following basic reasons (Urquhart *et al.*, 1996):

- (1) an increase in the number of infective stages on pasture
- (2) an alteration in host susceptibility
- (3) the introduction of susceptible stock into an infected environment
- (4) the introduction of infections into an environment
- (5) ineffective parasite removal from the host animals due to poor administration techniques, the use of sub-standard anthelmintic drugs and/or the development of anthelmintic resistance.

Practical, cost-efficient control of helminth infections is only possible after surveillance has provided enough information to understand the prevailing epidemiological factors influencing transmission. Thus, control programmes require knowledge of the most important sources of contamination, which result in the seasonal peaks of infection on pasture.

In Ethiopia, parasitological investigations of small ruminants in the humid central highland regions of the country have demonstrated that nematodes of the genera *Haemonchus*, *Trichostrongylus*, *Oesophagostomum*, *Bunostomum*, *Strongyloides*, *Cooperia*, *Nematodirus* and *Trichuris* are the most common (Bekele *et al.*, 1992; Tembely, 1995; Tembely *et al.*, 1997). The liver flukes of the genus *Fasciola* (*F. hepatica* and *F. gigantica*) are also of particular importance in ruminant livestock found in these regions (Over *et al.*, 1992; Ngategize *et al.*, 1993; Tilahun, 1993; Anon., 1994; Mezgebu, 1995). A study of lungworm infections in small ruminants in north-eastern Ethiopia by Alemu *et al.* (2006) showed the presence of three respiratory nematodes, *Dictyocaulus filaria*, *Muellerius capillaris* and *Protostrongylus rufescens*. Another study by Debela (2002) also reported that *H. contortus*, *Strongyloides papillosus* and *Trichostrongylus* spp. were the most prevalent gastro-intestinal (GI) nematode species infecting goats in the Rift Valley of southern Ethiopia. However,

information on the importance of helminth parasites of small ruminants in the semi-arid region of eastern Ethiopia is scanty.

Small-holder farmers and pastoralists of Ethiopia practice varying degrees of parasite control in their livestock. These practices range from the use of anthelmintic drugs of varying quality, to the use of traditional medicines (Adugna, 1990). In Kenya, ethno-veterinary remedies are widely used by pastoralists and small-holder farmers for treatment of their livestock against helminth parasites (Githiori, 2004). Treatments are generally given during the rainy season, ranging from occasional *ad hoc* treatments that are typical of the small-holder farmers, to more frequent administration on institutional (University and Research Station) farms. The consequence of inappropriate anthelmintic treatment procedures (e.g. poor quality drugs, poor dosing procedures, intensive use, etc.), has been the development of resistance to the three classes of broad-spectrum anthelmintic drugs (benzimidazoles, imidothiazoles and macrocyclic lactones) in countries that have significant small ruminant populations (Kaplan, 2004; Coles, 2005).

Anthelmintic resistance has been reported throughout Africa, being a particularly serious problem in South Africa and to a lesser extent in Kenya (Vatta & Lindberg, 2006). In Ethiopia, the presence of anthelmintic resistance has not been reported, although it may be expected to be much less of a problem, because of the almost universal practice of communal grazing, and the perceived low frequency of treatment practiced by the small-holder farmers. This was evidenced by a recent study by Asmare *et al.* (2005), which showed the absence of anthelmintic resistance in GI nematodes of sheep and goat flocks owned by small-holder farmers in the southern parts of Ethiopia. However, there is no published information on the status of anthelmintic resistance to GI nematodes of small ruminants owned by the research institute, small-holder farmers and pastoralist communities in eastern regions of Ethiopia. Thus, it is essential to detect anthelmintic resistance early in the course of its development, so that appropriate control strategies can be designed and implemented to prevent the further development and spread of resistant worms (Kaplan, 2006).

Therefore, this PhD study was aimed at overcoming the lack of knowledge of parasite infections of small ruminants raised in the semi-arid part of eastern Ethiopia. In addition, investigations into the efficacy of anthelmintics that are commonly used for treatments of sheep and goats in this region of the country were undertaken.

Background

Physico-geographical and climatic features of Ethiopia

Ethiopia is located in east Africa, bordering Eritrea in the north, Djibouti and Somalia in the east, Kenya in the south and Sudan in the west. The country lies between geographical co-ordinates of 3° 24' and 14° 53' North and 32° 42' and 48° 12' East (see Figure 1). It covers an area of 1,220,000 km², and varies in altitude from almost 110 m below sea level to over 4,600 m above sea level (m.a.s.l.). The country is divided into nine federal states: Tigray, Afar, Amhara, Oromia, Somalia, Benshangul-Gumuz, Southern Nations, Nationalities and Peoples, Gambella Peoples, and Harari People; and two city administrations (councils): Addis Ababa and Dire Dawa. The current population of Ethiopia is estimated to be 75 million, with an annual growth rate of 2.7% (Anon., 2007). Ethiopia has a diverse mix of people with different ethnic and linguistic backgrounds. There are more than 80 ethnic groups, each with its own language and dialect, culture and traditions. There are four main language groups: Semitic, Cushitic, Omotic and Nilo-Saharan.



Figure 1. Topographic map of Ethiopia (National Geographic Society, 2001).

Ethiopia has a wide range of climatic features as a result of its location in the African tropical zone, the varied topography and elevation, and the seasonal changes in atmospheric pressure systems that control the prevailing winds (Anon., 2000a). The topographic diversity and climatic heterogeneity have resulted in the formation of a multitude of agro-ecological zones with varied agricultural production systems. Eighteen major agro-ecological zones and 49 sub-zones have been identified based on homogeneity in terms of climate, physiography, soils, vegetation, land use, farming system and animal production (Anon., 1998; Anon., 2000a). The different agro-ecological zones are traditionally classified into five categories based on altitude and rainfall. The five zones, from the highest to the lowest altitudes with traditional names assigned to each zone, are: “wurch” (alpine or very cold), “dega” (moist cold or cold), “woyna dega” (moist-cool or dry-warm or semi-arid), “kolla” (sub-moist or arid), and “bereha” (dry-hot or desert) (Anon., 1998; Anon., 2000a).

Epidemiology of helminths of sheep and goats in Africa

Epidemiology is the study of diseases or infections in host populations and the factors that determine their occurrence. In addition, it includes investigation and assessment of other health-related events in livestock, such as productivity and resistance to the drugs used to control these infections. The study of the epidemiology of helminthoses in livestock thus encompasses the factors that affect the prevalence and intensity of helminth infections, and how these affect animals in terms of clinical disease, as well as the economic effects of productivity losses. The epidemiology of the helminth parasitic diseases therefore depends on factors such as the infection pressure in the environment and the susceptibility of the host species (or individual). The infection pressure, in turn, depends on factors that affect the free-living and intermediate stages, such as temperature, rainfall and moisture. Furthermore, the availability of large numbers of susceptible definitive and intermediate hosts will increase the parasites' ability to reproduce and result in high parasite abundance (Torgerson & Claxton, 1999).

Parasitic helminths of small ruminants belong to the phyla Platyhelminthes (flatworms) and Nematelminthes (roundworms) (Soulsby, 1982; Urquhart *et al.*, 1996). The former phylum has two classes, Cestoda (tapeworms) and Trematoda (flukes). The most important cestode parasites of small ruminants, both in terms of public health and veterinary medicine, belong to the family Taeniidae. These include cystic or larval stages of *Echinococcus granulosus*, *Taenia hydatigena*, *T. ovis* and *T. multiceps*. Moreover, adult cestode parasites of the genera *Moniezia*, *Avitellina*, *Thysanosoma* and *Stilesia* are also common parasites of small ruminants in African countries (Urquhart *et al.*, 1996). All of the trematode species that are parasitic in small ruminants belong to the subclass Digenea. The most important of these species in Africa are the liver flukes, *F. hepatica*, *F. gigantica* and *Dicrocoelium* spp., and rumen flukes (paramphistomes), *Paramphistomum* spp. (Soulsby, 1982; Anon., 1994; Hansen & Perry, 1994; Urquhart *et al.*, 1996).

The Nematelminthes (nematodes) include several superfamilies of veterinary importance. These are Trichostrongyloidea, Strongyloidea, Metastrongyloidea, Ancylostomatoidea, Rhabditoidea, Trichuroidea, Filarioidea, Oxyuroidea, Ascaridoidea and Spiruroidea. The GI nematodes of greatest importance in small ruminants are members of the order Strongylida, which contains the first four superfamilies, but most belong to the superfamily Trichostrongyloidea. All grazing sheep and goats are infected with a community of these strongylid nematodes, whose combined clinical effect is the condition known as parasitic gastroenteritis (PGE) (Zajac, 2006).

Helminth infections, or helminthoses, thus refer to a complex of conditions caused by parasites of the Nematoda, Cestoda and Trematoda. Although all grazing sheep and goats may be infected with the above-mentioned parasites, low worm burdens usually have little impact on animal health. But as the worm numbers increase, effects in the form of reduced weight gain and decreased appetite occur. With heavier worm burdens clinical signs such as weight loss, diarrhoea, anaemia, or sub-mandibular oedema (bottle jaw) may develop.

Gastro-intestinal nematodes

The most important strongylid nematodes of sheep and goats in African countries are: *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus* spp. (*T. axei*, *T. colubriformis* and *T. vitrinus*). Other species of lesser importance include *Pseudomaraschidia* (*Longistrongylus*) *elongata*, *Nematodirus* spp. (*N. spathiger* and *N. filicollis*), *Cooperia curticei*, *Bunostomum trigonocephalum*, *Gaigeria pachycelis*, *Oesophagostomum* spp. (*O. venulosum* and *O. columbianum*) and *Chabertia ovina* (Hansen & Perry, 1994). Other GI nematodes belonging to different taxonomic orders also commonly parasitize the small and large bowel of sheep and goats but are not considered to be important pathogens, and cause disease only in rare circumstances. These nematodes include *Strongyloides papillosus* and *Trichuris ovis*.

The life cycles are direct, requiring no intermediate hosts, which applies to all of the economically important strongylid parasites of small ruminants (Hansen & Perry, 1994; Urquhart *et al.*, 1996). In these cycles, adult female parasites in the GI tract produce eggs that are passed out with the faeces of the animal (see Figure 2). Development occurs within the faecal mass, the eggs embryonate and hatch into first-stage larvae (L1), which in turn moult into second-stage larvae (L2), shedding their protective cuticle in the process. During this time the larvae feed on bacteria. The L2 moult into third-stage larvae (L3), but retain the cuticle from the previous moult. The L3 constitute the infective stage, and these migrate onto surrounding vegetation where they become available for ingestion by grazing sheep and goats.

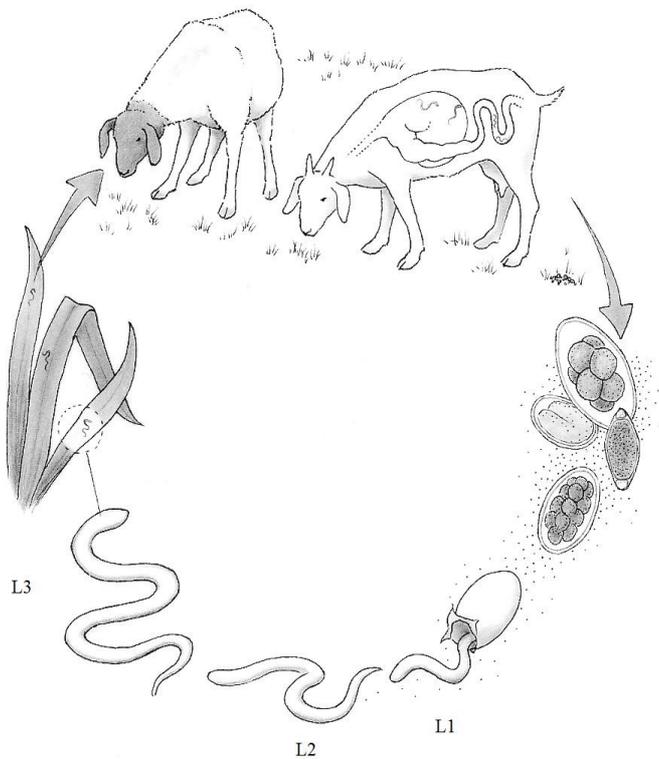


Figure 2. Principal life-cycle of GI nematodes. (Picture: Katarina Näslund).

