Evaluation of Anthelmintic Properties of Ethnoveterinary Plant Preparations Used as Livestock Dewormers by Pastoralists and Small Holder Farmers in Kenya

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2004

Acta Universitatis Agriculturae Sueciae
Veterinaria 173

ISSN 1401-6257
ISBN 91-576-6666-0

Tryck: SLU Service/Repro, Uppsala 2004

Abstract

Githiori, John B. 2004. Evaluation of Anthelmintic Properties of Ethnoveterinary Plant Preparations Used as Livestock Dewormers by Pastoralists and Small Holder Farmers in Kenya. Doctoral thesis
ISSN 1401-6257, ISBN 91 576 6666 0

Parasitic nematodes, especially *Haemonchus contortus*, are among the most common and economically important causes of infectious diseases of sheep and goats owned by pastoralists and small holder farmers in East Africa. In Kenya, control of these infections mainly relies on the use of anthelmintic drugs. However, ethnoveterinary medicine (EVM) preparations are widely used by pastoralists and small holder farmers (SHF) for treatment of their livestock against helminth parasites.

This thesis covers the evaluation of the anthelmintic efficacy of some EVM preparations used by pastoralists and SHF in Kenya. The plant species selected, and prepared for animal dosing with the help of traditional healers (THs) were: *Aframomum sanguineum*, *Albizia anthelmintica*, *Ananas comosus*, *Annona squamosa*, *Azadirachta indica*, *Dodonaea angustifolia*, *Hagenia abyssinica*, *Hildebrandtia sepalosa*, *Myrsine africana*, *Olea europaea* var. *africana*, and *Rapanea melanophloeos*. Evaluation was carried out in two *in vivo* infection models, namely *H. contortus* in sheep and *Heligmosomoides polygyrus* in mice. The anthelmintic efficacy of the EVM preparations was monitored through faecal egg count (FEC) reduction, at regular intervals for a period of 2 – 3 weeks post treatment in sheep. Monitoring in mice was done through FEC and total worm counts (TWC) one week after treatment. An *a priori* cut-off value of 70% reduction of FEC and TWC, to denote useful anthelmintic efficacy, was used for both sheep and mice.

Of the ten plant species tested in sheep, the largest decrease of 34% in FEC was measured from a bark preparation of *A. anthelmintica* collected from the Samburu District. None of the other plant species had a significant effect on FEC.

Similarly, the seven plant species and some of their related active constituents evaluated in mice did not significantly reduce FEC or remove parasites. Preparations of *A. anthelmintica* at doses above 0.5 g per mouse were toxic.

In conclusion, no reduction of FEC or TWC greater than the *a priori* value of 70% was observed in sheep or in mice. Therefore, the plants evaluated were ineffective as anthelmintics in the preparations and forms that were used.

Keywords: Ethnoveterinary preparations, sheep, mice, *Haemonchus contortus*, *Heligmosomoides polygyrus*, anthelmintic efficacy, pastoralists, smallholder farmers, Kenya

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Appendix

Papers I-IV

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

I. Githiori, J. B., Höglund, J., Waller, P. J. & Baker, R. L. 2002. Anthelmintic activity of preparations derived from *Myrsine africana* and *Rapanea melanophloeos* against the nematode parasite, *Haemonchus contortus*, of sheep. *Journal of Ethnopharmacology 80*, 187-191.

II. Githiori, J. B., Höglund, J., Waller, P. J. & Baker, R. L. 2003. The anthelmintic efficacy of the plant, *Albizia anthelmintica*, against the nematode parasites *Haemonchus contortus* of sheep and *Heligmosomoides polygyrus* of mice. *Veterinary Parasitology 116*, 23-34.

III. Githiori, J. B., Höglund, J., Waller, P. J. & Baker, R. L. 2003. Evaluation of anthelmintic properties of extracts from some plants used as livestock dewormers by pastoralist and smallholder farmers in Kenya against *Heligmosomoides polygyrus* infections in mice. *Veterinary Parasitology 118*, 215-226.

IV. Githiori, J. B., Höglund, J., Waller, P. J. & Baker, R. L. 2004. Evaluation of anthelmintic properties of some plants used as livestock dewormers against *Haemonchus contortus* infections in sheep. *Parasitology*, in press.

Approval has been obtained from each of the respective scientific journals for the inclusion of these manuscripts in this thesis.

Appendices

A. *In vitro* evaluation of plant preparations in different non parasitic and parasitic nematode species shown in Tables 1, 2 and 3.

B. *In vivo* evaluation of plant preparations against helminth parasites of different host animals including humans shown in Tables 4, 5 and 6.

Abbreviations

AEZ	agroecological zones
ANOVA	analysis of variance
AR	anthelmintic resistance
ASAL	arid and semi arid lands
bwt	body weight
BZs	benzimidazole/probenzimidazoles anthelmintic products
cm	centimetres
CE	cold method of aqueous extraction of plant material
CTs	condensed tannins
COWP	copper oxide wire particle
D	Dorper breed of sheep
EPG	trichostrongyle eggs per gram of faeces
ES	excretory/secretory proteins
EVM	ethnoveterinary medicine
FEC	nematode faecal eggs counts per gram of faeces
FECR	faecal egg count reductions
GABA	gamma aminobutyric acid
GI	gastrointestinal
g	grams
HE	hot method of aqueous extraction of plant material
HT	hydrolysable tannins
ILRI	International Livestock Research Institute
ITDG	Intermediate Technology Development Group
kg	kilograms
L_3	third or infective stage of nematode larvae
L_4	fourth stage of parasitic nematode larvae
LWT	live weight
MLs	macrocyclic lactones
m	metres
ml	millilitres
min	minutes
NGO	non-governmental organisation
PCV	packed red cell volume
RM	Red Maasai breed of sheep
SHF	small holder farmers
SLU	Swedish University of Agricultural Sciences
SVA	National Veterinary Institute
SWEPAR	Department of Parasitology, SVA/SLU
TWC	total worm counts of recovered nematodes
TWCR	total worm count reductions
TH	traditional healer
WAAVP	World Association for the Advancement of Veterinary Parasitology

Introduction

Diseases caused by helminth parasites in livestock continue to be a major productivity constraint, especially in small ruminants in the tropics and subtropics (Perry *et al.*, 2002). The parasitic diseases of production animals are widely distributed, but have different impacts in different parts of the world (Perry & Randolph, 1999). In the Developed world, with the exception of those countries in the southern hemisphere, the greatest impact is probably found in the costs of control, particularly in the case of the helminth parasitoses. In the Developing world, the greatest impact of parasitic diseases is in direct and potential productivity losses (Perry & Randolph, 1999).

Infections by gastrointestinal (GI) helminth parasites of livestock are among the most common and economically important diseases of grazing livestock (Perry *et al.*, 2002). They are characterised by lower outputs of animal products (meat, milk, hides and skins), manure and traction, which all impact on the livelihood of small holder farmers (SHF) (Perry & Randolph, 1999). As such, their effects, in terms of productivity losses and control costs, are generally dealt with at the producer rather than at the societal level (Perry & Randolph, 1999). The greatest losses associated with nematode parasite infections are sub-clinical, and economic assessments show that financial costs of internal parasitism are enormous (Preston & Allonby, 1979; McLeod, 1995). One exception to this is the highly pathogenic nematode parasite of small ruminants, *Haemonchus contortus*, which is also capable of causing acute disease and high mortality in all classes of stock (Allonby & Urquhart, 1975). Haemonchosis has been identified as one of the top ten constraints to sheep and goat rearing in East Africa (Perry *et al.*, 2002).

Consequently, there is an urgent and ever-present need to control infections caused by *H. contortus* in small ruminants in the tropics. Control is generally achieved by the use of synthetic anthelmintics in combination with grazing management. However, misuse and poor formulations of these products have led to the development of anthelmintic resistance (AR) (Lans & Brown, 1998). Novel approaches to nematode parasite control are needed for small ruminants in the tropics and sub-tropics, to counteract the problem of AR (Waller, 1997, 1999). There is a need to rethink the use of anthelmintics, as well as develop novel approaches, which will lead to sustainable control of parasites (Waller, 2003). Ideally, this may entail an integrated approach, including biological control, reduced frequency of anthelmintic treatments, parasite vaccines, livestock breeds that are resistant to parasites, and the use of plants with anti-parasitic properties as well as the use of traditional herbal remedies, or ethnoveterinary medicine (EVM) (Waller, 1999).

Adulteration of anthelmintics has been found to be a common practice in Kenya (Monteiro *et al.*, 1998). The use of such products, with inadequate amounts of the active ingredients, encourage the development of AR. Illiteracy and/or unfamiliarity with synthetic anthelmintics, resulting in incorrect usage, is also a problem leading to the same consequences (Danø & Bøgh, 1999). Moreover, these drugs are relatively expensive and often unavailable to farmers in rural areas.

These constraints indicate that the reliance on synthetic anthelmintics in many countries in the Developing world may present difficulties in the management of GI parasite infections in sheep and goats, necessitating novel alternative methods of helminth control.

As a consequence of these problems and difficulties, pastoralists and SHF have continued to use indigenous plants as livestock dewormers, drawing upon centuries of traditional belief and use of EVM (Danø & Bøgh, 1999). The use of EVM may present a cheaper, sustainable alternative if the compounds were demonstrated to work. These herbal preparations are much cheaper than synthetic drugs and have been used over time by pastoralists and SHF for treatment of their livestock against helminth parasites.

This thesis provides an experimental evaluation of anthelmintic efficacy of some of the EVM preparations used by pastoralists and SHF in Kenya. The evaluation was carried out in a ruminant host-parasite model using sheep artificially infected with *H. contortus*, and in a monogastric host-parasite model using mice infected with *Heligmosomoides polygyrus*. Although these parasites exhibit some striking biological differences, most notably in the class of host, they belong to the same nematode superfamily Trichostrongyloidea (Anderson, 1992).

Background

The parasitic helminths of grazing animals mainly belong to the phyla Platyhelminthes and Nemathelminthes (Soulsby, 1982).

- Platyhelminthes, which are otherwise referred to as flatworms, have two classes of parasites, the Cestoda (tapeworms) and Trematoda (flukes). These parasites are flattened dorsoventrally and hermaphroditic. The most common and abundant cestode parasite in grazing small ruminants is *Moniezia* spp. (Soulsby, 1982). The most important trematode in livestock in Kenya is *Fasciola gigantica*, which is endemic in marshy areas with poor drainage and high rainfall (Wamae *et al.*, 1990).
- Nemathelminthes in which the class Nematoda (roundworms) is found. Some of the superfamilies of veterinary importance in the phylum include Ancylostomatoidea, Ascaridoidea, Oxyuroidea, Rhabditoidea, Strongyloidea, and especially Trichostrongyloidea (Anderson, 1992).

Helminthoses refers to a complex of conditions caused by parasites of the classes Nematoda, Cestoda and Trematoda. In sheep and goats in Kenya, GI parasites are prevalent and especially the nematode *H. contortus*, and to a lesser extent the nematode parasites *Trichostrongylus colubriformis* and *Oesophagostomum* spp., with occasional infections of *Strongyloides* and *Trichuris* spp. (Preston & Allonby, 1979; Carles, 1993; Gatongi *et al.*, 1997; Maingi, Munyua & Gichigi, 2002).

Direct and indirect losses due to nematode infections are estimated to be high (Preston & Allonby, 1979), and control of these parasites is therefore considered important. In most areas in the tropics, animals continuously graze on pasture all

year round. This exposes the animals to continuous parasite pressure when climatic conditions are favourable for the development and survival of free living stages (Dinnik & Dinnik, 1958). In Kenya, infections caused by *H. contortus* were reported to affect all adult sheep and lambs in a flock, resulting in numerous deaths within six weeks after onset of clinical signs (Allonby & Urquhart, 1975). In the same study, other species were found to be less than 3% of the total GI nematode parasite burden in sheep (Allonby & Urquhart, 1975). More recently, it has been demonstrated that *H. contortus* was the dominant species, comprising 73% of the recovered parasites in sheep, in a semi arid area (Gatongi *et al.*, 2003).

The total economic loss to the Kenya agricultural sector due to haemonchosis in small ruminants was estimated at US$ 26 million per year (Allonby & Urquhart, 1975). Although this estimation is old, it may not have taken into consideration the losses incurred in pastoral animals (Iles, 1993). Mukhebi *et al.* (1985) calculated the benefit-cost ratios for helminth control in small holder farms of western Kenya to be 5.6, 5.0 and 3.4 for East African x Toggenburg cross breeds, Galla and East African breeds of goats, respectively. Similarly, a benefit-cost ratio of 3.3 was obtained as a result of endoparasite control in the Transmara district of Kenya (Muenstermann & Tome, 1989).

Small ruminants in Kenya

Importance to pastoralists and small holder farmers

Agriculture is the mainstay of the economy of Kenya, in which the small holder farming sector plays an important role in food production. These small holder farms (<5 ha) occupy about 90% of the land under agriculture (Masai, 1995), and are found in the humid to the semi humid agro-ecological zones (AEZ). The arid and semi arid lands (ASAL) in AEZ IV – VII (Fig. 1) cover over 80% of the total land surface, and provide subsistence economy to 25% of the population, who are mainly pastoralists and agropastoralists (Government of Kenya, 2002; Mwagore, 2003). Pastoralist communities typically practise transhumance or nomadic livestock husbandry and own animal herds of varying sizes, which are mainly communally grazed. For sheep and goats in Kenya, the total numbers and distribution are shown in Table 1 and Figure 1, respectively.

Table 1. *Total number of sheep and goats in Kenya between 1998 and 2002 (1000s)*

Year	1998	1999	2000	2001	2002	Mean
Goats	8945	9208	9647	9000	9000	9160
Sheep	8126	8535	8462	8000	8000	8225
Total	17071	17743	18109	17000	17000	17385

Adopted from FAO (2003)

In ASAL areas (AEZ IV – VII) of Kenya (see Fig. 1), it has been found that small ruminant production contributes up to 50% of the income used for daily and development needs by pastoralists and SHF (Mucuthi, 1992). Amongst these communities, small ruminants provide a means of generating and preserving wealth, and make a significant contribution to household assets (Perry *et al.*, 2002).

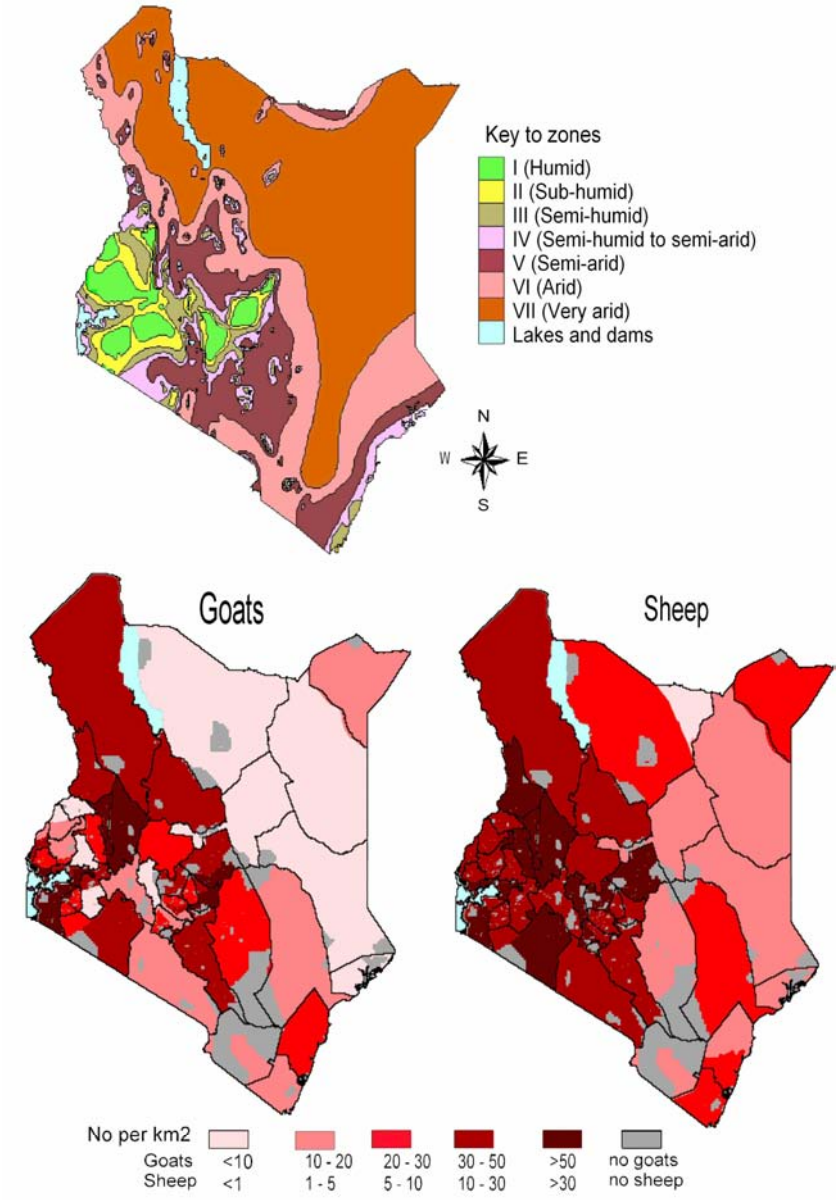

Fig. 1. Maps showing (top) agroecological zones (AEZ) (adopted from Kenya Soil Survey 2002), and (bottom) the distribution of goats and sheep in Kenya (adopted from FAO 2004)

Constraints to small ruminant productivity

The factors that affect the productivity of small ruminants in sub-Saharan Africa include:

- limited natural resources such as fragile ecosystems and water scarcity;
- scarcity and poor quality of forages for feeds;
- diseases such as contagious caprine pleuropneumonia, helminthosis, peste des pestis ruminants and sheep pox;
- inappropriate genotypes coupled with low productivity and poor characterisation;
- socio-economic factors such as poor extension services and infrastructure, deficient inputs and inefficiency in distribution of drugs (Ibrahim, 1998).

The importance of parasitic diseases as a constraint to the productivity and development of the livestock industry in developing countries is well recognized (Over, Jansen & van Olm, 1992; Perry *et al.*, 2002). In Kenya, GI parasitism has been recognised as a major constraint to small ruminant production (Carles, 1993).

Control strategies

The main methods for control of GI nematode parasites are prophylactic treatment with synthetic anthelmintics in combination with grazing management. However, alternative strategies have also been examined for the sustainable control of GI nematode parasites. A brief overview of the various methods of control with regards to their applicability to pastoralists and SHF in Kenya is described below.

Anthelmintics

The broad spectrum anthelmintics, which remove parasites in different stages of development within the host species, are the cornerstone of parasite control in GI nematode infections. The major classes of synthetic anthelmintics used for control of GI nematode parasites of ruminant livestock are:

- The benzimidazole/probenzimidazoles (BZs) group. The mode of action of BZs is by interference with polymerization of microtubules (Harder, 2002). These drugs bind to the protein tubulin of the parasite, therefore causing death by starvation (Roos, 1997).
- The tetrahydropyrimidines/imidazothiazoles group (levamisole/pyrantel–morantel). These drugs affect acetylcholine neuro-transmission by interfering with nicotinic acetylcholine receptors (Roos, 1997; Harder, 2002).
- The macrocyclic lactones (MLs) or avermectins/milbemycins group. The MLs are thought to interact with chloride channels on helminth gamma-aminobutyric acid (GABA) receptor complexes, and also inhibit pharyngeal pumping (and hence feeding), motility and fecundity in susceptible nematodes, resulting in paralysis and ultimately elimination from the host (Harder, 2002; Yates, Portillo & Wolstenholme, 2003).

There are other anthelmintics referred to as narrow spectrum compounds, which have activity against fewer species of parasites and/or lack high levels of efficacy against all stages of the parasites (Bowman, Lynn & Georgi, 2003). Examples of

these anthelmintics include naphthalophos, salicylanilides and substituted phenols (closantel, oxyclozanide and nitroxynil), and triclabendazole.

The control of GI parasites in small ruminants in Kenya has tended to rely on the use of anthelmintic drugs, mainly the BZs and levamisole (Kinoti, Maingi & Coles, 1994; Maingi, Munyua & Gichigi, 2002). Studies have shown that AR exists for all commonly used anthelmintics in Kenya (Kinoti, Maingi & Coles, 1994; Mwamachi *et al.*, 1995; Wanyangu *et al.*, 1996; Maingi *et al.*, 1998). The spread of AR may be attributed to the introduction of animals with nematode resistant parasites, purchased from government and large scale commercial farms (Kinoti, Maingi & Coles, 1994; Mwamachi *et al.*, 1995).

However, the use of anthelmintics at the same time is limited by their high costs, uncertain availability and poor quality in Kenya (Monteiro *et al.*, 1998). Still, there is an escalating problem with increasing frequency of AR, partly related to the spread of resistant parasite strains along with host movements (Maingi, 1991; Kinoti, Maingi & Coles, 1994; Mwamachi *et al.*, 1995; Wanyangu *et al.*, 1996; Maingi *et al.*, 1998; Waruiru, Ngotho & Mukiri, 1998).