Following ingestion, the L3 larvae pass to the abomasum or intestine, where they ex-sheathe. The L3 of the trichostrongyle worms penetrate the epithelial layer of the mucus membrane (in the case of *Haemonchus* and *Trichostrongylus*) or enter the gastric glands (*Teladorsagia*). In normal development, the L3 moult within 2–3 days to become fourth-stage larvae (L4), which remain in the mucous membrane or in the gastric glands for a further 10 to 14 days. Finally, the L4 emerge and moult to become young adult parasites. The time between ingestion of L3 and the parasite becoming mature adults (referred to as the prepatent period) varies between parasite species, but generally is between 3 and 5 weeks. *Nematodirus*, *Trichuris*, *Bunostomum*, *Gaigeria* and *Strongyloides* species are exceptions to the lifecycle described above, the details of which can be found in key texts (Soulsby, 1982; Urquhart *et al.*, 1996) but will not be considered here.

The development, survival and transmission of the free-living stages of nematode parasites are influenced by micro-climatic factors within the faecal pellets and herbage. These include sunlight, temperature, rainfall, humidity and soil moisture. Under optimal conditions (high humidity and warm temperature), the development process takes 7 to 10 days, but for *H. contortus* a more rapid translation of eggs to larvae can occur in warm wet conditions. In most African countries, the temperatures are permanently favourable for larval development in

the environment. Development of trichostrongylid larvae occurs in a temperature range of approximately 10–36 °C. The optimal humidity requirement for free-living stage development of most species is 85%. Although desiccation is lethal for the free-living stages of parasite worms, the important nematode parasites can survive such conditions either as embryonated eggs or as infective larvae (Donald, 1968; Tembely, 1998; O'Connor *et al.*, 2006). The L3 of trichostrongylid nematodes may survive for varying periods, depending on species and particularly the prevailing weather conditions. In the desiccated state L3 can survive for several months, but once hydrated they become active and rapidly exhaust their food reserves (Tembely, 1998; Torina *et al.*, 2004; O'Connor *et al.*, 2006).

In general, the combined effects of these factors are responsible for the seasonal fluctuations in the availability of L3 on pasture, and subsequently in the prevalence of worm burdens in the hosts. This seasonal variation of parasite population dynamics has been described in a number of studies in many African countries (Assoku, 1981; Vercruysse, 1983; Van Wyk, 1985; Faka, 1990; Fritsche *et al.*, 1993; Maingi *et al.*, 1993; Pandey *et al.*, 1994; Tilahun, 1995; Tembely *et al.*, 1997; Nginyi *et al.*, 2001; Debela, 2002). In general, rapid translation of eggs through to L3 occurs throughout most of the rainy seasons, and grazing animals acquire the highest infections during these times. However, in the humid tropical climate of West Africa, as well as in the regions surrounding Lake Victoria and parts of coastal eastern Africa, the climatic conditions permit development of eggs and larval stages more-or-less continuously throughout the year (Chiejina *et al.*, 1989; Hansen & Perry, 1994; Tembely, 1998; O'Connor *et al.*, 2006). In the arid tropical climates of lowland areas of Ethiopia, parts of Somalia and Sudan, there exists an environmental gradation which ranges from deserts, to extensive pasturelands and browse plants, to intensive grazing areas around permanent water courses (lakes and rivers) and to irrigation. Thus, there exists a range of environments, which range from being hostile to those that are most favourable for development and survival of free-living stages of the parasites.

The seasonal fluctuations in numbers and availability of the infective larval stages are also influenced by the level of contamination of the pasture. The latter is controlled by the biotic potential (fecundity) of the adult parasites in the host, the density of stocking, and the immune status of the host (Hansen & Perry, 1994; Urquhart *et al.*, 1996). Although different species of GI nematodes of small ruminants have varying egg-producing capacities, *H. contortus* is one of the most prolific nematodes. A female *H. contortus* may produce thousands of eggs each day, and larval numbers on pasture can rapidly increase during the wet seasons (Soulsby, 1982; Hansen & Perry, 1994; Urquhart *et al.*, 1996).

The number of eggs produced by an adult female nematode is influenced by the level of host immunity to the parasites. In sheep and goats, adult female nematodes may increase their egg output around the time of parturition. This phenomenon, known as the peri-parturient rise (PPR), is of great importance in the epidemiology of GI nematodes of small ruminants, and has been reported in different African countries (Connan, 1976; Agyei *et al.*, 1991; Tembely, 1995; Ng'ang'a *et al.*, 2006). A PPR in nematode egg excretion has been observed as early as 2 weeks

before lambing and kidding, and persisted up to 8 weeks post-partum when lambing and kidding took place during the wet seasons (Zajac *et al.*, 1988; Agyei *et al.*, 1991; Tembely, 1995; Ng'ang'a *et al.*, 2006). Thus, pregnant, or lactating, ewes and does become the major source of infection for the new-born lambs and kids.

Overstocking is a major problem in Africa outside the tsetse fly (*Glossinia* spp.) infested areas (Hansen & Perry, 1994). In addition to contributing to pasture degradation and soil erosion, this forces the animals to graze closer to faecal material, which results in the uptake of higher numbers of infective larvae. Thus, high stocking density also increases the level of contamination of the pasture with the free-living larval stages of the GI nematodes. This may be exacerbated when the majority of the flocks became susceptible to parasite infections as a result of inadequate nutrition, pregnancy, or lactation (Gibson & Everett, 1971; Connan, 1976; Le Jambre, 1984; Coop *et al.*, 1990). It is well known that adequately fed animals are more able to tolerate parasitism than are animals on a low plane of nutrition (Knox & Steel, 1996; Waruiru *et al.*, 2004; Knox *et al.*, 2006). Thus, small ruminants affected by blood-sucking parasites, such as *H. contortus*, may be able to maintain their haemoglobin levels as long as their iron and protein intakes are adequate. However, if the animals' iron reserves and protein intake are reduced then their haemopoietic systems become exhausted, and they may die (Abbott *et al.*, 1986; Gibbs & Barger, 1986; Rowe *et al.*, 1988; Vatta *et al.*, 2002).

The epidemiology of nematode parasites of small ruminants is also strongly influenced by aspects of host-parasite biology after infection occurs. Larvae of important GI nematodes are able to undergo a period of arrested development (hypobiosis) in the host (in the abomasal or intestinal mucosae). Following infection, larvae may become metabolically inactive for several months. Although the immune status of the host also has an influence on rates of hypobiosis, the greatest proportion of larvae usually becomes arrested at times when conditions in the external environment are least favourable for development and survival of eggs and larvae (Michel *et al.*, 1975; Ogunsusi & Eysker, 1979; Chiejina *et al.*, 1988; El-Azazy, 1995; Eysker, 1997). This suspension of development helps some nematode parasites to survive the dry seasons. Resumption of development usually coincides with the onset of the rainy seasons, the most favourable period for larval development and transmission on pasture (Agyei *et al.*, 1991; El-Azazy, 1995; Tembely, 1995). The stimuli for the onset of arrested development in tropical areas, which are linked to the dry conditions, are in contrast to those stimuli (e.g. falling temperatures) for nematode parasites of ruminant livestock found in temperate zones (Allonby & Urquhart, 1975; Vercruysse, 1985; Chiejina *et al.*, 1988; El-Azazy, 1995).

Liver and gastro-intestinal trematodes

The life-cycles of important trematode species (*Fasciola hepatica*, *F. gigantica* *Parampistomum* spp. and *Dicrocoelium* spp.) of small ruminants all involve intermediate hosts such as different species of aquatic or terrestrial snails, and ants for *Dicrocoelium* species. The details of these life-cycles are found in key texts (Urquhart, *et al.*, 1996) and will not be considered here. The epidemiology of

fluke infections in small ruminants depends on many variables, including the presence of suitable intermediate hosts as well as favourable climatic and ecological conditions for them. The factors influencing the development and survival of both the larval stages of the flukes and their intermediate hosts are similar to those of the nematode parasites described earlier. These environmental factors include temperature, rainfall, humidity and moisture (Anon., 1994; Hansen & Perry, 1994; Urquhart *et al.*, 1996; Torgerson & Claxton, 1999).

Sufficient moisture is required for the fluke eggs to separate from the faecal material, and for development and hatching to occur. The ideal moisture conditions for snail breeding and the development of flukes within snails are provided when rainfall exceeds transpiration, and field saturation is attained. A mean day/night temperature of 10 °C or above is necessary, both for snails to breed and for the development of flukes within the snail. Activity ceases at temperatures below 5 °C or above 30 °C (Anon., 1994; Hansen & Perry, 1994; Urquhart *et al.*, 1996; Torgerson & Claxton, 1999).

In most parts of Africa, the temperatures and moisture are favourable for embryonation and hatching of the fluke eggs, development and survival of fluke larvae in the intermediate host, as well as for breeding of the snail host. In those African countries with distinct wet and dry seasons, it appears that optimal development of eggs to miracidia occurs at the beginning of the wet season, and development within the snail is complete by the end of the rains. Shedding of cercariae commences at the start of the dry season, when the water level is still high, and continues as the water level drops. The animals then ingest the metacercariae while grazing on these areas during the dry season, and clinical problems, occur at the end of that season or at the beginning of the next wet season, depending on the rate of infection (Urquhart *et al.*, 1996).

Diagnosis of helminth infections in small ruminants

The diagnosis of helminth parasites of small ruminants is based on demonstrating the presence of helminth eggs, or larvae, in faecal samples, or the presence of parasites recovered from the digestive tracts or other viscera of the animals. Although a great variety of methods and modifications have been described for such diagnosis, standardization of techniques, such as egg or larval counts, worm counts, pasture larval counts, etc., does not exist. Therefore, in practice, most diagnostic laboratories as well as teaching and research establishments apply their own set and protocols of test procedures (Kassai, 1999). The following diagnostic procedures for helminth infections of small ruminants are relevant to African conditions.

Faecal examination by means of the modified McMaster technique for the enumeration of worm eggs and larval differentiation by faecal culture methods are the most common routine means for the diagnose helminthosis in small ruminants. The strongylid nematode genera produce eggs that are similar in appearance and cannot be easily discriminated, which means that genus identification cannot accurately be made by faecal examination alone. To identify nematodes in faecal samples, faecal cultures are required to yield L3 larvae, which generally can be

differentiated to genus level (Anon., 1986; Hansen & Perry, 1994; Urquhart *et al.*, 1996; Kassai, 1999; Van Wyk *et al.*, 2004). *Nematodirus*, *Strongyloides* and *Trichuris* species have eggs that can be differentiated by their distinct morphological features.

Post-mortem examinations and identification of adult worms and arrested larvae (EL4) in animals are the definitive means of identifying the parasite infection status of animals. Similar to faecal egg counts, there are many procedures that are described for post-mortem examination for nematode parasites (Anon., 1986; Hansen & Perry, 1994; Urquhart *et al.*, 1996; Kassai, 1999).

In areas where haematophagous (blood-sucking) worm species, particularly *H. contortus*, are prevalent, estimation of clinical anaemia by examining ocular mucous membranes of sheep and goats using the FAMACHA[®] chart (Bath *et al.*, 1996) is becoming a common diagnostic procedure. This procedure of estimation of clinical anaemia can give a relatively reliable approximation of the haematological status of sheep and goats suffering from the haematophagous worm infections, especially at lower haematocrit values (Van Wyk & Bath, 2002).

Anthelmintic resistance and its detection

Despite success in the development of anthelmintics in the later part of the last century, helminth infections continue to play a significant role in limiting livestock productivity, particularly that of small ruminants, worldwide (Waller, 2006). The appearance over the last decades of populations of parasitic worms that have developed resistance to one or more of the available anthelmintic groups has threatened livestock productivity globally (Sangster, 1999; Jackson & Coop, 2000; Kaplan, 2004; Miller & Waller, 2004; Waller, 2006). Although many factors are involved in the evolution of anthelmintic resistance, the proportion of the parasite population under drug selection is believed to be the single most important factor in determining how rapidly resistance will develop (Kaplan, 2004).