Grazing management

Pasture management is designed to prevent infection of ruminants with internal parasites, and requires long-term planning. The main methods of parasite control through exploiting pasture management have been defined as preventive, evasive and diluting (Michel, 1985).

Grazing management appropriate for Kenya includes the rapid rotational grazing systems applied elsewhere in the humid tropics (Banks *et al.*, 1990). Tethering (Fig. 2) and zero grazing, which separate the host from infective larvae on pasture, are also widely practiced by SHF in Kenya (Semenye *et al.*, 1992).

Areas with cool temperatures, such as the highlands of Kenya, favour rapid hatching and larval development, and a 12 – 16 week survival period on pasture can be expected (Waruiru, Ngotho & Mukiri, 1998). This short duration of survival of infective larvae has further been demonstrated in arid and semi arid regions of Kenya, where tracer lambs did not acquire larvae from pasture for three months during the dry season, and only animals permanently grazing on these pastures had low numbers of parasites (Wanyangu *et al.*, 1997).

Rotational grazing may not be feasible due to limitations in farm/plot size in small holder farms in Kenya. Furthermore, communal grazing (Fig. 2) and common watering points, which are shared in most pastoralist and small holder flocks in Kenya, are a source of parasitic infection (Iles, 1993, Nginyi, 2001). Land ownership is also not defined in pastoral areas, therefore making grazing management difficult. Moreover, grazing (herding) strategies in pastoralist flocks/herds are determined by the rainfall pattern (Iles, 1993). In addition, the stocking density is difficult to control in pastoral areas due to social issues involved in animal ownership.

Fig. 2. Grazing management practices amongst pastoralists and SHF in Kenya. Top picture shows communal grazing of sheep and goats in pastoral flocks, and bottom is tethering of sheep (top arrow in bottom picture) owned by SHF. The sheep in the bottom picture is also supplemented with farm and household vegetable waste (lower arrow), while sheep and goats owned by pastoralists do not receive any supplementation.

Targeted drenching

A general feature of parasitic infections in animal flocks/herds is that the distribution of parasites is skewed (Roberts & Swan, 1982; Wilson & Grenfell, 1997). That is, a few individuals in a flock/herd are infected with the majority of parasites and many animals have few or no parasites (Shaw & Dobson, 1995). Targeted drenching is based on selective treatment of those individual animals that are diagnosed as heavily infected, and with clinical symptoms of the disease. The FAMACHA system was founded on this concept, and developed in South Africa for treatment of small ruminants infected with *H. contortus* (Malan & van Wyk, 1992). The system utilises clinical identification of anaemia in animals infected with this parasite by use of a chart indicating the degree of anaemia. Thus, instead of treating the whole flock, only those animals with infections inducing anaemia are treated with an effective anthelmintic (van Wyk, Bath & Malan, 1998; van Wyk & Bath, 2002; Vatta *et al.*, 2002).

This system is only suitable in situations where anaemia is attributable to infection by *H. contortus*. However, most of Kenya's rangelands are infested with *Glossina* spp. (tsetse flies), which transmit trypanosomes to livestock (Griffin & Allonby, 1979). Clinical trypanosomosis is also a common cause of anaemia (Griffin & Allonby, 1979). Furthermore, infection with *Fasciola* spp. (liver flukes) in small ruminants also results in blood loss and anaemia. Therefore these other conditions need to be eliminated as the cause of anaemia for the correct use of the FAMACHA system. This system has also not been fully evaluated in goats (van Wyk & Bath, 2002), and labour requirements in pastoral areas where flocks of sheep and goats may be large could be a drawback to its use.

Biological control

Biological control of parasitic nematodes in livestock aims at establishing a situation where grazing animals are exposed to a low level of infective larvae, but at a level that will secure the development of naturally acquired immunity in the same animals (Thamsborg, Roepstorff & Larsen, 1999). Current research has focused almost exclusively on *Duddingtonia flagrans*, largely because the fungus is able to survive gut passage as the resistant resting stages (chlamydospores), its ability to grow rapidly in fresh dung, and its voracious nematophagous ability (Larsen, 1999, 2000). This microfungus has been shown to occur in the same environment that favours larval development and translation (Thamsborg, Roepstorff & Larsen, 1999). Field evaluation of this concept has been on-going for the last decade in sheep, goats, cattle, horses and pigs in a range of geoclimatic conditions worldwide (FAO, 2002; Chandrawathani *et al.*, 2003; Dimander, Höglund & Waller, 2003).

Although some studies have been carried out in Kenya on the use of *D. flagrans* to control nematode parasites of sheep (S. Githigia, University of Nairobi, personal communication), this technology is highly unlikely to be appropriate for use by pastoralists and SHF. This is mainly because there is a lack of a suitable application system, and the method needs to be combined with other control strategies. Moreover, the nematode trapping fungi are only effective against larvae

in faecal pats but not those that have migrated to vegetation nor on worm burdens in the animals (Githigia *et al.*, 1997).

Copper oxide wire particles (COWP)

In studies conducted in sheep in New Zealand (Bang, Familton & Sykes, 1990) and Australia (Knox, 2002), and in goats in France (Chartier *et al.*, 2000), COWP administered as capsules was shown to be effective in reducing the establishment and fecundity of *H. contortus*. The mechanism of action is assumed to be based on the lethal effects on the parasite of ionic copper liberated from the COWP by the acid secreting mucosa of the abomasum. However, the concentration necessary for an anthelmintic effect and the potential for toxicity in copper sufficient animals, or those exposed to copper accumulating plants, are yet to be established.

In some pastoral areas, lambs were found to suffer from copper deficiency while in other areas toxicity was a problem (C. Mwendia, Egerton University, personal communication). As such, the use of COWP boluses would have to be evaluated in both areas. Additionally, current high costs of these boluses make them unaffordable and unavailable to pastoralists and SHF in Kenya.

Utilisation of immune system

Immunity, or host resistance, as it applies to nematode parasites is the ability of an animal to prevent infection with parasites, and/or reject established parasites in the animals, by utilizing both innate and acquired immune responses (McClure, 2000). Innate immunity is inherent, whereas acquired immunity gradually develops in the animals after exposure to parasites. Most adult ruminants exhibit naturally acquired protective immunity to gastrointestinal nematodes (McClure, 2000). However, unlike in bacterial infections, where vaccination and natural boosting often produces high levels of acquired immunity, young sheep infected by GI nematodes are unable to respond as early or as profoundly (McClure, 2000). In addition, this protection is only partial (incomplete) as parasites are also present after immunity has developed. The speed with which immunity develops is influenced by factors such as the dose of larvae ingested and the species of parasites (Dobson *et al.*, 1990). The protective effect is finally expressed by the ability of an animal to reject incoming larvae, to depress worm fecundity and/or to expel adult parasites (McClure, 2000). Immunity is also subject to physiological and external factors such as age, pregnancy and lactation, health, sex, genotype, nutrition and stress of the animal (for review see McClure (2000)). The use of vaccines, nutrition and breeding for resistance are all influenced by immunity, and their use as control strategies against nematodes is discussed below.

Vaccines

Current mathematical models on worm control have shown that a vaccine yielding 60% protection in 80% of the herd, or flock, would be a highly valuable control tool (Barnes, Dobson & Barger, 1995). Following the success of the irradiated larval vaccine against bovine lungworm, *Dictyocaulus viviparus*, a similar approach using irradiated *H. contortus* L_3 was found to consistently offer good

protection in sheep older than six months (Gray, 1997). However, hopes for commercial production of an irradiated *H. contortus* vaccine disappeared when it became clear that high levels of protection could not be achieved in young lambs, and useful protection only developed if sheep were worm free prior to vaccination (Gray, 1997; Bain, 1999).

Current research on helminth vaccines has generally concentrated on the production of synthetic or recombinant vaccines using either natural or hidden (concealed) antigens (Schallig, van Leeuwen & Cornelissen, 1997; Smith *et al.*, 2003). A number of reviews has been published on possible recombinant vaccines developed against nematode parasites of ruminants (Smith, 1999; Knox & Smith, 2001; Claerebout, Knox & Vercruysse, 2003; Dalton *et al.*, 2003; Meeusen & Piedrafita, 2003; Newton & Meeusen, 2003).

Irrespective of the progress that has been made, and the promise for the future, it will take a long time before these vaccines have a place in control of *H. contortus* in the flocks of pastoralists and SHF.

Resistant and resilient breeds

Resistance has been described as the ability of the host to prevent or limit the establishment or development of infection. Resilience is the ability of an animal to maintain reasonable levels of production when subjected to parasitic challenge (van Houtert & Sykes, 1996). Considerable research over decades has been undertaken in many sheep rearing countries to identify breeds that have natural resistance to GI nematode parasites infections (Woolaston & Baker, 1996; Raadsma, Gray & Woolaston, 1998).

In Kenya, it has been demonstrated that the local Red Maasai (RM) sheep and small East African goat are more resistant than the Dorper sheep and Galla goat, respectively (Mugambi *et al.*, 1997; Baker, 1998; Baker *et al.*, 2003). Although these breeds were shown to be resistant, studies elsewhere indicate that when the plane of nutrition is low, the immune responses of sheep are inadequate, and these animals can then succumb to effects of GI nematode infections (Kahn, 2003). It has been observed in pastoral areas that animals sometimes succumb to haemonchosis before the onset of rains, due to malnutrition during drought and dry seasons (Iles, 1993; Gatongi *et al.*, 1997).

Strategic nutritional supplementation

GI nematodes impair animal productivity through reduction in voluntary food intake and/or reduction in the efficiency of food use, especially inefficient use of absorbed nutrients (Coop & Kyriazakis, 2001). Disturbances in protein metabolism and reduced absorption and/or retention of minerals are significant during parasite infection (Coop & Kyriazakis, 2001). The magnitude of these effects is influenced by the size of the larval challenge and the number and species of parasites present. A common feature of GI infections is the increased loss of endogenous proteins into the GI tract (Coop & Kyriazakis, 2001). Considerable research has shown that nutritional supplementation, especially improved dietary protein supply, reduces production losses and mortality from GI nematode

parasites (van Houtert & Sykes, 1996; Coop & Kyriazakis, 2001; Sykes & Coop, 2001).

Feed scarcity and thus malnutrition is a common feature in livestock kept by pastoralists and SHF in Kenya (Carles, 1986; Semenye *et al.*, 1992). Severe weight loss and death due to chronic haemonchosis was observed during the dry season when pasture quality was very poor, with crude protein falling below 2% (Allonby & Urquhart, 1975). Preston & Allonby (1979) demonstrated that sheep and goats on a high plane of nutrition in a semi arid area in Kenya displayed less severe signs of clinical haemonchosis, and self cure occurred in these animals compared to those on a low plane of nutrition. In AEZ IV – VII in Kenya (see Fig. 1), there is wide variation of quality and quantity of feed available to goats and sheep due to variability of rain (Blackburn & Field, 1990).

Plants with antiparasitic properties

Considerable research has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects (Waghorn & McNabb, 2003). For example, plants that contain condensed tannins (CTs), a class of phenolic secondary metabolites, have these effects. Tannins are mainly subdivided into two groups:

- hydrolyzable tannins (HT) are polymers esterified to a core molecule, commonly glucose or a polyphenol such as catechin. HT are potentially toxic to ruminants (Reed, 1995);
- proanthocyanidins (condensed tannins) are relatively stable in the digestive tract of the animal, and rarely have toxic effects (Reed, 1995).

CTs are powerful antimetabolites, and in amounts exceeding 5%, which is common in tropical forages, can impair microbial functioning of ruminants (Singh & Bhat, 2003). At high concentrations, the CTs act as anti-feedants because they bind in tight chemical complexes with proteins, as well as microbial enzymes, thereby reducing fermentation and degradation of fibrous tissue in the rumen (Reed, 1995; Singh & Bhat, 2003). Additionally, they may also bind to digestive enzymes, thus reducing their activity, and also have an astringent taste (D'Mello, 1992; Reed, 1995; Min & Hart, 2003). When they bind to proteins, these compounds are not broken down during passage through the digestive tract. This results in lowering of the nutritive value of plants containing CTs. Consequently, a huge amount of research in the tropics has been directed towards overcoming these undesirable qualities of tanniferous plants in grazing animals (Butler, 1992; Norton, 2000).

The vast majority of studies investigating the effects of CTs on GI nematode parasites, either in experimental or in grazing conditions, have been conducted using sheep (Niezen *et al.*, 1996; Niezen *et al.*, 1998; Molan *et al.*, 2000; Athanasiadou *et al.*, 2001; Waghorn & McNabb, 2003). Studies have also shown that CTs had an effect on GI parasite infections in goats (Kabasa, Opuda-Asibo & ter Meulen, 2000; Kahiya, Mukaratirwa & Thamsborg, 2003; Paolini *et al.*, 2003). Some investigations were conducted on tropical forages such as *Acacia karoo*, which was fed to goats infected with *H. contortus*, leading to significant

reductions of faecal egg counts (FEC) and the number of parasites in the abomasa (Kahiya, Mukaratirwa & Thamsborg, 2003). The possible modes of action of CTs against GI nematodes have been reviewed by Kahn & Diaz-Hernandez (2000) and Min & Hart (2003). During the dry season in Kenya, the Samburu and Maasai pastoralists feed goats and sheep on pods from *Acacia tortilis* trees, which are known to contain CTs (Gowda, 1997).

Ethnoveterinary medicine

Ethnoveterinary medicine (EVM) refers to people's beliefs, knowledge, skills and practices relating to care of their animals (McCorkle, 1986). EVM covered in this thesis relates only to those aspects of herbal medicine with emphasis on anthelmintic effects.

History

Traditional veterinary practice is based on indigenous knowledge passed on from generation to generation. EVM is important in Kenya, particularly in the pastoral communities such as the Rendille, Turkana, Maasai, Samburu, Marakwet and Pokot, who have cultures that are rich in traditional knowledge of disease control (Lindsay, 1978; Ohta, 1984; Wanyama, 1997a, 2000; Ole-Miaron, 2003). The traditional systems of veterinary services are useful where modern techniques are nonexistent, or when available are too expensive or difficult for the community to access (Wanyama, 1997a, b; Danø & Bøgh, 1999). There is a difference in practice of EVM between pastoralists and SHF (agropastoralists). In pastoral areas, general knowledge about EVM is shared freely to all group members and becomes part of the general knowledge of the community. However, there are exceptions, where opinions are sought from specialists (*e.g.* Laibon, "diviners" amongst the Samburu) who claim that they can predict outbreaks of disease, and also have a better knowledge of disease control (Fratkin, 1996; Wanyama, 1997b). In contrast, amongst agropastoralists, EVM knowledge is a preserve of a few people and this knowledge is more-or-less a guarded secret, which is passed down the lineage line (Wanyama, 2000). However, there are a few records of animal husbandry methods used by SHF. For example, the animal husbandry methods as well as a botanical index of plants used for different ailments in livestock and humans by the southern Kikuyu before 1903, have been recorded (Leakey, 1977a, b).

The use of EVM is limited by the seasonal availability of certain plants, the scarcity of treatment against infectious disease, the ineffectiveness of some treatments, the existing harmful practices and the often inadequate ethno-diagnosis (Mathias-Mundy & McCorkle, 1989; Martin, McCorkle & Mathias, 2001). There is normally a lack of pathophysiological understanding of disease, which results in poor diagnosis (Schillhorn van Veen, 1997). The understanding of the causes of disease is poorly developed, and therefore treatment and prevention may be inappropriate. In many cases, disease classification by pastoralists and SHF is

based on observed signs and abnormalities in the animal, and treatment is usually offered to alleviate these symptoms (Ohta, 1984; Iles, 1993; Catley & Mohammed, 1996; Hammond, Fielding & Bishop, 1997; Wanyama, 1997a; Namanda, 1998; Tamboura *et al.*, 2000; Nfi *et al.*, 2001; Alawa, Jokthan & Akut, 2002; Ole-Miaron, 2003). Although EVM is widely used in many parts of East Africa, the lack of wider acceptance by scientists and veterinarians is because it is believed to be associated with superstition, without a place in reality, and to be the domain of "quacks" (Mesfin & Obsa, 1994; Danø & Bøgh, 1999). This is mainly because EVM does not follow the paradigms of scientific evidence-based demonstration of efficacy.

In this context, it is important to realise that plant/herbal remedies were also extensively used as anthelmintics in the Developed world before the era of broad spectrum drugs (British Veterinary Codex, 1953, 1965). Many currently available therapeutic compounds are plant derived and/or synthetic analogues derived from those compounds (Farnsworth *et al.*, 1985). Specifically in relation to herbal anthelmintics, some plants and remedies in the British veterinary codex (1965) include:

- Oil of chenopodium (frequently combined with a laxative) derived from *Chenopodium ambrosioides* (de Bairacli Levy, 1991), was used for many years in the UK and US to treat nematode parasite infections (*Strongylus*, *Parascaris* and *Ascaris* spp.*)* in monogastric animals including humans (Gibson, 1965). A monoterpene (ascaridole) is believed to be the active ingredient in the oil of this plant (Okuyama *et al.*, 1993; Anonymous, 2001; Ketzis *et al.*, 2002). However, the use of this oil was discontinued in the 1960s. Recently it was shown that short-term administration of the oil, or freshly ground plant material, administered to infected goats was ineffective in reducing adult *H. contortus* populations (Ketzis *et al.*, 2002).
- Male fern *Dryopteris filix-mas* was used against the cestode *Moniezia* spp., the nematode *Ascaridia* spp., as well as other gastro intestinal nematodes of ruminants such as *Cooperia*, *Haemonchus*, *Nematodirus*, *Ostertagia* and *Trichostrongylus* spp.
- Plants of the genus *Artemisia* were used against the nematodes *Ascaris suum* and *Toxocara* spp. as well as cestodes of poultry.

Several books have been written on ethnoveterinary medicine (Mathias-Mundy & McCorkle, 1989; Anonymous, 1994; Bizimana, 1994; Anonymous, 1996; McCorkle, Mathias & Veen, 1996; Köhler-Rollefson, Mundy & Mathias, 2001; Martin, McCorkle & Mathias, 2001) and a few databases and web sites on the subject exist (CIRAN, 2001; Ethnovetweb, 2003; PRELUDE, 2003; SEPASAL, 2004). However, in most of these sources, there is only a brief description of the plants used, and the purported conditions that they treat, and often no validation of the effect against these conditions is provided. Most of the research on testing of EVM preparations has so far been carried out in Asia (Akhtar *et al.*, 2000). Conversely, a number of publications has been produced that relate to diseases that affect humans and livestock in Africa (Gachathi, 1993; Kokwaro, 1993; Bizimana, 1994).

In Kenya, a manual was recently published that identified a number of plants used against all major groups of helminths (i.e. cestodes, trematodes and nematodes) (Anonymous, 1996). In this book, an attempt was made to identify the method of preparation of the plants listed. Similarly, (Kokwaro, 1993) listed 21 plants used against hookworms (*Ancylostoma spp*.), six against roundworms, (without specifying whether nematodes or ascarids), 22 against tapeworms (cestodes), one plant used against thread worm (*Strongyloides spp*.) and a list of 79 plants that are used as general anthelmintics in humans in East Africa.

Five plants (*Dryopteris inaequalis, Albizia anthelmintica, A. gummifera, Olea africana* and *Myrsine africana*) were reportedly used by the Marakwet people of Kenya as anthelmintics (Lindsay, 1978). The Kikuyu of Central Kenya purportedly use *Cissampelos pareira* roots, *Vernonia lasiopus* roots and leaves, *Myrsine africana* fruits, *Rapanea melanophloeos* fruits, *Ficus thonningi* sap, *Albizia anthelmintica* roots and *Ficus sycomorus* sap for treatment of GI parasites of small ruminants. They also use the bark of *Acacia mellifera* against the GI parasites of cattle (Gachathi, 1993).

Elsewhere in Africa, Ibrahim *et al.* (1984) screened 18 plant species used in West Africa for their anthelmintic activity, while Kasonia *et al.* (1991) reported 11 plants used for the same purpose in Zaire. From Nigeria, 92 species of plants were identified to be used in traditional veterinary practice, with 15 reported to be used against general worm infestation and three against fasciolosis in cattle (Nwude & Ibrahim, 1980). However, no description was given of the type of parasite infestation referred to (Nwude & Ibrahim, 1980). A study in the Madikwe area of South Africa identified eight plants that were used as dewormers of cattle, although cattle owners in that area had a poor understanding of helminth infections of cattle (van der Merwe, 2000). In the Sanaag area in Somalia, six plants were reported to be used for treatment of helminths in livestock (Catley & Mohammed, 1996). Tagboto & Townson (2001) in their review listed 39 plants used against cestodes, 16 against trematodes and 45 against nematodes in humans worldwide. There are also recent reports in Africa of plants that had anthelmintic activity when fed to livestock due to their CTs content (Kabasa, Opuda-Asibo & ter Meulen, 2000; Kahiya, Mukaratirwa & Thamsborg, 2003).