In most regions of Africa, the development of anthelmintic resistance could be expected to be slow, because of limited availability and infrequent use of anthelmintics by most small-scale farmers. The exception is South Africa, where on large-scale commercial sheep farms the intensive use of anthelmintics for several decades has led to very high levels of multiple anthelmintic resistance (Van Wyk *et al.*, 1999). Although the overall prevalence of anthelmintic resistance has not been extensively investigated throughout the African continent, anthelmintic resistance in sheep and goat parasites has been reported from at least 14 countries (Vatta & Lindberg, 2006).

The growing importance of anthelmintic resistance has led to an increased need for reliable and standardized detection methods. A variety of *in vivo* (faecal egg count reduction tests (Coles *et al.*, 1992) and controlled test (Wood *et al.*, 1995)) and *in vitro* (egg hatch assays (Dobson *et al.*, 1986), larval paralysis, migration and motility tests (Martin & LeJambre, 1979; Varady & Corba, 1999), larval development assay (Taylor, 1990), adult development test (Stringfellow, 1988; Small & Coles, 1993), bio-chemical tests (Lacey & Snowden, 1988) and

molecular techniques (Roos *et al.*, 1990)) have been developed for the detection of resistance to the main anthelmintic groups. However, each test has some shortcomings, which may include high cost, poor reliability, reproducibility, sensitivity and ease of interpretation (Torgerson *et al.*, 2005; Coles *et al.*, 2006). These methods have been reviewed by Taylor (2002).

The faecal egg count reduction test (FECRT) provides an estimation of anthelmintic efficacy by comparing faecal egg counts before treatment with those taken 10–14 days after treatment (Boersema, 1983; Presidente, 1985). The arithmetic mean faecal egg counts are used for the interpretation of data, and resistance is considered to be present if the percentage of reduction is less than 95% and the 95% lower confidence limit is less than 90%. If only one of the two criteria is met, resistance is suspected (Anon., 1989). To conduct an FECRT, a minimum of 10–15 animals should be randomly selected for the untreated control group, as well as for each drug to be tested. Guidelines that give precise details and recommendations on the use of the FECRT have been produced (Anon., 1989, Coles *et al.*, 1992).

A series of *in vitro* tests have been developed to detect anthelmintic resistance (e.g. egg hatch and larval motility tests; O'Grady & Kotze, 2004; Kotze *et al.*, 2006), but these are largely inappropriate for African conditions. However there has been an *in vitro* kit that has been commercially developed (DrenchRite[®] test), and which has been modified for surveying the prevalence of anthelmintic resistance in nematode parasites of small ruminants in various south-east Asian countries (Anon., 1996).

Aims of the study

This PhD project aimed to address some of the gaps in knowledge of parasite infections of small ruminants raised in the semi-arid region of eastern Ethiopia. The study was intended to provide a better understanding of the importance of these parasites and their environmental interactions in the management of livestock, and to suggest possible control options for the small-holder farmers of this region. The study was planned and executed as four subprojects, each of which is presented in the form of a separate publication.

Specific objectives were:

- To determine the prevalence, seasonal dynamics and intensity of gastro-intestinal nematode infections in communally grazing sheep in the Haramaya district of eastern Ethiopia.
- To determine the prevalence and seasonal incidence of gastro-intestinal nematode and trematode infections in sheep and goats slaughtered at four abattoirs located at different sites in eastern Ethiopia.
- To identify the optimal times of the year for strategic worm control.
- To assess the anthelmintic resistance status of the Haramaya University goat and sheep flocks, which are used to supply breeding stock to surrounding small-holder farmers.
- To explore the possibility of reducing the problem of anthelmintic resistance by replacing resistant worm populations with anthelmintic-susceptible parasite populations on communal pastures.
- To evaluate the efficacy of anthelmintics used for treatment of parasites by small-holder farmers in eastern Ethiopia.

Methodological considerations

Design of the study

This series of studies was designed and subsequently executed as four separate studies (papers I–IV). The first two investigations (papers I & II) focused on describing the epidemiology of the GI nematode and fluke infections of sheep and goats in the semi-arid areas of eastern Ethiopia. An experimental study on the epidemiology of GI nematode parasite infections in sheep was conducted for 2.5 years (May 2003 to September 2005) utilizing the Haramaya University (HU) sheep flock (paper I). A parallel 2-year abattoir survey of the prevalence of helminth infections of sheep and goats was carried out from May 2003 to April 2005, on animals slaughtered at four abattoirs located in the towns of Haramaya, Harar, Dire Dawa and Jijiga, in eastern Ethiopia (paper II).

A further two investigations (papers III & IV) involved an evaluation of the efficacy of anthelmintics that are commonly used for treatment of GI nematodes in small ruminants in eastern Ethiopia. This also included the elimination of anthelmintic-resistant parasite populations in the HU goat flock, by exploiting refugia (paper III). These studies were conducted on the HU flocks (paper III) and communal flocks owned by small-holder farmers in neighbouring communities (paper IV).

Study area

The study was carried out in the Haramaya, Harar, Dire Dawa and Jijiga farming areas of eastern Ethiopia. These regions are located 500–600 km east of Addis Ababa. The areas differ in both topography and climate. The towns of Haramaya and Harar are approximately 2000 m above sea level and the surrounding farming areas are semi-arid, although there are large areas of communal grazing around ephemeral water sources and lakes in the Haramaya district. Jijiga is located at a lower elevation (1600 m), and Dire Dawa is in the Rift Valley at 1100 m. Dire Dawa and Jijiga districts are hotter and drier and are generally more arid than those of the Haramaya and Harar districts. Details of each of the study areas are found in the material and methods sections of papers I–IV.

Study animals

Haramaya University flocks

The HU has a sheep flock of approximately 400 head of the indigenous Black Head Ogaden (BHO) breed, which are managed separately from all other livestock owned by the University. Similarly, a goat flock of approximately 300 head of Anglo Nubian and local breeds (Short-eared Ogaden, Long-eared Ogaden and Hararghe Highland), as well as assorted cross-breed animals, is separately maintained.

During the day, the sheep flock is herded on permanent communal grazing pasture together with animals (sheep, goats, cattle, equines and camels) owned by local small-holder farmers. Animals are housed at night to prevent losses by theft and predators. In contrast, the university goat flock had grazed on a permanent natural pasture designated solely for the goat flock. Similarly, during the night all of the goats were housed. Each year there were two lambing and kidding periods, which were synchronised with the rain seasons, but mainly concentrated in August and September.

An experimental flock was used for the epidemiology study from May 2003 to September 2005 (paper I), consisting of 60 BHO sheep that grazed together with the main HU sheep flock. This experimental flock comprised 4 groups of 15 animals, namely young male, young female, adult male and adult female sheep. At the start of the experiment, the ages of the adult sheep were 1–1.5 years, and the young sheep were less than 6 months. A new experimental flock was established each year of the study period. Further details of the experimental sheep flock are found in the material and methods section of paper II.



Figure 3. Experimental Black Head Ogaden sheep grazing on communal pasture at Haramaya.

The investigations of anthelmintic resistance status in the HU sheep and goat flocks utilized 100 animals (50 BHO sheep and 50 local breed goats) which were matched for age (6–9 months) and randomly allocated into treatment groups to conduct FECRTs. Animals were randomly divided into 5 treatment groups, each consisting of 10 animals: albendazole (ABZ), tetramisole (TET), a combination (ABZ + TET) and ivermectin (IVM), at the manufacturers' recommended dose rates, and untreated control. A total of 4 separate FECRTs were conducted. Details of the procedures of FECRTs are described below and in paper III.

Tracer lambs

A total of 112 young male BHO sheep, 4–6 months of age, were used as tracer lambs from June 2003 to September 2005, in the epidemiology study described in paper I. These animals were purchased from neighbouring small-holder farmers at three different times of the year (16 male BHO sheep at a time; 48 animals per year). All of the purchased sheep were treated on arrival at HU with both albendazole (Exiptol, ERFAR Pharmaceutical Laboratories) and tetramisole (Tetramisole, ERFAR, Pallini-Attiki, Greece), each at the manufacturer's recommended dose rates, and then housed in a quarantine facility for 2 weeks. Faecal egg counts were carried out on each animal 14 days after treatment to confirm their worm-free status.

Subsequently, the tracers were maintained in a separate house with an area of helminthologically clean pasture, and fed hay and concentrate each day. Four weeks after anthelmintic treatment, sub-groups of 4 animals were introduced into the HU flock, and allowed to graze with the flock (and local farmers' animals) for 1 month. After this tracer-grazing period, the animals were re-housed for a period of 2 weeks, before slaughter and post-mortem worm recovery.

Abattoir examination

During the 2-year (May 2003 to April 2005) abattoir survey (paper II), visceral organs (including liver, lungs and whole GI tracts) were collected from a total of 1,287 animals (655 sheep and 632 goats), and examined for the presence of helminth parasites. The age of each animal was determined by dental inspection, whereby animals having temporary incisors (milk teeth) were classified as young, and those with permanent incisors were recorded as adults. All animals were raised in the farming areas located within the community boundaries for each town. Details of the numbers of animals examined at each abattoir are described in paper II.

Small-holders' flocks

There is considerable variation in the number of livestock owned by local small-holder farmers in the eastern Hararghe zone of Ethiopia, but typically the number of animals in a household comprises approximately 3 cattle, 6 sheep and 7 goats. In general, management of ruminants is by communal herding of all livestock species (sheep, goats, cattle, equines and camels), which graze on areas of natural

pasture, with each family supervising their own animals. This procedure is followed during the dry season, but animals are usually tethered during the wet season, when crops are grown. There is widespread use of maize and sorghum thinning and crop residues as a feed supplement. Sweet potato vines are fed as a dry-season supplement, and leftover “khat” (*Catha edulis*) leaves, known locally as “geraba”, is widely fed to semi-urban sheep and goats. Many farmers are only able to water their sheep and goats every second day during the dry season, and some water every third day, with only a few farmers managing to water their animals daily. Sheep and goats are housed at night, mainly in the owner’s house. In some cases the animals are housed in specially constructed buildings. Generally, there are two kidding and lambing periods per year, which are synchronized with the rain seasons, but concentrated in August and September.

Parasitological measurements

Faecal egg counts

Faecal samples were collected directly from the rectum of each animal during the experimental epidemiology (paper I) and the anthelmintic-resistance (papers III & IV) studies. Numbers of faecal nematode eggs were determined using a modified McMaster technique with saturated sodium chloride solution as the floating medium (Anon., 1986; Hansen & Perry, 1994; Urquhart *et al.*, 1994; Kassai, 1999). In each case, 3 g of faeces were mixed in 42 ml of saturated salt solution, and the number of nematode eggs per gram of faeces (EPG) was obtained by multiplying the number of nematode eggs counted in two squares of the McMaster slide by a dilution factor of 50 (Anon., 1986; Hansen & Perry, 1994; Urquhart *et al.*, 1994; Kassai, 1999). The nematode eggs present were identified in general terms as strongylid eggs, since relevant nematode genera produce eggs that are similar in appearance and cannot be discriminated easily, except for the eggs of *Nematodirus*, *Strongyloides* and *Trichuris* species.

Faecal cultures, larval identification and enumeration

Duplicate composite faecal cultures, consisting of 2 g of faeces from each animal, were done for each group of animals in the experimental epidemiology (paper I) and anthelmintic-resistance (papers III and IV) studies. The pooled faecal materials were incubated at room temperature (approximately 22–25 °C) for 14 days, and then the nematode larvae were harvested, identified to species level and quantified according to Anon. (1986) and Van Wyk *et al.* (2004). Where possible, 100 larvae were identified and counted for each experimental group of animals.

Post-mortem worm recovery, identification and enumeration

In the experimental epidemiology study (paper I), 4 tracer lambs were slaughtered each month from June 2003 to September 2005. The entire GI tract, liver and lungs of each lamb were collected and processed separately. Similarly, in paper II the viscera (including the liver, lungs and GI tract) were collected from 10–15 sheep and 10–15 goats slaughtered each month from May 2003 to April 2005 from

each of the 4 abattoirs. Collected materials were transported within 24 h to the parasitology laboratory of HU, where they were processed upon arrival. The sampling procedures and the identification of nematode and trematode species were based on methods described by Anon. (1986), Soulsby (1982) and Urquhart *et al.* (1996). Details of these are found in the material and method sections of papers I and II.

Estimation of anaemia

Haematocrit (packed red cell volume)

Every 4 weeks during the 2.5-year experimental epidemiology study (paper I), a jugular venous blood sample (about 2 ml) was taken from each animal, using a vacutainer needle and tube containing sodium heparin as anticoagulant. After each blood sample was gently mixed for 2 min, the blood was filled up into a 75 X 1.5 mm capillary tube up to $\frac{3}{4}$ of its length and one end sealed with sealant. Then, all of the blood filled tubes were centrifuged at 12,000 rpm for 4 min using a micro-haematocrit centrifuge. Finally, each tube was placed in a micro-haematocrit reader, to determine the percentage of packed red cell volume (PCV) for each animal. The procedure followed was according to Hansen & Perry (1994) and Urquhart *et al.* (1996).

FAMACHA eye-colour scores

General clinical examination and the FAMACHA eye-colour score estimation (Bath *et al.*, 1996; Van Wyk & Bath, 2002) were also performed every month for each study animal during the 2.5-year experimental epidemiology study (paper I). The colour of the mucous membrane of the lower eyelid of each animal was examined in good natural light and compared to the colours of the FAMACHA[®] chart (Bath *et al.*, 1996; Van Wyk & Bath, 2002). Each sheep was scored on a scale of 1–5 (1 = red, non-anaemic; 2 = red-pink, non-anaemic; 3 = pink, mildly anaemic; 4 = pink-white, anaemic; 5 = white, severely anaemic). Each month all animals were scored by the same persons who had been previously trained to use the FAMACHA technique. While it appears to be simple and straightforward to examine the ocular mucous membranes and assign animals to the proper category, my experience has shown that training and experience is required to use this system effectively.

Faecal egg count reduction tests

In paper III, two series of FECRTs were conducted on the HU sheep and goat flocks during May-June 2003 and November-December 2003, to evaluate the efficacy of the commonly used anthelmintics in this region. Anthelmintics tested were albendazole (ABZ), tetramisole (TET), a combination of ABZ and TET and ivermectin (IVM), at the manufacturers' recommended dose rates. The procedures followed were in general accordance with those described by Anon. (1989) and Coles *et al.* (1992). Each test included a total of 50 animals of similar age (6–9 months), which were previously untreated with anthelmintics (or at least not for

the previous 3 months), and were randomly selected from the university flocks. Arithmetic mean, percentage of reduction, and 95% upper and lower confidence limits were used as criteria to determine anthelmintic efficacy.

Similarly, 16 separate FECRTs (8 tests for sheep and 8 for goats) were also conducted between July and September 2004, on sheep and goat flocks owned by small-holder farmers that received breeding stock from the HU flocks (paper IV). Eight Peasant Associations (PA) located in the Haramaya district were selected. In each PA, a total of 50 local breed sheep and 50 local breed goats, with an age range of 6–9 months, were used for each FECRT. These animals were randomly divided into five treatment groups (ABZ, TET, ABZ + TET, IVM and untreated control), and distinguished by marking with colour paint. The names of the sheep and goat owners were also registered.

Weather conditions

The climatic elements, such as temperature, rainfall and humidity, were recorded at four stations (HU, Harar, Dire Dawa and Jijiga) of the National Meteorological Services Agency. Thus, all of the required climatic data (*viz.* minimum and maximum temperatures, rainfall and humidity) for all of the study areas during the study period (2003–2005) were obtained every month from these units. The descriptions of the climatic features (temperature, rainfall and humidity) of the study sites are presented in papers I and II.

Data analyses

All of the parasitological and performance data (EPG, faecal culture larval counts, tracer and abattoir post-mortem worm counts, PCV, FAMACHA eye scores, and body weight change) were first entered into the Microsoft Excel program.

The fixed effects of age and sex of animals, seasons and years on EPG were analysed with repeated measures analyses according to the SAS mixed procedures (Statistical Analysis System Institute, version 9.1, Cary, NC, USA). The EPG and tracer worm counts (TWC) were first logarithm-transformed in the form of $\log_{10}(\text{EPG} + 1)$ and $\log_{10}(\text{TWC} + 1)$, respectively, to stabilize the variances before analyses. Antilogarithms of the means of EPG and TWC were used to summarize the results in the graphs. Correlation tests were also conducted to ascertain whether there was any association between EPG, PCV, FAMACHA eye scores and LBW.

Similarly, the abattoir post-mortem data for adult GI nematode counts were analyzed using the General Linear Model (GLM) of the SAS system (Statistical Analysis System Institute, version 9.1). The fixed effects of location (abattoir), host animal species, age, sex, and season, and all possible interactions, on each adult nematode species were analyzed using the univariate analyses of the SAS GLM procedures. However, analyses of the fluke data, such as basic descriptive statistics, cross tabulation and chi-square tests in relation to region, animal species, age, sex, and season, were done using the Minitab program, version 14 (Minitab Inc., Quality Plaza, State College, USA).

The efficacy of anthelmintics was evaluated based on the reduction in faecal egg count and percentage of larvae found in the cultures. Calculation of the arithmetic mean, percentage of reduction and 95% upper and lower confidence limits were conducted according to the procedures described by Coles *et al.* (1992). Resistance was declared when the percentage of reduction was less than 95% and the 95% lower confidence limit was less than 90%. If only one of the two criteria was met, resistance was suspected.

Results and discussion

Transmission dynamics of gastro-intestinal nematodes of sheep

Nematode faecal egg counts and infective larval availability on pasture

The nematode faecal egg counts of the HU sheep flock during the experimental epidemiology study (paper I) showed that the effects of the age of the animal and the season of the year on the EPG were highly significant (all $P < 0.0001$). However, the sex of the sheep and the years of the study had no significant effects on the EPG of the experimental sheep flock (all $P > 0.05$). In all groups (young males, young females, adult males and adult females), the EPG increased during the short rain period (beginning of March to mid May) and reached a peak just after the end of the short rains, in May of each year, except in 2003. Thereafter, the EPG decreased during the short dry period (end of May to mid June) of each year. Similarly, in all of the groups the EPG increased during the long rain period (end of June to mid October), to peak approximately in September of each year, but the EPG then decreased during the long dry period (end of October to end of February). Although the EPG of all 4 groups followed similar patterns over the entire study period, which was associated with the bi-modal annual rainfall pattern of the region, the young sheep had a significantly higher mean EPG than did the old sheep, particularly during the dry seasons, short rains and the latter part of the long rain seasons. No PPR in faecal egg counts was evident in the lactating females during the study period. The characteristic eggs of *Nematodirus* and *Strongyloides* were occasionally recorded at low numbers in the faecal counts of young sheep.

In addition, the results of the composite faecal cultures revealed that *H. contortus* was the dominant nematode species of sheep, representing 60–85% of the total larval recovery from cultures throughout the entire study period. The percentage of this parasite was highest during the time of the highest faecal egg counts in the wet seasons. *Trichostrongylus* spp. were the next most prevalent parasites, which represented 13–26% of the total infective larvae harvested from the composite faecal cultures during the study period. Others, including *Oesophagostomum*, *Strongyloides*, *Bunostomum*, *Nematodirus*, *Cooperia*, and *Chabertia* spp. (in order of dominance), were found at varying percentages, but together only comprised 3–15% of the larval cultures. Individually, these latter

genera never comprised more than 3% of the total amount of infective larvae on any occasion.

Availability of infective larvae of GI nematodes on pasture (or infective larval pickup) as measured by the monthly tracer tests (paper I), showed similar patterns to the nematode faecal egg counts, with the highest worm burdens of the tracer lambs being recorded in the wet season ($P = 0.003$). *H. contortus* was again established as the predominant nematode, accounting for 50–65% of the total worm burden recovered from each tracer lamb during the study period. Next were *Trichostrongylus* spp. and other GI nematode species (*Oesophagostomum*, *Bunostomum*, *Nematodirus*, *Strongyloides*, *Cooperia*, *Chabertia*, and *Trichuris* spp.), recorded with broadly the same abundance as observed in the faecal cultures of the experimental sheep flock. Interestingly, *Teladorsagia circumcincta* was not found in this study, and no lungworms or liver flukes were found in tracers.

The observed results, of highest EPGs with the largest percentages of *H. contortus* L3 in faecal cultures during the rainy seasons, were in accordance with studies in other countries in Africa with distinct rainy and dry seasons (*Ghana*: Agyei, 1991; *Kenya*: Maingi *et al.*, 1993; Nginyi *et al.*, 2001; *Zimbabwe*: Pandey *et al.*, 1994). This can be attributed to the high biotic potential of *H. contortus*, resulting in this parasite rapidly take up dominance at times when environmental conditions on pasture are favourable for the development and survival of the free-living stages. In addition, overstocking, which is a major problem in many African communal pastures (Hansen & Perry, 1994), may have contributed to the observed increased availability of infective larvae of GI nematodes on pasture during the wet season.

Similar to my investigation, other studies in Africa have shown that the age and immune status of the host animal have significant influences on nematode egg output (Assoku, 1981; Ashraf & Nepote, 1990; Magona & Musisi, 2002). It is generally recognized that young sheep are more susceptible to parasite infection than are sheep older than 1 year of age (Gamble & Zajac, 1992; Watson *et al.*, 1994; Colditz *et al.*, 1996). However, in this study the apparent lack of regulation in egg output of the adult sheep during the latter part of each year (when they are approaching 2–2.5 years of age) was likely to be due to a failure of their naturally acquired immune responses to parasitism, possibly because of the inadequate nutritional status that is a feature of small ruminant flocks in Ethiopia. However, with the need for *ad hoc* salvage anthelmintic treatment of individual young animals, then this may well have reduced the magnitude of group mean egg counts for the young male and female groups during the wet seasons.

Other studies in Africa have reported PPR in trichostrongylid nematode faecal egg output in ewes lambing during the wet season (Agyei *et al.*, 1991; Tembely *et al.*, 1998; Ng'ang'a *et al.*, 2006). However, there was no apparent PPR in the faecal egg counts of the lactating adult females during the wet seasons in my study. This may have been due to the fact that lactating ewes also received a greater proportion of salvage anthelmintic treatments, compared with adult male sheep, during the short and long rains (which are the lambing/lactating periods) of each experimental year.

Inhibited (EL4) larval development of *H. contortus* was also recorded in tracer lambs. No attempt was made to estimate any arrested larvae of other GI nematodes from the small intestines of the tracer lambs. The numbers of EL4 increased to account for up to 20% of the total *H. contortus* worm burdens during the dry seasons, and then decreased during the wet seasons to approximately 0.2% of the total *H. contortus* worm counts. Such developmental suspension during the dry seasons, and its resumption during the onset of the rainy seasons, may have contributed to the observed highest EPG, highest percentage of *H. contortus* L3 from faecal cultures, and highest worm burdens from tracer lambs during the wet season of each of the study years. This result differs from that of the study by Tembely *et al.* (1997), conducted in the cool highlands of Ethiopia, where they found the greatest numbers of *H. contortus* EL4 during the long rainy seasons (August to October/November) and no inhibition recorded during the dry season. However, my findings are similar to other epidemiological studies on nematode parasites of small ruminants in semi-arid regions of Africa, where inhibition of *H. contortus* is a feature during the dry periods of the year (*Kenya*: Allonby & Urquhart, 1975; *Nigeria*: Ogunsusi & Eysker, 1979; *Senegal*: Verduyck, 1985; *Nigeria*: Chiejina *et al.*, 1988; *Zimbabwe*: Pandey *et al.*, 1994).

PCV and FAMACHA

The PCV of the experimental sheep in the epidemiology study (paper I) had a strong negative correlation ($R^2 = -0.72$, $P < 0.0001$) with the FAMACHA eye-colour scores. That is, as the PCV value decreased during the peak rainy seasons, the percentage of sheep with FAMACHA scores of 3, 4 and 5 (clinical signs of anaemia) increased, and vice versa. The number of animals that received anthelmintic treatment, based on the FAMACHA scores of 3, 4 and 5, which was the trigger for immediate dosing, reached up to 25% during May and September of each year. Of these animals, the majority were young sheep of both sexes and the adult female sheep. However, the FAMACHA score had a strong positive correlation ($R^2 = 0.8$, $P < 0.001$) with EPG, i.e., the percentage of sheep with FAMACHA scores of 3, 4 and 5 increased as the EPG increased. But the PCV of the experimental sheep had a strong negative correlation with the EPG ($R^2 = -0.85$, $P < 0.001$).