In many instances, EVM remedies have been identified for treatment of different conditions, but without validation. In such studies, plant species that are purportedly used by traditional healers (TH) are listed, often without giving a proper description of the method of preparation (Weiss, 1979; Wilson & Mariam Woldo, 1979; Anonymous, 1994, 1996; Danø & Bøgh, 1999; Guarrera, 1999).

However, some evaluations have been carried out to validate the putative anthelmintic properties. Some *in vitro* evaluations of plant preparations have utilised the nematode parasites *H. contortus* (Table 2), or in other instances mixed GI nematode infections have been tested in these assays (Asuzu & Onu, 1993). Non-parasitic nematodes have been used in many *in vitro* assays for determination of anthelmintic efficacy. For example, in several trials, *in vitro* effects of plant extracts on the free-living nematode *Caenorhabditis elegans* (Table1, appendix A) and *Rhabditis pseudoelongata* (Okpekon *et al.*, 2004) were evaluated. An investigation in South Africa tested the *in vitro* activity of 72 species of plants

from 18 families against *C. elegans* (McGaw, Jager & van Staden, 2000), while in the Ivory Coast 17 species from 13 families were evaluated against *R. pseudoelongata* (Okpekon *et al.*, 2004).

The *in vitro* effects of EVM preparations against ascarids of humans and their livestock have similarly been tested (Table 2, appendix A). *In vitro* investigations have evaluated the effects of plant preparations against the rodent nematode *Heligmosomoides polygyrus* (Table 3, appendix A), and in assays the anthelmintic effects of 57 plant extracts were evaluated against the trematode *Schistosoma mansoni* and the cestode *Hymenolepis diminuta* (Molgaard *et al.*, 2001).

Table 2. In vitro *assay of plant preparations evaluated against the nematode* Haemonchus contortus *(adult parasite, eggs or infective larvae)*

Plant species	Active principles[1]	Parts used[1a]	Target[b]	Reference
Adhatoda vesica		R	A	Lateef *et al.*, 2003
Annona glabra	kaurenoic acid	B	A	Padmaja *et al.*, 1995
Annona senegalensis	diterpenoids	B	E	Alawa *et al.*, 2003
Alstonia boonei		B	L_3	Fakae *et al.*, 2000
Calotropis procera	triterpenoid, anthocyanin, alkaloids, resins	Lx	L_3	Al-Qarawi *et al.*, 2001
Piliostigma thonningii	D-3-O-methylchiroinositol[2]	B	L_3	Asuzu, Gray & Waterman, 1999; Fakae *et al.*, 2000
Chenopodium ambrosioides	ascaridole	O, L	E	Ketzis *et al.*, 2002
Vernonia amygdalina		L	E	Alawa *et al.*, 2003
Cucurbita mexicana		Fr	A	Iqbal *et al.*, 2001
Ocimum gratissimum	oleanolic acid	L	L_3	Njoku & Asuzu, 1998; Fakae *et al.*, 2000
Allium sativum		Bb	A	Iqbal *et al.*, 2001
Spigelia anthelmia		Sh	E, L_3	Assis *et al.*, 2003
Melia azedarach		L		Nirmal, Sangwan & Sangwan, 1998
Nauclea latifolia		L	L3	Fakae *et al.*, 2000
Ficus religiosa		B	A	Iqbal *et al.*, 2001
Zingiber officianale		Rh	A	Iqbal *et al.*, 2001

[1] Where specified, [2] Active principles evaluated

[a] Parts used (if specified): B = bark, Bb = bulbs, Fr = fruits, Lx = latex, L = leaves, O = oil, Rh = rhizomes, R = root, Sh = shoots

[b] Target (if specified): A = adult parasites, E = eggs, L_3 = infective larvae

Several investigations have assessed *in vivo* plant efficacy against different helminth parasites, in animal and human hosts. In a few studies, the anthelmintic

properties of plants in naturally or artificially infected sheep, goats and cattle, have been determined against the nematode *H. contortus* (Table 3), as well as against mixed trichostrongyle nematode infections in ruminants (Table 4). Similarly, *in vivo* efficacy of plant preparations was investigated against cestode infected hosts such as humans (Desta, 1995), rodents (Galal *et al.*, 1991b, a; Vishnyauskas *et al.*, 1993; Ghosh *et al.*, 1996; de Amorin *et al.*, 1999; Molgaard *et al.*, 2001), and ruminants (Akhtar & Riffat, 1986), as well as against trematodes of ruminants and rodents (Table 3, appendix B). In addition, some studies have focused on effects of plant preparations against ascarids of humans, pigs, and chicken (Table 4, appendix B). Likewise, the *in vivo* effects of some plant extracts on nematode parasites of rodents have also been investigated (Table 5, appendix B).

Table 3. In vivo *evaluation of plant preparations against* Haemonchus contortus *in ruminant hosts*

Plant species	Parts used[a]	Active principles[1]	Host[b]	Reference
Acacia nilotica *Acacia karoo*	L	condensed tannins	G	Kahiya, Mukaratirwa & Thamsborg, 2003
Allium sativum	Bb	allicin	G	Vieira *et al.*, 1999
Annona squamosa	L	anthraquinone terpenoids	G	Vieira *et al.*, 1999
Artemisia herba-alba	Sh	santonin	G	Idris, Adam & Tartour, 1982
Calotropis procera	L	triterpenoids, anthocyanins, alkaloids	S	Al-Qarawi *et al.*, 2001
Canavalia brasiliensis	S		G	Vieira *et al.*, 1999
Carica papaya	S	benzyl isothiocyanate	G	Vieira *et al.*, 1999
Chenopodium ambrosioides	L	ascaridole	G	Vieira *et al.*, 1999
Chrysophyllum cainito	St		B	Fernandez, 1991
Hymenaea courbaril	B		G	Vieira *et al.*, 1999
Menta spp.	L		G	Vieira *et al.*, 1999
Momordica charantia	St		G	Vieira *et al.*, 1999
Musa acuminate	L		G	Vieira *et al.*, 1999
Tinospora rumphii	St		G	Fernandez, 1999

[1] Where specified

[a] Parts used (if specified): B = bark, Bb = bulbs, L= leaves, RB = root bark, S = seeds, Sh = shoots, St = stem

[b] Host (if specified): B = bovids, G = goats, S = sheep

Table 4. In vivo *evaluation of plant preparations against mixed GI nematode infections in ruminant hosts*

Plant species	Parts used[a]	Active principles[1]	Host[b]	Reference
Adhatoda vesica	R	alkaloids, glycosides	S	Lateef *et al.*, 2003
Albizia anthelmintica	B, RB	sesquiterpene, kosotoxins	S	Grade & Longok, 2000; Gakuya, 2001; Gathuma *et al.*, 2004
Ananas comosus	L	bromelain	S, B	Jovellanos, 1997; Baldo, 2001; Hördegen *et al.*, 2003
Annona squamosa	L	anthraquinone terpenoids	G, B	Jovellanos, 1997; Vieira *et al.*, 1999
Azadirachta indica	S L	azadirachtin	S, B	Pietrosemoli *et al.*, 1999; Chandrawathani *et al.*, 2003; Hördegen *et al.*, 2003
Chenopodium ambrosioides	L, S O	ascaridole	S	Ketzis *et al.*, 2002
Chrysanthemum cinerariaefolium	Fl	pyrethrins[2]	S	Mbaria *et al.*, 1998
Caesalpinia crista	S		S	Hördegen *et al.*, 2003
Embelia ribes	Fr		S	Hördegen *et al.*, 2003
Fumaria parviflora	W		S	Hördegen *et al.*, 2003
Hagenia abyssinica	Fr	kosotoxin	G	Abebe *et al.*, 2000
Hildebrandtia sepalosa	RB		S	Gathuma *et al.*, 2004
Khaya anthotheca	B		B	Nfi *et al.*, 1999
Maerua edulis	Tb		S	Gakuya, 2001
Myrsine africana	Fr	benzoquinone	S	Gathuma *et al.*, 2004
Nauclea latifolia	B	resin, tannins, alkaloids	S	Onyeyili *et al.*, 2001
Solanum aculeastrum	R		B	Nfi *et al.*, 1999
Terminalia glaucescens	B	anthraquinone	B	Nfi *et al.*, 1999
Vernonia anthelmintica *Vernonia amygdalina*	S L		S B	Nfi *et al.*, 1999; Hördegen *et al.*, 2003

[1] Where specified, [2] Active principle(s) evaluated

[a] Parts used (if specified): B = bark, Fl = flowers, Fr = fruits, L = leaves, R = root, RB = root bark, O = oil, S = seeds, Tb = Tuber, W = whole plant

[b] Host (if specified): B = bovids, G = goats, S = sheep

Active components

There is a dearth of good quality trials and documentation of selective toxicity of EVM preparations against internal parasites (Tagboto & Townson, 2001). However, in a number of studies, the active ingredients have been purified and characterised from plant extracts. Examples include:

- embelin extracted from *Embelia schimperi*, which was evaluated *in vivo* in mice and rats infected with the cestodes *Hymenolepis microstoma* and *H. diminuta*, and mice infected with the trematode *Echinostoma caproni*, and the nematode *Heligmosomoides polygyrus.* This compound was found to be effective *in vivo* against the cestodes but not against the other parasites (Bøgh, Andreassen & Lemmich, 1996);
- atanine, a quinolone alkaloid extracted from *Evodia rutaecarpa* dried fruits. *In vitro* tests demonstrated inhibition of motility of free-living stages of the trematode *Schistoma mansoni*, and against the nematodes *C. elegans* adult stage and larvae of *Teladorsargia circumcincta* (Perrett & Whitfield, 1995);
- β-sitosterol, from leaves of *Mentha cordifolia*. This was tested in an *in vitro* assay against the adult parasites of the porcine roundworm *Ascaris suum*, displaying similar activity to synthetic anthelmintic mebendazole on contact of the parasite with the preparations (Villasenor *et al.*, 2002);
- mangiferin, a major polyphenol in the aqueous extract vimang acquired from *Mangifera indica.* The polyphenol and the aqueous extract were tested in an *in vivo* mouse model against enteric and parenteral stages of *Trichinella spiralis.* Moderate effects were reported against the larval stages in the muscles but not against adult parasites (Garcia *et al.*, 2003);
- flavan-3-ols (the monomer units of CT), and their galloyl derivatives were evaluated *in vitro* on the viability of eggs, the development and the viability of the free living stages of the sheep nematode *Trichostrongylus colubriformis*. The flavan-3-ol was found to have an effect on egg hatching as well as the development of larvae (Molan *et al.*, 2003).