Thus, for the first time in Ethiopia, this study has demonstrated the utility of the FAMACHA anaemia guide as a reliable pen-side procedure for the indirect diagnosis of haemonchosis. Infection with *H. contortus* may cause severe anaemia and hypo-proteinemia, leading to depression, loss of condition, reduced productivity, and eventual death. The disease tends to be more severe in young kids and lambs, and particularly in lactating females where their immune status is compromised. The FAMACHA monitoring of the animals in this trial triggered the need for salvage anthelmintic treatment of individual lactating ewes, and, as mentioned, this was likely to be responsible for the fact that a PPR in nematode egg output was not recorded in these animals.

However, it is important to remember that in many regions of Africa endemic haemonchosis overlaps with other important parasitic causes of anaemia in small ruminants, notably fasciolosis and trypanosomosis. In the case of eastern Ethiopia,

fasciolosis can be an important problem locally (see paper II). Thus, there could well be a confounding of the diagnosis as solely being haemonchosis, which is particularly important when recommending treatment, as many anthelmintics that are effective against *H. contortus* have no effect on liver flukes.

Body weight change

The body weights (BW) of the young and adult sheep at the beginning of each experimental year (paper I) ranged from 15–20 kg and 21–25 kg, respectively. There was no significant association between the EPG and the mean BW of the experimental sheep. However, the season of the study years had a significant effect on the mean BW of the study sheep. The mean BW of each group of experimental animals followed similar patterns to the faecal egg count in each year, with weights increasing during the wet seasons and then decreasing during the dry seasons. Overall, the average daily weight gains for years 1 and 2 of the study were: 23 and 31 g for young males; 21 and 30 g for young females; 36 and 43 g for adult males; 27 and 40 g for adult females, respectively. Although these performance data were poor, it is well known that ruminants kept on natural pasture and crop residues, which are typical for the semi-arid regions of Ethiopia, exhibit poor performance on such nutrition. The improved performance of the experimental animals during the wet seasons is likely to be compensatory weight gains due to the availability of fresh grass during these times.

Prevalence, intensity and seasonal incidence of helminth parasites of sheep and goats

The adult worm counts conducted on sheep and goats slaughtered in the four abattoirs (paper II) established that infections with helminth parasites in small ruminants occurred throughout the year. In both sheep and goats the predominant GI nematode species were: *H. contortus*, *T. axei*, *T. vitrinus*, *T. colubriformis*, *N. filicollis*, *N. spathiger*, *O. columbianum* and *O. venulosum*. Other nematode species, such as *C. curticei*, *B. trigonocephalum*, *S. papillosus*, *C. ovina* and *T. ovis*, were recorded, but their numbers were sporadic and insignificant. Although all of these nematodes can contribute to the overall prevalence of gastrointestinal parasitism or PGE in small ruminants, it was the highly pathogenic blood-sucking parasite *H. contortus* that was by far the most prevalent, abundant and important, representing more than 60% of the total worm burdens recorded in the sheep and goats. Intestinal *Trichostrongylus* species were the next most prevalent nematodes, followed by *Nematodirus* and *Oesophagostomum* species in both sheep and goats.

However, similar to the results of the composite faecal cultures and TWC in the epidemiological study on the HU sheep flock (paper I), *T. circumcincta* was also not recorded in the abattoir investigation (paper II). It is known that *T. circumcincta* has the ability to survive adverse conditions, both within the host and on pasture, which suggests that the sheep and goat flocks in this region in eastern Ethiopia have remained isolated from introductions of small ruminants (and their parasites) from locations where this parasite is common. However, the presence of

T. circumcincta has previously been recorded in small ruminants in the mid-lowland areas of the Rift Valley, southern Ethiopia (Tilahun, 1995; Debela, 2002).

In addition, lungworms were not recorded in either sheep or goats in this investigation. Nevertheless, the lungworm species *Dictyocaulus filaria*, *Muellerius capillaris* and *Protostrongylus rufescens* were recorded in sheep and goats in the cool north-eastern highlands of Ethiopia by Alemu *et al.* (2006).

Similar to the study on the HU sheep flock (paper I), the influences of the sex and age of the animals on nematode worm burdens was not significant, but the worm burdens during the wet seasons were significantly higher than during the dry seasons. During the wet seasons, a gradual build up of the nematode worm populations were observed both in the sheep and goats, with the greatest burdens recorded around the peaks of the rainy seasons. Thereafter, the nematode worm populations declined, with the lowest numbers being recorded around the middle of the dry season, indicating lower levels of larval pickup from pasture during this period. These seasonal variations in the nematode worm burdens were similar to studies in other tropical countries with distinct rainy and dry seasons (Fritsche *et al.*, 1993; Nginyi *et al.*, 2001; Magona & Musisi, 2002; Tsotetsi & Mbatia, 2003; Nwosu, 2006). In general, moist and warm environmental conditions are favourable for the development, survival and transmission of the pre-parasitic stages of parasitic nematodes (Donald, 1968; Hansen & Perry, 1994; Urquhart *et al.*, 1996).

In addition, in both sheep and goats there were significant differences in the mean worm burdens and abundance of the different nematode species between the four geographic locations, with worm burdens in the Haramaya and Harar areas being greater than those observed in the Dire Dawa and Jijiga locations. However, there were no significant differences (all $P > 0.05$) in the mean adult worm counts of each of the nematode species between sheep and goats in all of the abattoirs during the study period. A similar effect of agro-climatic zones on the prevalence and intensity of GI nematodes of goats was reported in Uganda by Magona & Musisi (2002).

The prevalence and seasonal incidence of fluke infections in sheep and goats are also presented in paper II. Four trematodes species (*F. hepatica*, *F. gigantica*, *P. microbothrium* and *D. dendriticum*) were recorded in both sheep and goats during the study period. The overall prevalence of *P. microbothrium*, *F. hepatica*, *F. gigantica* and *D. dendriticum* in sheep were 25%, 26%, 20% and 7%, respectively. Similarly, the overall prevalences in goats were 21%, 3%, 10% and 2%, respectively.

Similar to the GI nematodes in both sheep and goats, there were significant differences in the prevalence of each of the above fluke species between wet and dry seasons (all $P < 0.05$). However, no significant differences were observed in the prevalence of each of the trematode species between the four abattoir locations, the two sexes and the two age groups (all $P > 0.05$). In goats, the prevalences of *F. hepatica* and *D. dendriticum* were low, and no significant differences were observed in any of the above comparisons. The preferred browsing behaviour of goats, rather than grazing like sheep, would account for the lower prevalence of

liver flukes in goats, but it is difficult to explain the observed high prevalence of *P. microbothrium* as compared to the prevalence of liver flukes in goats.

In general, the expansions of small-scale traditional irrigation schemes, and the seasonal occurrences of ephemeral water sources in these semi-arid regions of Ethiopia, may have facilitated the development and transmission of the aquatic and amphibious (mud-dwelling) intermediate snail hosts (for example *Lymnaea* spp. for *Fasciola* spp. and *Bulinus* spp. for *Paramphistomum* spp.), which in turn may have increased the prevalence of *F. hepatica*, *F. gigantica*, and *P. microbothrium* both in sheep and goats in eastern Ethiopia. It is rare that *F. hepatica* and *F. gigantica* are sympatric (Soulsby, 1982; Urquhart *et al.*, 1996). A forecast model for fasciolosis, based on geographic information systems in East Africa by Yilma & Malone (1998), indicated that in Ethiopia *F. gigantica* could be found at altitudes below 1800 m.a.s.l., whereas *F. hepatica* could be found at altitudes between 1200–2500 m.a.s.l., and mixed infections with the two species could be encountered at altitudes between 1200–1800 m.a.s.l.

Anthelmintic resistance and its management

There have been no studies published on the efficacy of the most commonly used anthelmintic drugs in small ruminants in eastern Ethiopia. However, I was concerned about the possibility of the presence of anthelmintic resistance in the university flocks, as relatively intensive use of anthelmintics had been applied to both the sheep and goat flocks, particularly during the wet seasons. In addition, the university goat flock had been traditionally managed as a closed flock for many years. The university flocks are also a source of breeding animals that are supplied to the small-holder farming communities at low prices. Thus, the threat of exporting anthelmintic resistant parasites, together with animals, to the small-holder communities was a concern.

However, the results of the four series of FECRTs on the HU sheep flock indicated that there was no anthelmintic resistance (paper III). In contrast, the initial two FECRTs on the HU goat flock showed resistance to all of the drugs, including the combination of albendazole and tetramisole (paper III). Composite faecal cultures of all of the treatment groups showed that *H. contortus* was the dominant nematode species (paper III).

Following this result in the goat flock, the following management procedures were implemented:

1. The entire goat flock was treated using a combination of three anthelmintics (albendazole, tetramisole and ivermectin), each drug being given at the recommended sheep dose rates within a 12 h interval, when the goats were housed. 10–14 days after this time, faecal egg counts were conducted on 50 randomly selected goats. The results of these faecal egg counts showed that the majority of the animals had no detectable nematode eggs, and only a few individuals had very low EPGs. This combined use of the anthelmintics from the three different action families (i.e. benzimidazole, imidothiazole and macrocyclic lactone) during a 12 h

period when animals were withheld from feed, resulted in a high level of efficacy. It is known that anthelmintic efficacy is directly related to the duration of contact between the drug and the parasite, and hence restricting feed intake for 24 h before treatment decreases the digesta transit and extends the contact time, which in turn increases the drug availability and efficacy (Hennessy, 1997; Kaplan, 2006).

2. A different daily management plan was practiced on the HU goat flock for a period of 9 months (January–September 2004), whereby grazing on the former goat pastures was prevented, and all of the goats were herded on the communal grazing pasture together with the HU sheep flock and animals of the local small-holders
3. The goat pasture was protected from all livestock grazing for 3 months (January–March 2004). Thereafter, the pasture was cut and collected for making hay to feed to the HU cattle, but the area was still protected from all livestock grazing for the following 3 months (April–June 2004). Fire was also applied to the pasture during the dry season. After this 6-month period, only the HU cattle were allowed to graze the newly grown pasture for a further 3 months (July–September 2004).
4. During the course of this new management program, the health and particularly the anaemia status of the goats were monitored using the FAMACHA eye-colour chart (Bath *et al.*, 1996; Van Wyk & Bath, 2002). Faecal egg excretion was also monitored from 15 randomly selected goats every month during this period. Only those goats that showed anaemia and/or bottle jaw were treated with combinations of the three anthelmintic drugs albendazole, tetramisole and ivermectin, with each drug given at the recommended sheep dose rates within a 12 h interval, when the goats were housed.

Following the implementation of this change in management of the goat flock, the results of subsequent FECRTs have shown that anthelmintic resistance in the goat flock had disappeared. This new management of the goat flock resulted in major changes in the susceptibility of their nematode parasite populations to all of the tested drugs. The degree of efficacy of the tested drugs in the goat flock was initially 57–70%. These figures increased to 95–98% after the implementation of the new management system, which confirmed that reversion back to high-level anthelmintic efficacy had occurred by exploiting natural refugia in the parasite populations of this study site.

The initial difference in the resistance status between the HU sheep and goat flocks was most likely caused by the contrasting systems of management. Although the sheep flock was treated routinely with anthelmintics, generally just before the two rain seasons each year, they were communally grazed on a large area of natural pasture together with the local small-holders' animals. It was established through interviews with local farmers that they used anthelmintics very infrequently. In contrast, the university goat flock received not only anthelmintic treatments prior to the two rain seasons each year but also additional treatments, when a diagnosis of parasitism in individual animals was the trigger for dosing the

whole goat flock. In addition, and most relevant from the standpoint of the resistance status of parasites in the goat flock, these animals grazed solely on a specially designated pasture to which no other livestock were introduced.

Thus, these different animal management practises had resulted in the occurrence of multiple resistances in the goat flock, in contrast to the preservation of anthelmintic susceptibility in the nematode parasite population of the sheep flock, which in turn contributed to differences in refugia. The size of the unselected proportion of the parasite populations (refugia) is one of the most important factors affecting the rate of selection of anthelmintic resistance (Van Wyk, 2001). This unselected parasite population (including unexposed eggs and larvae on pastures and worms inside the host that are left untreated with anthelmintics) provided a pool of drug-sensitive genes, thus diluting the frequency of resistant genes in a population of worms (Kaplan, 2006). The occurrence of large refugia in the communal pastures may slow down the development of resistance (Van Wyk, 2001). Any parasites that survive anthelmintic treatments given to the university sheep flock would be quickly diluted, or replaced, by parasite populations derived from the continuous intake of larvae (Barger *et al.*, 1985; Dobson *et al.*, 1990). In contrast, the much greater proportions of the parasite population within the goat-raising unit would be exposed to anthelmintic selection, particularly when dosing was conducted during the dry season. It is known that if treatments are given when few infective larvae are on pasture during the dry seasons, then eggs shed by the resistant worms that survived these treatments would not be diluted by eggs produced by susceptible female parasites (Kaplan, 2006). Field studies have also shown that the combination of anthelmintic treatment to all animals plus extreme weather conditions on pasture (unfavourable for the survival of the free-living stages of nematode parasites) imposes strong selection for the development of anthelmintic resistance (Besier, 1996; Papadopoulos *et al.*, 2001).

In addition, when goats are kept intensively at high stocking rates and forced to graze, rather than allowing their natural habit to browse, problems with nematode parasites can be particularly severe. Under these relatively newly contrived management systems, managers of goat flocks tend to resort to very frequent use of anthelmintics. This has precipitated some of the earliest and worst cases of anthelmintic resistance recorded in ruminant livestock (Jackson *et al.*, 1992; Cabaret, 2000; Chandrawathani *et al.*, 2004). Clearly, the frequency of treatments is largely responsible for this situation, but because goats have a more rapid metabolism of anthelmintics than do sheep (Sangster *et al.*, 1991; Hennessy *et al.*, 1993), this may promote more rapid selection for resistance in nematode parasites in goats (Hennessy, 1997). However, for ivermectin at least, the dose rate of 200 µg/kg shows the same level of efficacy in both sheep and goats (Swan & Gross, 1985), and increasing the dose rate of anthelmintics for which there is no product registration claim can cause toxicity in goats, particularly for the imidothiazoles and organophosphate anthelmintics. The management of the HU goat flock, although well intentioned, provoked the development of high-level multiple anthelmintic resistance, which does not normally occur under the prevailing small-ruminant management systems in eastern Ethiopia.

Further anthelmintic-resistance studies were undertaken on the flocks owned by the small-holder farmers in communities surrounding the HU campus (paper IV), because they have been the recipient of animals from both the HU sheep and goat flocks. This study was performed on sheep or goats owned by small-holder farmers of each of 8 separate PAs as one unit. This enabled sufficient numbers of suitable stock (young, previously untreated animals) to be selected for 16 separate FECRTs in these PAs.

The results of the 16 separate anthelmintic resistance tests (8 FECRTs for sheep and 8 for goats) showed that there was no evidence of anthelmintic resistance in nematode parasites of either sheep or goats in any community. The reason for this result was likely to be due to a combination of factors. First was the dilution of resistant parasites present in the few imported animals by the large pool of parasites in the communal grazing systems that characterize livestock management in the PAs. Second was the very low selection pressure for the development of anthelmintic resistance that is applied by the small-holder farmers. Some farmers use anthelmintic drugs, particularly those who reside in close proximity to veterinary clinics, where they seek advice and medications. However, there is little evidence of synchronization of anthelmintic treatment of all small-ruminant livestock within any PA. Thus, at any one time the majority of the nematode parasite populations of the sheep and goats were unexposed to anthelmintic selection, because either they exist in the external environment as free-living stages, or inside untreated animals.

Summary and concluding remarks

In this study, important parasites of sheep and goats were identified, and those factors affecting the epidemiology of these parasites of small ruminants in eastern Ethiopia were elucidated. The experimental epidemiology study of the HU sheep flock (paper I) and the abattoir survey (paper II) showed well-defined seasonal transmission dynamics of the GI nematode and fluke infections, which corresponded with the bi-modal annual rainfall pattern of the study areas. These studies indicated that *Haemonchus*, *Trichostrongylus*, *Nematodirus*, *Oesophagostomum*, *Fasciola* and *Paramphistomum* species were common helminths of sheep and goats in this part of Ethiopia. Of these parasites, *H. contortus* was the most prevalent, representing more than 60% of the total worm burdens recorded in tracer tests. In the sheep and goats examined at the four abattoirs, mean *H. contortus* worm counts exceeded 4000 during the peak time.

High levels of prevalence, intensity and abundance of these parasites were generally observed around the middle of the two rain seasons, with peaks occurring in May and September of each year. This confirmed that the weather conditions of the wet seasons were generally favourable for the development, survival and transmission of the free-living stages of nematodes and the intermediate stages of trematodes, *i.e.* their snail hosts, as compared to the more unfavourable conditions of the dry seasons. Thus, rainfall and moisture status appear to be the major limiting environmental factors for the development and

survival of the infective stages of nematodes and flukes of small ruminants in eastern Ethiopia. However, further studies on the ecology of the free-living and intermediate stages of nematodes and trematodes, as well as their snail hosts, are required in these regions to further qualify these phenomena. The present studies also demonstrated the roles of other factors (age, sex and susceptibility of host animals, parasite fecundity, stocking density, peri-parturient rise, hypobiosis and agro-climatic zones) in the epidemiology of GI nematode and fluke infections of small ruminants in eastern Ethiopia.

Studies on anthelmintic resistance of nematode parasites in the HU sheep and goat flocks, which are used to supply breeding stock to the surrounding small-holder communities, showed the absence of anthelmintic resistance to albendazole, tetramizole and ivermectin in the sheep flock. However, high levels of anthelmintic resistance to albendazole, tetramisole and ivermectin were evident in the HU goat flock. Thus, in order to restore the anthelmintic efficacy in the HU goat flock, by exploiting the opportunity to replace the resistant worm populations with anthelmintic-susceptible parasite populations in the surrounding communal grazing pastures, a new management system was implemented in the goat flock for a period of 9 months. The results of the two consecutive FECRTs conducted after the implementation of the new management plan showed high levels of efficacy of all three drugs. This is the first field study to demonstrate that anthelmintic efficacy can be restored by exploiting refugia.

Further assessment of anthelmintic resistance to nematode parasites of the small ruminant flocks owned by small-holder farmers who received breeding stock from the HU, established that there was no evidence of resistance in these communities. This confirmed that dilution of the resistant parasite populations imported with the breeding stock received from the HU, and the low selection pressure imposed by the small-holder farmers themselves, had prevented anthelmintic resistance from emerging in the nematode parasite populations of small ruminants in these communities.

It is known that a prerequisite for the development and implementation of sustainable parasite management programs is to have an epidemiological knowledge of the parasites present in a specific area. The sustainability of helminth control practices also relies on the prevention of resistance and preservation of anthelmintic effectiveness, as well as effective utilization of the locally available feed resources. Thus, it is hoped that this study will provide a starting point for the development of epidemiologically based control strategies for GI nematode and fluke parasites of small ruminants in eastern Ethiopia.

Recommendations

Efforts to control parasites of small ruminants in eastern Ethiopia should be based on practical and cost-effective solutions, which rely on combinations of different approaches. These involve integration of traditional animal health management practices of small-holders and pastoralists with control methods founded on knowledge of parasite epidemiology. Based on the findings of the present investigations as well as field observations and literature studies, relevant approaches for helminth control could include:

- Improvement of basic animal management systems (housing, watering, control of lambing and kidding times, identification of animal groups most at risk, manure management) as well as grazing management (burning the pasture during the dry season, avoiding muddy and polluted grazing areas).
- Effective utilization of available feed resources such as agricultural by-products, natural pastures and browses, and appropriate nutritional supplementation programs for young and lactating animals.
- Optimized anthelmintic usage to preserve anthelmintic efficacy and prevent resistance to occur in GI nematodes. Recommendations include the use of the most suitable drugs, correct dosage, possible combination of drugs, treating selectively rather than the entire flocks, avoiding high frequency of treatments, and proper planning by expertise.
- Provision of animal health extension services, which includes regular monitoring of faecal egg output of selected animals, assessment of anaemia using the FAMACHA chart, and treatment of animals based on the outcome of these analyses.
- Education of the small-holder communities regarding correct ways to improve animal management systems, the importance of parasites and major signs of worm infections in their animals.

Future research could include:

- Scientific evaluation of ethno-veterinary remedies used by small-holders and pastoralists in the region.
- Continued research on the ecology of the free-living stages of helminths.
- Continued research on the relative importance of helminth parasites on productivity of small ruminants.
- Initiation of breeding programs to select for breeds of small ruminants that can resist the effects of parasites and adapt well to the environment of the region.

References

- Abbott, E. M., Parkins, J. J. & Holmes, P. H., 1986. The effect of dietary protein on the pathophysiology of acute ovine haemonchosis. *Veterinary Parasitology* 20, 291-306.
- Adugna, G., 1990. Black Head Ogaden sheep under traditional management practices in south-eastern Ethiopia. In: Rey, B. Lebbie, S. H. B. and Reynolds, L., July 1992 (eds), *Small Ruminant Research and Development in Africa*. Proceedings of the first Biennial Conference of the African Small Ruminant Research Network, 10-14 December 1990, ILRAD, Nairobi, Kenya.
- Ageyi, A. D., Sapong, D. & Probert, A. J., 1991. Periparturient rise in faecal nematode egg counts in west African dwarf sheep in southern Ghana in the absence of arrested strongyle larvae. *Veterinary Parasitology* 39, 79-88.
- Alemu, S., Leykun, E. G., Ayelet, G. & Zeleke, A., 2006. Study on small ruminant lungworms in north-eastern Ethiopia. *Veterinary Parasitology* (in press).
- Allonby, E. W. & Urquhart, G. M., 1975. The epidemiology and pathogenic significance of haemonchosis in a merino flock in east Africa. *Veterinary Parasitology* 1, 129-143.
- Anon., 1986. *Manual of Veterinary Parasitological Laboratory Techniques. Reference Book*. Her Majesty's Stationary Office, London. 160 pp.
- Anon., 1989. *Anthelmintic resistance*. Report of working party for the Animal Health Committee of the Standing Committee on Agriculture (SCA). SCA *Technical Report Series* No. 28, Canberra, Australia. 26 pp.
- Anon., 1992. *Ethiopia: Livestock Sector Development Project*. FAO (Food and Agriculture Organization of the United Nations), Report No. 107/92. FAO, Rome, Italy.
- Anon., 1994. *Diseases of Domestic Animals Caused by Flukes: Epidemiology, Diagnosis and Control of Fasciola, Paramphistome, Dicrocoelium, Eurytrema and Shistosome Infections of Ruminants in Developing Countries*. FAO (Food and Agriculture Organization of the United Nations), Report. Rome, Italy. 49 pp.
- Anon., 1996. *Drenchrite[®], Larval Development Assay Standard Operating Procedures*. Horizone Technology Pty Ltd, Roseville, NSW, Australia.
- Anon., 1997. *National Livestock Development Programme*. Ministry of Agriculture (MoA), Animal and Fisheries Resource Development Department, Addis Ababa Ethiopia.
- Anon., 1998. *Agro-ecological Zones of Ethiopia*. Natural resources management and regulatory department, Ministry of Agriculture, Addis Ababa, Ethiopia (Draft).
- Anon., 2000a. *Agro-ecological Zonations of Ethiopia*. Ministry of Agriculture, Addis Ababa, Ethiopia.
- Anon., 2000b. *Small Ruminant Research Strategy*. Ethiopian Agricultural Research Organisation (EARO). Animal Science Research Directorate, Addis Ababa, Ethiopia.
- Anon., 2004. *State of Ethiopian's Animal Genetic Resources- Country Report. A Contribution to the First Report on the State of the World's Animal Genetic Resources*. Institute of Biodiversity Conservation (IBC). Addis Ababa, Ethiopia. 74 pp.
- Anon., 2005. *Agricultural Sample Survey, 2004/2005 (1997 E.C.) Volume II. Report on Livestock and Livestock Characteristics (Private Peasant Holdings)*.