Previous research and shortcomings

In numerous literature surveys on EVM from various parts of the world, there is only anecdotal evidence for the usage of plants as anthelmintics in animals (Than *et al.*, 1993; Anonymous, 1994; Minja, 1994; Anonymous, 1996; Catley & Mohammed, 1996; Wanyama, 1997a, b; Lans & Brown, 1998; Namanda, 1998; Danø & Bøgh, 1999; Minja, 1999; Vieira *et al.*, 1999; Tamboura *et al.*, 2000; Nfi *et al.*, 2001; Uncini Manganelli, Camangi & Tomei, 2001; Waller *et al.*, 2001; Alawa, Jokthan & Akut, 2002). In such a survey in Asia, 23 plants were identified as potential anthelmintics (Anonymous, 1994). Of these plants, six were claimed to have an anthelmintic effect on poultry parasites, 10 on parasites of pigs and 11 against ruminant parasites. Some plants, like *Areca catechu*, were claimed to have an effect on parasites of all host species (Anonymous, 1994). In Tanzania, Minja (1994) classified seven plants as having anthelmintic properties. In Nigeria, 18 plants were identified to have anthelmintic effects, although no target animal host species was indicated (Ibrahim *et al.*, 1984). Recently in Northern Nigeria, four plants were identified as being active against helminth infections of livestock

(Alawa, Jokthan & Akut, 2002). However, in that study, neither the livestock species nor the target parasites to be treated were mentioned, although the dosages and methods of plant preparation were described (Alawa, Jokthan & Akut, 2002). Nevertheless, in both studies in Nigeria, *Khaya senegalensis* was identified to have anthelmintic properties. In another study in South Africa, no respondents had knowledge of the problem of GI parasites of cattle, although some plants were identified to treat the condition (van der Merwe, 2000).

A rather common problem in the few validation studies that have been carried out is that the number of animals per treatment group is low. In virtually all these studies, the number of animals in the treatment and control groups was less than the minimum number of six per group, recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP), for *in vivo* testing of anthelmintic products (Wood *et al.*, 1995; Vercruysse *et al.*, 2001). In a study to evaluate the anthelmintic properties of *Mallotus philippensis* (Kamala) in sheep artificially infected with a mixed infection of *Teladorsargia, Chabertia, Strongyloides, Haemonchus, Dictyocaulus* and *Trichostrongylus* spp., four animals were used per treatment and control group (Jost *et al.*, 1996). Similarly, Grade & Longok (2000) evaluated the effect of *A. anthelmintica* in village livestock, using two sheep, two goats and four calves in the treatment groups, and one sheep, one goat and two calves in the control groups. Furthermore, in that same study, a qualitative method of faecal egg floatation, instead of a quantitative one (*e.g.* McMaster method), was used to determine the efficacy of the plant preparation. In some other studies, control groups were missing, or when included, too few animals were used in order to make any valid statistical comparison (Idris, Adam & Tartour, 1982; Gakuya, 2001).

On some occasions, naturally infected animals shedding very few eggs (< 400 FEC) in the faeces have been tested (Akhtar & Riffat, 1984; Mbaria *et al.*, 1998; Gathuma *et al.*, 2004). For example, in a study carried out in northern Kenya to evaluate anthelmintic effects of different plant preparations, the mean FEC in treatment groups ranged from 250 to 450 in five groups of 11 animals each (Gathuma *et al.*, 2004). Correct interpretation is compromised because any small reduction in the number of eggs being shed translates into a disproportionately large percentage reduction, consequently implying high anthelmintic activity of any plant being tested. Furthermore, the FEC procedure is a relatively insensitive test, influenced by factors such as feed intake (and faecal output), and host induced effects (age and immunity) (McKenna & Simpson, 1987). For instance, Fernandez (1991) classified EPG into light (60 – 80 EPG), moderate (81 – 100 EPG) and high (>101 EPG), whereas the sensitivity of the McMaster test is generally in steps of 50 EPG. In that investigation, seven plants were classified as effective against *H. contortus*, despite the fact that the EPG values were based on the sensitivity of the McMaster method. Thus frequent sampling (pre and post treatment) is important, and interpretation of results should be made with caution. In addition, some of these mixed infections were not properly characterised through larval cultures, to ascertain the percentage of the infecting species (Jost *et al.*, 1996; Grade & Longok, 2000; Gathuma *et al.*, 2004).

In some investigations evaluating the effect of plant extracts against cestodes, the number of eggs excreted in faeces has been used as the endpoint (Akhtar, Chattha & Chaudry, 1982; Gathuma *et al.*, 2004). However, cestode eggs are normally released with gravid proglottids in the faeces. Therefore, unless the scolex is identified (Desta, 1995), it is impossible to interpret the effect of these extracts as they may simply induce destrobilation without parasite removal.

Furthermore, some *in vitro* studies have been based on the effects of EVM preparations on adult parasite motility in petri-dishes (Iqbal *et al.*, 2001; Lateef *et al.*, 2003), making unjustified extrapolation to *in vivo* efficacy. Many studies have also utilised free-living nematodes such as *C. elegans*, although the suitability of this nematode as a model for parasitic nematodes has been questioned (Geary & Thompson, 2001).

Aims of the study

The objective of this thesis was to evaluate the anthelmintic efficacy of plants commonly used by pastoralists and SHF in Kenya to treat their livestock. Since *H. contortus* has been identified as the most important parasite in these farming communities, an *in vivo* model based on experimentally infected sheep was developed. In order to evaluate whether there were any differences in the anthelmintic efficacy in monogastric animals, some plants were also tested in a mouse model infected with the nematode parasite *H. polygyrus*.

Methodological considerations

Identification of candidate plants

A three-step approach to selection of the candidate plants was taken. This involved:

1. literature search for available information on plants;
2. identification of sites where plants are available and arranging their collection;
3. obtaining information on methods of traditional preparation of these plants for deworming of livestock.

As a background source of information, the book “Ethnoveterinary Medicine in Kenya: A field manual for traditional animal health care practices” was used (Anonymous, 1996). Various databases (PRELUDE, 2003; NAPRALERT, 2004; SEPASAL, 2004) and MEDLINE were searched for further information on the plants. Information was retrieved on the purported anthelmintic activity of 11 plants that were identified as the “best bet” candidates. It was found that several of these plants had been identified as having anthelmintic activity (Minja, 1989; Galal *et al.*, 1991a; McCorkle, Mathias & Veen, 1996), but conclusive evidence

for the purported activity against nematodes of small ruminants was generally missing.

It also became clear that for some plants, such as *Albizia anthelmintica*, *Myrsine africana* and *Rapanea melanophloeos*, there were various methods used for making preparations or extracts for livestock dosing. In addition, it was also realised that different parts of the plants were used, such as leaves and/or fruits of *Myrsine africana* (Gachathi, 1993; Kokwaro, 1993; Anonymous, 1996). Anthelmintic properties possibly could differ depending on the method of preparation, the part of the plant used (root, stem, bark, leaf, flower, *etc.*) as well as the growth habitat (Martin, McCorkle & Mathias, 2001). Consequently, it was decided to evaluate all of these factors, such as the different methods of extraction and different growth habitat for some plants tested, *e.g. Myrsine africana* leaves and bark, *Albizia anthelmintica* root and trunk bark and cold and hot extracts (Papers I and II).

To overcome some of the problems associated with contact and confidence building with traditional healers (THs) in the pastoralist communities, collaboration was established with the Intermediate Development Group (ITDG). This is an international non-governmental organisation (NGO) with a regional office for East Africa, based in Nairobi. The NGO had a close working relationship with the communities and some individuals, and had established interactions with THs. The NGO wanted EVM incorporated into community based animal health care in Kenya.

To obtain information from THs, a series of workshops was held between the researchers and the THs. Informal key informant interviews were conducted with 46 healers from three locations in the Baragoi Division of Samburu district, which involved two ethnic groups (Samburu and Turkana). In the first workshop, the healers described livestock problems in their small ruminants. They then identified the problems that they claimed could easily be managed using traditional methods, and described how these conditions were controlled. Parasitic infections, or general helminth problems in livestock, were amongst conditions identified by the THs as those that they could confidently treat. In a further workshop, the THs as a group identified plants that they thought had the highest de-worming activity. They then came to consensus opinions on the methods of preparation of those plants which had been identified. This was considered an important step because different doses were applied depending on where the plant was collected, and on the ethnic group that was using the plant. After the third workshop, the THs demonstrated the methods of collection and preparation of these plants. Subsequently, THs selected sheep that they suspected to be infected with helminth parasites, and applied the preparations that were freshly made. The animals identified and treated by the healers were also tested for FEC using a modified McMaster method on the day of treatment and on days 3, 7 and 10 after treatment. The method, drug preparations and quantities used were all recorded. During treatment of animals by TH, one sheep died from asphyxiation. The THs were requested to slaughter the animal and demonstrate the location of the ‘worms’ that the plants were active against. They were also asked to describe the parasites they expected the plant preparations to be active against. It became clear that most of

the THs only attributed anthelmintic activity to plants that caused expulsion of 'worms' that were clearly visible. Of the 46 respondents in the workshop, only one TH described small reddish 'worms' (by inference *H. contortus*) in the abomasum of goats, but these were described not to occur in any other animals. Further investigations revealed that most THs considered 'worms' to be represented by tapeworms in the gut, or their segments in faeces. The plant preparations evaluated by the THs were described as successful if tapeworm segments were observed in faeces immediately after treatment, or the next morning.

Additional information was sought from a TH from Nyeri, 150 km north of Nairobi, who was marketing his products. A commercial preparation, whose contents were not disclosed to the author, was purchased and tested in two groups of 16 sheep each, artificially infected with 3000 *H. contortus* L_3. The dose for every animal consisted of 200 g of the product mixed with 100 g of soda-ash, and boiled in 500 ml of water. After cooling, the animals were drenched with the preparation. However, the product only resulted in an average FEC reduction of 32% in the treated group compared to untreated infected lambs (see Table 7).

Plant collection and preparations

Collection of a number of plants was undertaken with the help of THs. The districts where the plants were collected, the parts of plant used, as well as the traditional dose in sheep and the median dose used in the mouse model are shown in Table 5.

The plants collected were prepared as per the information provided by the THs and finally tested under controlled conditions at the International Livestock Research Institute (ILRI) in sheep experimentally inoculated according to a standard protocol with 3000 *H. contortus* L_3. As a rule, the animals were monitored daily for a period of two to three weeks post treatment for behavioural changes. FEC and PCV were determined every two to three days in the first week and twice weekly thereafter. Some of the plants were also tested later in a mouse–parasite model.