- Statistical Bulletin, 331, Central Statistical Authority, Addis Ababa, Ethiopia, 142 pp.
- Anon., 2007. *The World Factbook*. CIA Directorate of Intelligence. <http://www.cia.gov/ciapublicatins/pubs.html>. (Accessed in March 2007).
- Ashraf, M. & Nepote, K. H., 1990. Prevalence of gastro-intestinal nematode, coccidian and lungworms in Maryland dairy goats. *Small Ruminant Research* 3, 291-298.
- Asmare, K., Gelaye, E. & Ayelet, G., 2005. Anthelmintic resistance test in gastrointestinal nematodes of small ruminants in southern Ethiopia. *Bulletin of Animal Health and Production in Africa* 53, 89-95.
- Assoku, R. K., 1981. Studies of parasitic helminths of sheep and goats in Ghana. *Bulletin of Animal Health and Production in Africa* 29, 1-10.
- Barger, I. A., 1985. The statistical distribution of trichostrongylid nematodes in grazing lambs. *International Journal for Parasitology* 15, 645-649.
- Bath, G. F., Malan, F. S. & Van Wyk, J. A., 1996. The FAMACHA[®] Ovine Anaemia Guide to assist with the control of haemonchosis. In: *Proceedings of the 7th Annual Congress of the Livestock Health and Production Group of the South African Veterinary Association*. Port Elizabeth, South Africa, 5-7 June 1996, p. 5.
- Bekele, T., Woldeab, T., Lahlou-Kassi, A. & Sherington, J., 1992. Factors affecting morbidity and mortality on-farm and on-station in the Ethiopian highland sheep. *Acta Tropica* 52, 99-109.
- Besier, R. B., 1996. Ivermectine resistance in *Ostertagia* on Western Australia sheep farms. In: *Proceedings of the Sheep Sessions, Second Pan-Pacific Veterinary Conference*, Christchurch, New Zealand. Veterinary Continuing Education, Massey University, New Zealand, p. 195.
- Boersema, J. H., 1983. Possibilities and limitations in the detection of anthelmintic resistance. In: *Facts and Reflection IV: Resistance of Parasites to Anthelmintics*. A Workshop held at the Central Veterinary Laboratory, pp. 207-218.
- Cabaret J., 2000. Anthelmintic with nematode resistance in goats: from fiction to facts. In: *Proceedings of 7th International Conference on Goats*. Tours, France, 15-18 May, pp. 793-794.
- Chandrawathani, P., Yusoff, N., Wan, L. C., Ham, A. and Waller, P. J., 2004. Total anthelmintic failure to control nematode parasites of small ruminants on government breeding farms in Sabah, East Malaysia. *Veterinary Research and Communication* 28, 479-489.
- Chiejina, S. N., Fakae, B. B. & Eze, B. O., 1988. Arrested development of gastrointestinal trichostrongylids in goats in Nigeria. *Veterinary Parasitology* 28, 103-113.
- Chiejina, S. N., Fakae, B. B. & Eze, P. I., 1989. Development and survival of free-living stages of gastrointestinal nematodes of sheep and goats on pasture in the Nigerian derived savanna. *Veterinary Research and Communication* 13, 103-112.
- Colditz, I. G., Watson, D. L., Gray, G. D. and Eady, S. J., 1996. Some relationships between age, immune responsiveness and resistance to parasites in ruminants. *International Journal for Parasitology* 26, 869-877.
- Coles, G. C., Bauer, C., Borgsteede, F. H. M., Geerts, S., Klei, T. R., Taylor, M. A. and Waller, P. J., 1992. World Association for the Advancement of Veterinary Parasitology (WAAP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* 44, 35-44.
- Coles, G. C., 2005. Anthelmintic resistance--looking to the future: A UK perspective. *Research in Veterinary Science* 78, 99-108.
- Coles, G. C., Jackson, F., Pomroy, W. E., Prichard, R. K., Von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M. A. & Vercruyse, J., 2006. The

- detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology* 136, 167-185.
- Connan, R. M., 1976. Effect of lactation on the immune response to gastro-intestinal nematodes. *The Veterinary Record* 99, 476-477.
- Coop, R. L., Mellor, D. J., Jackson, E., Jackson, F., Flint, D. J. & Vernon, R. G., 1990. *Teladorsagia circumcincta* egg output at the onset of natural and induced lactation in ewes. *Veterinary Parasitology* 35, 295-305.
- Debela, E., 2002. Epidemiology of gastro-intestinal helminthiasis of Rift Valley goats under traditional husbandry system in Adami Tulu district, Ethiopia. *Ethiopian Journal of Science* 25, 35-44.
- Dobson, R. J., Donald, A. D., Waller, P. J. & Snowdon, K. L., 1986. An egg-hatch assay for resistance to levamisole in trichostrongyloid nematode parasites. *Veterinary Parasitology* 19, 77-84.
- Dobson, R. J., Waller, P. J. and Donald, A. D., 1990. Population dynamics of *Trichostrongylus colubriformis* in sheep: the effect of infection rate on the establishment of infective larvae and parasite fecundity. *International Journal for Parasitology* 20, 347-352.
- Donald, A. D., 1968. Ecology of the free-living stages of nematode parasites of sheep. *Australian Veterinary Journal* 44, 139-144.
- El-Azazy, O. M., 1995. Seasonal changes and inhibited development of the abomasal nematodes of sheep and goats in Saudi Arabia. *Veterinary Parasitology* 58, 91-98.
- Eysker, M., 1997. Some aspects of inhibited development of trichostrongylids in ruminants. *Veterinary Parasitology* 72, 265-283.
- Fakae, B. B., 1990. The epidemiology of helminthosis in small ruminants under the Traditional husbandry system in eastern Nigeria. *Veterinary Research and Communication* 14, 381-391.
- Fritsche, T., Kaufmann, J. & Pfister, K., 1993. Parasite spectrum and seasonal epidemiology of gastro-intestinal nematodes of small ruminants in The Gambia. *Veterinary Parasitology* 49, 271-283.
- Gamble, H. R. and Zajac, A. M., 1992. Resistance of St. Croix lambs to *Haemonchus contortus* in experimentally and naturally acquired infections. *Veterinary Parasitology* 41, 211-225.
- Gatongi, P. M., Scott, M. E., Ranjan, S., Gathuma, J. M., Munyua, W. K., Cheruiyot, H. & Prichard, R. K., 1997. Effects of three nematode anthelmintic treatment regimes on flock performance of sheep and goats under extensive management in semi-arid Kenya. *Veterinary Parasitology* 68, 323-336.
- Gibbs, H. C. & Barger, I. A., 1986. *Haemonchus contortus* and other trichostrongylid infections in parturient, lactating and dry ewes. *Veterinary Parasitology* 22, 57-66.
- Gibson, T. E. & Everett, G., 1971. The seasonal fluctuations of the larval populations of four species of trichostrongylid nematode on pasture herbage. *Research in Veterinary Science* 12, 602-604.
- Githiori, J. B., 2004. *Evaluation of Anthelmintic Properties of Ethno-veterinary Plant Preparations used as Livestock Dewormers by Pastoralists and Small-holder Farmers in Kenya*. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden
- Hansen, J. & Perry, B., 1994. *The Epidemiology, Diagnosis and Control of Helminth Parasites of Ruminants. A Handbook*. 2nd ed. ILRAD (International Laboratory for Research on Animal Diseases), Nairobi, Kenya, 171 pp.
- Hennessy, D. R., 1997. Physiology, pharmacology and parasitology. *International Journal for Parasitology* 27, 145-152.
- Hennessy, D. R., Sangster, N. C., Steel, J. W. and Collins, G. H. 1993. Comparative pharmacokinetic behaviour of albendazole in sheep and goats. *International Journal for Parasitology* 23, 321-325.
- Jackson F. & Coop, R. L., 2000. The development of anthelmintic resistance in sheep nematodes. *Parasitology* 120, S95-S107.

- Jackson, F., Jackson, E. & Coop, R. L., 1992. Evidence of multiple anthelmintic resistance in a strain of *Teladorsagia circumcincta* isolated from goats in Scotland. *Research in Veterinary Science* 53, 371-374.
- Kaplan, R. M., 2004. Drug resistance in nematodes of veterinary importance: a status report. *Trends in Parasitology* 20, 477-481.
- Kaplan, M., 2006. Update on parasite control in small ruminants: Addressing the challenges posed by multiple-drug resistant worms. In: *Proceedings of the American Association of Bovine Practitioners*. Saint Paul, MN, USA, September 21-23.
- Kassa, H. B., 2003. *Livestock and Livelihood Security in the Harar Highlands of Ethiopia: Implications for research and development*. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Kassai, T., 1999. *Veterinary Helminthology*. Butterworth-Heinemann, Reed Educational and Professional Publishing Ltd., Oxford, USA. 260 pp.
- Knox, M. & Steel, J., 1996. Nutritional enhancement of parasite control in small ruminant production systems in developing countries of South-East Asia and the Pacific. *International Journal for Parasitology* 26, 963-970.
- Knox, M. R., Torres-Acosta, J. F. & Aguilar-Caballero, A. J., 2006. Exploiting the effect of dietary supplementation of small ruminants on resilience and resistance against gastro-intestinal nematodes. *Veterinary Parasitology* 139, 385-393.
- Kotze, A. C., Le Jambre, L. F. & O'Grady, J., 2006. A modified larval migration assay for detection of resistance to macrocyclic lactones in *Haemonchus contortus*, and drug screening with Trichostrongylidae parasites. *Veterinary Parasitology* 137, 294-305.
- Lacey, E. & Snowden, K. L., 1988. A routine diagnostic assay for the detection of benzimidazole resistance in parasitic nematodes using tritriated benzimidazole carbamates. *Veterinary Parasitology* 27, 309-324.
- LeJambre, L. F., 1984. Stocking rate effects on the worm burdens of Angora goats and Merino sheep. *Australian Veterinary Journal* 61, 280-282.
- Magona, J. W. and Musisi, G., 2002. Influence of age, grazing system, season and agroclimatic zone on the prevalence and intensity of gastro-intestinal strongylosis in Ugandan goats. *Small Ruminant Research* 44, 187-192.
- Maingi, N., Gichanga, E. J. & Gichohi, V. M., 1993. Prevalence of gastro-intestinal helminths and coccidian parasites and frequency distribution of some nematode genera of goats on some farms in four districts of Kenya. *Bulletin of Animal Health and Production in Africa* 41, 285-290.
- Martin, P. J. & LeJambre, L. F. 1979. Larval paralysis test as *in vitro* assay of levamisole and morantel tartrate resistance in *Ostertagia*. *Veterinary Science and Communication* 3, 159-164.
- Mezgebu, M., 1995. *A Survey on Ovine Fascioliasis and Lungworm Infection in Addis Ababa and the Surrounding Highland Areas*. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.
- Michel, J. F., Lancaster, M. B. & Hong, C., 1975. Arrested development of *Ostertagia ostertagi* and *Cooperia oncophora*. Effect of temperature at the free-living third stage. *Journal of Comparative Pathology* 85, 133-138.
- Miller, E. and Waller, P. J., 2004. Novel approaches to control of parasites – a workshop. Edited by Miller, J. E. and Waller, P. J. *Veterinary Parasitology* 125, 59-68.
- Mukasa-Mugerwa, E., Lahlou-Kassi, A., Anindo, D., Rege, J. E. O., Tembely, S., Tibbo, M. & Baker, R. L., 2000. Between and within breed variation in lamb survival and the risk factors associated with major causes of mortality in indigenous Horro and Menz sheep in Ethiopia. *Small Ruminant Research* 37, 1-12.
- Nari, A., Salles, J., Castell, D. & Hansen, J. W., 1997. Control of gastro-intestinal nematodes in farming systems of Uruguay. In: Hansen, J. W., Waller P. J. (Eds.), *Proceedings of Biological Control Workshop*. FAO Technical Series Report No. 141. pp. 89-94.