Further collection of other plants was conducted in the highlands of Kenya (AEZ I – III), where the majority of the SHF are located (see Fig. 1). A healer from the Mbeere district, 200 km northeast of Nairobi, was identified, and an informal interview was held to obtain information on some plants used to treat nematode parasites of sheep and goats. A series of visits was undertaken before the TH identified the plants used, as well as demonstrating the methods of preparation of *D. angustifolia* and *O. europaea* ssp. *africana*.

The plants selected for evaluation were those that were confidently identified by THs and most frequently used in single plant formulations to treat animals on single occasions. Proximity to Nairobi was considered to be important for selection of plants. The East African Herbarium at the National Museums of Kenya was used as a reference point for identification of the plants collected. The plants selected were: *Aframomum sanguineum, Albizia anthelmintica, Ananas comosus, Annona squamosa, Azadirachta indica, Dodonaea angustifolia*

(D. viscosa), Hagenia abyssinica, Hildebrandtia sepalosa, Myrsine africana, Olea europaea ssp. *africana,* and *Rapanea melanophloeos*. Evaluation of preparations made from *A. anthelmintica*, *H. abyssinica*, *H. sepalosa* and *Rapanea melanophloeos* was based on information initially acquired from local literature (Anonymous, 1996) and later from information obtained from THs on their usage. *Aframomum sanguineum* was collected and validated in collaboration with the Traditional Medicines Research Centre of the Kenya Medical Research Institute. Amongst these plants, *Ananas comosus*, *Annona squamosa* and *Azadirachta indica* were introduced into Africa from Asia.

Table 5. *Plant parts used, district of collection, and traditional and median dose used in sheep and mice*

No	Species	Parts used[a]	Place of collection	Traditional dose in sheep[b]	Median dose in mice[b]
1	*Aframomum sanguineum*	S	1. Meru Central	NT[c]	1 g kg^{-1}
2	*Albizia anthelmintica*	1. B	2.1 Machakos	50 g	NT
		2. B	2.2 Kajiado	50 g	0.5 g
		3. RB	2.3 Samburu	50 g	0.25 g
3	*Ananas comosus*	L	3. Thika	1 g kg^{-1} bwt[d]	NT
4	*Annona squamosa*	L	4. Kiambu	1 g kg^{-1}	NT
5	*Azadirachta indica*	1. L	5.1 Kilifi	0.5 g kg^{-1}daily	5 g kg^{-1}
		2. L	5.2 Kajiado	NT	NT
		3. L	5.3 Malaysia[e]	NT	5 g kg^{-1}
6	*Dodonaea angustifolia*	L	6. Mbeere	30 g	10g kg^{-1}
7	*Hagenia abyssinica*	Fl	7. Nyandarua	1 g kg^{-1}	NT
8	*Hildebrandtia sepalosa*	RB	8. Samburu	50 g	2 g kg^{-1}
9	*Myrsine africana*	1. L	9.1 Nyandarua	250 g	NT
		2. Fr	9.2. Samburu	50 g	4 g kg^{-1}
10	*Olea europaea* ssp. *africana*	B	10. Kiambu	50 g	NT
11	*Rapanea melanophloeos*	1. Fr	11.1 Nyandarua	125 g	NT
		2. Fr	11.2 Samburu	50g	4g kg^{-1}

[a] Part used: B = bark, Fl = female flowers, Fr = dry fruits, L = leaves, RB = root bark, S = seeds
[b] Dose per animal where not indicated as g kg^{-1} bwt
[c] NT = not tested in the host-parasite model
[d] bwt = body weight
[e] Tested in mouse model only

The methods used for preparing the various plants were specific to each plant. Some plants were administered as crude aqueous extracts, while others such as *A. indica* leaves were dispensed to animals without processing, and others such as *M. africana* and *R. melanophloeos* were administered to animals as milled preparations. For aqueous extracts, either a cold or hot method of extraction was carried out, depending on the method used by the THs.

Aframomum sanguineum K. Schum. (Zingiberaceae)

Synonym: *A. angustifolium* (Sonn.) Schum. This herb grows in dense stands, with erect leafy shoots consisting of long tubular sheathing leaf bases. Leaves arise in two ranks from underground rhizomes. It has a tough orange to red black berry with oval dark brown seeds which are hard and smooth. These seeds are reportedly used in gruel to make a powerful anthelmintic for humans. The plant was also reportedly used as a treatment for stomach-ache and the root decoction taken as a remedy for dysentery and snake bites in humans (Kokwaro, 1993).

The fruits of this tree were collected from the Meru district, and the seeds recovered from the fruit were air dried under shade. The dried seeds were then ground using a hammer mill. From the milled preparation, 25 g was mixed with 200 ml and boiled for 30 min. The extract was then sieved, frozen and lyophilised overnight. From the lyophilised preparation, each mouse was treated with 1 g kg^{-1} bwt as the median dose, which was administered once (Paper III).

Albizia anthelmintica Brongn. (Mimosaceae)

A. anthelmintica is a deciduous shrub or small tree, ranging in height from 3 – 11 m. It has a characteristic grey bark, which can be smooth or deeply reticulated in texture. The fruits are glossy pale brown and glabrous. The tree is commonly found throughout most of the dry bushland of Kenya, less often in bushed grassland or woodland, and is rarely found in evergreen coastal bushlands (Beentje, 1994). The Pokot in Kenya (Beentje, 1994), and various communities in Ethiopia (Desta, 1995), were reported to use an infusion of bark decoction as an emetic against cestodes. Bark decoction was also reported to be a popular treatment for intestinal parasites amongst the Samburu of Kenya (McCorkle, Mathias & Veen, 1996), and in many parts of East Africa, in both humans and livestock (Galal *et al.*, 1991b, a; Minja, 1994; Grade & Longok, 2000; Koko, Galal & Khalid, 2000).

The bark of this plant was collected from three districts in Kenya. The tree bark was collected from the Machakos and Kajiado districts, while root bark was collected from the Samburu district. The plant collection was done by using a machete to remove the bark in strips of 30 x 5 cm wide (see Fig. 3), which were then transported to Nairobi. The bark was dried under the shade for two weeks and stored at room temperature for a further three weeks. Root bark collection was done with the help of THs who identified trees that were supposed to have “stronger’ medicine, in a similar fashion to that of the tree bark. The root bark was then dried and subsequently transported to Nairobi.

Two methods of preparation for treatment of sheep were used, using methods described in Anonymous (1996) and those demonstrated by THs in the Samburu district. The methods involved a hot extraction (HE) and a cold extraction (CE) of crude aqueous preparations. The CE method involved soaking a 50 g strip of stem bark collected from the Machakos district in 300 ml of water overnight, and sieving the extract to provide the traditional dose per sheep (Anonymous, 1996). Another CE which utilised root bark collected from the Samburu district was prepared by milling the dried bark in a hammer mill, and mixing 50 g of the

ground material in 300 ml of water, which was used to drench one sheep according to the traditional dosing practices of the THs. In the HE method, a 50 g strip of bark was immersed in 300 ml of water, and the mixture was heated to 70 °C while stirring and pounding the bark, which resulted in foaming of the mixture. This preparation was heated for 15 min, and then cooled to room temperature by whisking. After cooling, the mixture was sieved using a tea strainer (250 µm aperture), and then administered to lambs as the traditional dose.

The preparation administered to mice was prepared as a CE in a similar way to that of sheep, and thereafter sieved using a tea strainer. The filtrate was then freeze-dried overnight, and stored at -20 °C before administration to mice. The dose for mice was reconstituted in a volume of 0.2 ml of water to give a median dose of 500 mg per mouse in the first experiment and half that in the second experiment (Paper II).

Ananas comosus (L.) Merr. (Bromeliaceae).

Synonyms: *A. ananas* (L.) Korst, *A. sativus* Schulf.f., *Ananassa sativa* Lindl., *Bromelia ananas* L., *B. comosa* L. and *B. pigna* Perr. Commonly known as the pineapple, large plantations are found in the central and coastal regions of Kenya. The leaves and skin of the fruit have been used in Asia as anthelmintic preparations for livestock (Jovellanos, 1997; Baldo, 2001). The pineapple plant contains cysteine proteases (bromelain) believed to have some anthelmintic properties.

The leaves of *A. comosus* were collected from a commercial plantation in the Thika district. The leaves were collected from mature plants with fruits that were ready for harvesting. The leaves were then transported to Nairobi, and dried under shade. The dry leaves were then pounded with a hammer mill and stored in this form at room temperature. The milled leaves provided a traditional dose of 1 g kg^{-1} bwt. To ease application, the plant preparation was lyophilised and reconstituted at the time of treatment, and administered as a single dose (Paper IV).

Annona squamosa L. (Annonaceae)

This is commonly known as sugar apple, or custard apple. It is a small tree 3 – 5 m in height. The leaves are somewhat hairy when young, oblong, and 8 – 15 cm in length with petioles 1 – 1.5 cm long. The flowers occur singly in the axils of the leaves and are about 2.5 cm long. They are pendulous, hairy, three-angled, and greenish-white or yellowish. The fruit is somewhat heart-shaped, and 6 – 9 cm in length. The outside of the fruit is marked by polygonal tubercles. When the fruit is ripe it is a light yellowish green. The flesh is white, sweet, soft and juicy, and has a mild very agreeable flavour. The leaves of the tree were reportedly used in Asia (Anonymous, 1994; Jovellanos, 1997; Anonymous, 2003) and South America (Vieira *et al.*, 1999) as an anthelmintic for livestock.

Annona squamosa leaves were collected from Muguga, Kiambu district and air-dried. The leaves dried within a week after which they were ground using a hammer mill. The preparation was lyophilised before application, and

reconstituted in 20 ml of water to give a dose of 1 g kg^{-1}, which was administered once to the animals (Paper IV).

Azadirachta indica A.Juss. (Meliaceae)

Synonyms: *Antelaea azadirachta* (L.) Adelb., *Azedarach fraxinifolia* Moench, *Melia azadirachta* L., *M. fraxinifolia* Salisb., *M. indica* (A.Juss.) Brandis, *M. pinnata* Stokes. This plant is commonly referred to as neem, neem-tree, Indian lilac, or white cedar. It is a hardy tree growing to a height of 15 – 20 m, with a dense leafy, oval-shaped canopy. The bark is rough, pale grey-brown in colour. The plant has shiny compound leaves, with 5 – 8 pairs of leaflets, crowded towards the end of branches. The flowers are scented, small creamy white and hang down in long sprays, while the fruits are oval and yellow when ripe, yielding aromatic oil. The tree is evergreen except in the driest areas, and is usually found throughout the tropics and subtropics. It flourishes also in arid and semi-arid areas of Eastern Africa (Dharani, 2002). *Azadirachta indica* is used throughout the tropics against various ailments including helminth parasites (Deka, Majumdar & Dutta, 1983).