- Ng'ang'a, C. J., Kanyari, P. W., Maingi, N. & Munyua, W. K., 2006. The effect of weather on the occurrence and magnitude of periparturient rise in trichostronglyid nematode egg output in Dorper ewes in a semi-arid area of Kajiado District of Kenya. *Tropical Animal Health and Production* 38, 389-395.
- Ngategize, P. K. J., Bekele, T. & Tilahun, G., 1993. Financial losses caused by ovine fascioliasis in the Ethiopian highlands. *Tropical Animal Health and Production* 25, 155-161.
- Nginyi, J. M., Duncan, J. L., Mellor, D. J., Stear, M. J., Wanyangu, S. W., Bain, R. K. & Gatongi, P. M., 2001. Epidemiology of parasitic gastro-intestinal nematode infections of ruminants on smallholder farms in central Kenya. *Research in Veterinary Science* 70, 33-39.
- Nwosu, C. O., Madu, P. P. and Richards, W. S., 2006. Prevalence and seasonal changes in the population of gastro-intestinal nematodes of small ruminants in the semi-arid zone of north-eastern Nigeria. *Veterinary Parasitology* (in press).
- O'Connor, L. J., Walkden-Brown, S. W. & Kahn, L. P., 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. *Veterinary Parasitology* 142, 1-15.
- O'Grady, J. & Kotze, A. C., 2004. *Haemonchus contortus*: in vitro drug screening assays with the adult life stage. *Experimental Parasitology* 106, 164-172.
- Ogunsusi, R. A. & Eysker, M., 1979. Inhibited development of trichostrongylids of sheep in northern Nigeria. *Research in Veterinary Science* 26, 108-110.
- Over, H. J., Jansen, J. & Von Olm, P. W., 1992. *Distribution and Impact of Helminth Diseases of Livestock in Developing Countries*. FAO Animal Production and Health Paper 96. FAO (Food and Agriculture Organization of the United Nations), Rome, Italy, pp. 221.
- Pandey, V. S., Ndao, M. & Kumar, V., 1994. Seasonal prevalence of gastro-intestinal nematodes in communal land goats from the highveld of Zimbabwe. *Veterinary Parasitology* 51, 241-248.
- Papadopoulos, E., Himonas, C. and Coles, G. C., 2001. Drought and flock isolation may enhance the development of anthelmintic resistance in nematodes. *Veterinary Parasitology* 97, 253-259.
- Perry, B. D. & Randolph, T. F., 1999. Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. *Veterinary Parasitology* 84, 145-168.
- Perry, B. D., Randolph, T. F., McDermott, J. J., Sones, K. R. & Thornton, P. K., 2002. *Investing in Animal Health Research to Alleviate Poverty*. International Livestock Research Institute (ILRI), Nairobi, Kenya, pp. 148.
- Presidente, P. J. A., 1985. Methods for the detection of resistance to anthelmintics. In: Anderson, N., Waller, P. J. (Eds.), *Resistance in Nematodes to Anthelmintic Drugs*. Division of Animal Health, CSIRO, Australia, pp. 13-27.
- Roos, M. H., Boersema, J. H., Borgsteede, F. H. M., Cornelissen, J., Taylor, M. & Ruitenbergh, E. J., 1990. Molecular analysis of selection for benzimidazole resistance in the sheep parasite *Haemonchus contortus*. *Molecular Biochemistry and Parasitology* 43, 77-88.
- Rowe, J. B., Nolan, J. V., de Chaneet, G., Teleni, E. & Holmes, P. H., 1988. The effect of haemonchosis and blood loss into the abomasum on digestion in sheep. *British Journal of Nutrition* 59, 125-139.
- Sangster N. C., 1999. Anthelmintic resistance: past, present and future. *International Journal for Parasitology* 29, 115-24.
- Sangster, N. C., Rickard, J. M., Hennessy, D. R., Steel, J. W. and Collins, G. H. 1991. Disposition of oxfendazole in goats and efficacy compared with sheep. *Research in Veterinary Science* 51, 258-263.
- Small, A. J. & Coles, G. C., 1993. Detection of anthelmintic resistance by culture in vitro of parasitic stages of ovine nematodes. *Veterinary Parasitology* 51, 163-166.

- Soulsby, E. J. L., 1982. *Helminths, Arthropods and Protozoa of Domesticated Animals*, 7th Ed. The English Language Book Society and Ballière Tindall, London, 809 pp.
- Stringfellow, F., 1988. An in vitro test for drug resistance in *Haemonchus contortus*. *Proceedings of the Helminthology Society of Washington* 50, 19-23.
- Swan G. E, & Gross, S. J. 1985. Efficacy of ivermectin against gastrointestinal nematode infections in goats. *Veterinary Record* 117, 147-149.
- Taylor, M. A., 1990. A larval development test for the detection of anthelmintic resistance in nematodes of sheep. *Research in Veterinary Science* 49, 198-202.
- Taylor, M. A., Hunt, K. R. & Goodyear, K. L., 2002. Anthelmintic resistance detection methods. *Veterinary Parasitology* 103, 183-194.
- Tembely, S., 1995. The effect of genotype, lactation and season on the periparturient rise in faecal egg counts in Ethiopian highland ewes: Preliminary observations. Pp. 351-363. In: Gray, G. D., Uilenberg, G. and Jacobsen, L. L. (Eds.), *Parasitology Research in Africa*. Proceedings of an IFS (International Foundation for Science) workshop, Bobo-Dioulasso, Burkina Faso, pp 351-363.
- Tembely, S., 1998. Development and survival of infective larvae of nematode parasites of sheep on pasture in a cool tropical environment. *Veterinary Parasitology* 79, 81-87.
- Tembely, S., Lahlou-kassi, A., Rege, J. E., Sovani, S., Diedhiou, M. L. & Baker, R. L., 1997. The epidemiology of nematode infections in sheep in a cool tropical environment. *Veterinary Parasitology* 70, 129-141.
- Tibbo, M., Woldemeskel, M. & Gopilo, A., 2001. An outbreak of respiratory disease complex in sheep in central Ethiopia. *Tropical Animal Health and Production* 33, 355-365.
- Tibbo, M., Mukasa-Mugerwa, E. Woldemeskel, M. & Rege, J. E. O., 2003. Risk factors for mortality associated with respiratory disease among menz and Horro sheep in Ethiopia. *The Veterinary Journal* 165, 276-287.
- Tibbo, M., Aragaw, K., Philipsson, J., Malmfors, B., Nasholm, A., Ayalew, W. & Rege, J. E. O., 2006. Economics of sub-clinical helminthosis control through anthelmintics and nutrition in indigenous Menz and Awassi-Menz crossbred sheep in Ethiopia. Unpublished.
- Tilahun, G., 1993. A survey of fascioliasis in cattle, sheep and goats slaughtered at Addis Ababa abattoir. In: Arosenius, C., Jacobsen, L., Lindberg, R. and Nilsson, P. O. (Eds.), *Animal Diseases of the Gastro-intestinal Tract and Liver: An African Perspective*. Proceedings of a seminar held at ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia, pp 125-133.
- Tilahun, G., 1995. Epidemiology of helminth parasites of small ruminants in mid-lowland Ethiopia. In: Gray, G. D., Uilenberg, G. and Jacobsen, L. L. (Eds.), *Parasitology Research in Africa*. Proceedings of an IFS (International Foundation for Science) workshop, Bobo-Dioulasso, Burkina Faso, pp 255-269.
- Torgerson, P. & Claxton, J., 1999. Epidemiology and control. In: *Fasciolosis* (ed. Dalton, J. P.). CAB International.
- Torgerson, P. R., Schnyder, M. & Hertzberg, H., 2005. Detection of anthelmintic resistance: a comparison of mathematical techniques. *Veterinary Parasitology* 128, 291-298.
- Torina, A., Ferrantelli, V., Sparagano, O. A., Reale, S., Vitale, F. & Caracappa, S., 2004. Climatic conditions and gastro-intestinal nematode egg production: observations in breeding sheep and goats. *Annals of New York Academy of Science* 1026, 203-209.
- Tsotetsi, A. M. & Mbat, P. A., Parasitic helminths of veterinary importance in cattle, sheep and goats on communal farms in the north-eastern Free State, South Africa. *Journal of South African Veterinary Association* 74, 45-48.
- Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M. & Jennings, F. W., 1996. *Veterinary Parasitology*, 2nd ed. Blackwell Science, United Kingdom, p. 307.
- Van Wyk, J. A., 1985. The epidemiology and control of gastro-intestinal nematode infestation of sheep and cattle in South Africa. I. The historic role of

- Onderstepoort and a short discussion of present research priorities. *Onderstepoort Journal of Veterinary Research* 52, 215-219.
- Van Wyk, J. A., 2001. Refugia – overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. *Onderstepoort Journal of Veterinary Research* 68, 55-67.
- Van Wyk, J. A. & Bath, G. F., 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Veterinary Research* 33, 509-529.
- Van Wyk, J. A., Cabaret, J. & Michael, L. M., 2004. Morphological identification of nematode larvae of small ruminants and cattle simplified. *Veterinary Parasitology* 119, 277-306.
- Van Wyk, J. A., Stenson, M. O., Van der Merwe, J. S., Vorster, R. J. & Viljoen, P. G., 1999. Anthelmintic resistance in South Africa: surveys indicate an extremely serious situation in sheep and goat farming. *Onderstepoort Journal of Veterinary Research* 66, 273-284.
- Vatta, A. F., Krecsek, R. C., Letty, B. A., Van der Linde, M. J., Grimbeek, R. J., de Villiers, J. F., Motswatswe, P. W., Molebiemang, G. S., Boshoff, H. M. & Hansen, J. W., 2002. Incidence of *Haemonchus* spp. and effect on haematocrit and eye colour in goats farmed under resource-poor conditions in South Africa. *Veterinary Parasitology* 103, 119-131.
- Vatta, A. F. & Lindberg, A. L., 2006. Managing anthelmintic resistance in small ruminant livestock of resource-poor farmers in South Africa. *Journal of South African Veterinary Association* 77, 2-8.
- Varady, M & Corba, J. 1999. Comparison of six *in vitro* tests in determining benzimidazole and levamisole resistance in *Haemonchus contortus* and *Ostertagia circumcincta* of sheep. *Veterinary Parasitology* 80, 239-249.
- Vercruyse, J., 1983. A survey of seasonal changes in nematode faecal egg count levels of sheep and goats in Senegal. *Veterinary Parasitology* 13, 239-244.
- Vercruyse, J., 1985. The seasonal prevalence of inhibited development of *Haemonchus contortus* in sheep in Senegal. *Veterinary Parasitology* 17, 159-163.
- Waller, P. J., 2006. From discovery to development: current industry perspectives for the development of novel methods of helminth control in livestock. *Veterinary Parasitology* 139, 1-14.
- Waruiru, R. M., Ngotho, J. W. & Mutune, M. N., 2004. Effect of urea-molasses block supplementation on grazing weaner goats naturally infected with gastrointestinal nematodes. *Onderstepoort Journal of Veterinary Research* 71, 285-289.
- Watson, D. L., Colditiz, I. G., Andrew, M., Gill, H. S. and Altmann, K. G., 1994. Age-dependent immune response in merino sheep. *Research in Veterinary Science* 57, 152-158.
- Wood, I. B., Amaral, N. K., Bairden, K., Duncan, J. L., Kassai, T., Malone, J. B., Pankavich, J. A., Reinecke, R. K., Slocombe, O., Taylor, S. M. & Vercruyse, J., 1995. World Association for the Advancement of Veterinary Parasitology (WAAVP) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Veterinary Parasitology* 58, 181-213.
- Yilma J. & Malone, J. B. 1998. A geographical information system forecast model for strategic control of fasciolosis in Ethiopia. *Veterinary Parasitology* 78, 103-127.
- Zajac, A. M., 2006. Gastro-intestinal nematodes of small ruminants: life cycle, anthelmintics, and diagnosis. *Veterinary Clinics, Food Animal Practice* 22, 529-541.
- Zajac, A. M., Herd, R. P. & McClure, K. E., 1988. Trichostrongylid parasite populations in pregnant or lactating and unmated Florida Native and Dorset/Rambouillet ewes. *International Journal for Parasitology* 18, 981-985.

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