The fresh leaves used for evaluation in sheep were collected from Magadi town, Kajiado district, three times per week, and molasses was added to increase their palatability. The leaves were offered to the lambs every morning after all feed had been withdrawn. Having consumed the leaves, the lambs were provided with the normal ration of pellets and hay in the afternoon. Animals receiving the same dose were housed in one pen and fed together daily for three weeks. The amount of waste each day was calculated and that amount added to top up the feed the next day so that by the end of week three the required daily dose rate of 0.5 g kg^{-1} bwt for the traditional dose had been achieved (Paper IV).

For evaluation of *A. indica* in mice, leaves were collected from the Kilifi district, on the coast of Kenya, and from the Veterinary Research Institute, Ipoh, Malaysia. For a median dose for mice, 50 g of fresh leaves collected in Kenya were blended in 300 ml of water. The extract was then sieved and the filtrate freeze dried overnight. The freeze dried material was reconstituted in 0.2 ml of water to give a single dose to a mouse of 5 g kg^{-1} bwt. The preparation from Malaysia was made in a similar manner using 50 g of leaves blended for five min in 300 ml of distilled water. The extract was sieved through a tea strainer and the supernatant sieved through five layers of gauze and one layer of cotton wool. The filtrate was then dispensed in volumes of 1 ml into vaccine bottles and freeze dried overnight (P. Chandrawathani, Veterinary Research Institute, Ipoh, Malaysia, personal communication). The preparation was then consigned to the ILRI laboratory, Nairobi, for evaluation. Both the Malaysian and Kenyan preparations of *A. indica* were administered once to mice, in similar dosages (Paper III).

Dodonaea angustifolia L.f. (Sapindaceae)

Synonyms: *D. viscosa* (L.) Jacq sensu KTS or *D. viscosa* spp. *angustifolia* (L.f.) J. G.West. This is an evergreen, shrubby tree attaining a height of 3 – 8 m and distributed widely from sea level to 2700 m on rocky, stony or lava sites and forest

margins in East Africa. It is fire tolerant, and this increases its abundance in areas that are regularly burnt. A decoction of the leaves and twigs was reported to be a remedy for stomach disturbances and diarrhoea in humans (Dharani, 2002). The leaf juice from this plant has been reported to be a remedy for cestodes in humans in Ethiopia (Desta, 1995).

The plant tested was collected in the Mbeere district, 200 km north-east of Nairobi, with the help of a TH from the Mbeere ethnic group who are mainly agropastoralists. Leaves of the plant were collected from bushes in a natural forest next to the TH's home, and transported to Nairobi. For treatment of sheep, the traditional dose was prepared by blending 30 g of leaves in 300 ml of water. The mixture was filtered using a tea strainer, and then freeze-dried. The freeze-dried material was reconstituted in 200 ml of water to give a single dose of 1 g kg^{-1} bwt for each sheep (Paper IV).

To make the preparation for mice, leaves were collected and dried. To each 100 g of dried leaves was added 300 ml of hot water. The leaves were left immersed for 30 min, and the mixture was sieved, and the filtrate freeze-dried. The single dose of 10 g kg^{-1} bwt for a mouse was reconstituted in 0.2 ml of water (Paper III).

Hagenia abyssinica (Bruce) J.F.Gmelin (Rosaceae)

Synonyms: *Banksia abyssinica* Bruce, *Brayera anthelmintica* Brayer, *H. abyssinica* var. *viridifolia* Hauman, and *H. anthelmintica* (Kunth) Eggleling. This is a dioecious tree that grows up to 25 m in height, in upland and high montane forests in East Africa. The flowers are greenish, or white, turning reddish with maturity in female flowers. The tree is dominant in the woodland zone just above the bamboo zone as well as in moist forests below the bamboo. The dried female flower heads and the bark infusion serve as a reputed, powerful remedy for intestinal parasites, especially against cestodes (Beentje, 1994). It is also claimed that the bark cures diarrhoea and stomach ache in humans; however it is also reputed to cause abortions (Dharani, 2002). *H. abyssinica* has been used as an anthelmintic in ruminants (Anonymous, 1996; Abebe *et al.*, 2000) and also against tapeworms in humans in Ethiopia (Desta, 1995; Giday *et al.*, 2003). The plant tested herein (Paper IV) was collected from the Aberdares forest, about 100 km north-west of Nairobi. Only the female inflorescences were utilised for evaluation, as these were identified by the THs from the Aberdares forest as possessing de-worming activity. The flowers were cut when pink in colour and about to dry on the trees, and transported to Nairobi. The flowers were subsequently kept in a carton at 4 $^{\circ}$C for a week before use. Thirty grams of the flowers were immersed in 500 ml water and shredded in a blender. The preparation was sieved and administered orally to each lamb at a dose rate of 1 g kg^{-1} bwt for those lambs receiving the traditional dose. This preparation was administered as a single dose to the animals.

Hildebrandtia sepalosa Rendle (Convolvulaceae)

This is a small shrub of 0.5 – 2 m in height that grows in arid and semi-arid bush land of Kenya. *Hildebrandtia sepalosa* was reported to be used as an anthelmintic

for livestock by the Samburu ethnic community of Northern Kenya (unpublished data) and against stomach problems in humans (Heine, Heine & König, 1988; Fratkin, 1996).

The root bark of the shrub *H. sepalosa* was collected from the Samburu district with the help of THs. The root was dug up, sun dried and the bark then removed by pounding the dried root. The bark was then milled into a powder that was transported to Nairobi and used to make the preparation for dosing animals.

The traditional dose of *H. sepalosa* used in each sheep consisted of 50 g of sun-dried root bark blended in 300 ml of water. For ease of administration to the animals, the plant preparation was lyophilised and the traditional dose given by reconstituting the material with 100 ml of water to provide a dose rate of 1.6 g kg^{-1} bwt, for those lambs receiving the traditional dose. This preparation was administered as a single dose (Paper IV).

The preparation for mice was made by immersing 500 g of the dried milled root bark in three litres of water for one hour. The extract was sieved and the filtrate was freeze-dried overnight. The freeze-dried material was reconstituted in 0.2 ml of water per mouse to give a single dose of 2 g kg^{-1} bwt, which was administered once (Paper III).

Myrsine africana L. (Myrsinaceae)

Synonym: *M. retusa* Sol. This is a small shrub or tree (1 – 5 m high) that is widespread particularly in upland dry forests and rocky hillsides in Kenya (Beentje, 1994). The fruits are globose and have a dark purple colour. There are reports that concoctions made from the bark, fruits, leaves and roots of the tree have been widely used as anthelmintics in humans and livestock (Kokwaro, 1993; Beentje, 1994; Desta, 1995; Anonymous, 1996). Particular emphasis on their role for livestock medication was made by Gachathi (1993), who reported the use of fruits as anthelmintics in sheep and goats. The plant is also used as a multipurpose cure-all for humans and livestock conditions; for example, an aqueous suspension of powdered seeds of the plant is used for treatment of east coast fever, intestinal parasites and as a body tonic for young men (McCorkle, Mathias & Veen, 1996).

Two preparations of *M. africana* were tested in two experiments in sheep (Paper I). In the first experiment, *M. africana* leaves were collected from the Aberdares forest, in the Nyandarua district, Kenya. The leaves were plucked from the shrub and kept at 4 $^{\circ}$C and used within a week. The preparation was made as described in Anonymous (1996).

In a second experiment, the collection and preparation was done as described by Samburu and Turkana THs (Paper I). This preparation was made from the dried fruits. The ripe fruits of *M. africana* were collected from Tuum at the foot of the Nyiro Mountain in the Samburu district, and dried under shade. For each animal, 50 g of the dried fruits was ground using a coffee mill and mixed with 250 ml of water, and the mixture was heated to 70 $^{\circ}$C for five min. The mixture was then stirred to cool for 10 min and used to drench each animal at the traditional dose, which was administered once to the animals. A third method which involved

giving the milled dried fruit to the animal followed by drenching with water to make sure that the animal had taken the fruits was not tested in sheep, but used in mice (Paper III). However, in order to administer the preparation to mice, the ground dried fruit was mixed in peanut butter and the mixture was fed for four days. The final preparation resulted in a dose of approximately 4 g kg^{-1} bwt.

Olea europaea ssp. *africana* (Mill.) P.S.Green (Oleaceae)

Synonyms: *O. africana* Mill., *O. chrysophylla* Lam. or *O. europaea* var. *nubica* L.Schweinf. ex Baker. The tree grows to a height of 20 m and has a rough dark, brown bole and spreading grey branches. *Olea europaea* ssp. *africana* bark decoction was reported to be used for treatment of tapeworms and as a general anthelmintic in humans (Kokwaro, 1993; Fratkin, 1996). The bark decoction is reputedly added to baths to alleviate itching in humans (Dharani, 2002). A decoction prepared by soaking bark of the plant in water for 30 min, was described as an anthelmintic in livestock amongst the Samburu (Fratkin, 1996).

The stem bark was collected from the Muguga forest, Kiambu district, in strips of about 60 g measuring 30 x 5 cm. The preparation of *O. europaea* ssp. *africana* traditional dose for each lamb was made by pounding and soaking overnight a 60 g strip of bark in 500 ml of water. Each strip provided a traditional dose of 2 g kg^{-1} bwt, administered as a single dose (Paper IV).

Rapanea melanophloeos (L.) Mez (Myrsinaceae)

Synonyms: *R. pulchra* (Gilg & Schellemb.) or *R. rhododendroides* (L.) Mez. This tree grows to a height of 20 m, is evergreen, with grey brown bark. It is widespread in Kenya in upland forests to the edge of moorlands. The fruits, like those of *M. africana,* are globose and purple in colour when ripe. The bark, roots and fruits are reputed to have anthelmintic properties (Beentje, 1994). The fruits were reported to be used as an anthelmintic in livestock (Gachathi, 1993), and in humans, to be used primarily against cestodes but also as a general anthelmintic (Kokwaro, 1993).

The fruits of the plant were collected from two locations and prepared in two different ways for drenching by the traditional dose methods (Paper I). In the first experiment, *R. melanophloeos* fruits were collected from the Aberdares forest and were dried under shade. Preparation followed the directions described in Anonymous (1996). Briefly, 125 g of dried fruits were ground in a coffee mill and mixed with 500 ml of water. The mixture was boiled for 15 min and then allowed to cool for 30 min. Each animal that received the traditional dose was then drenched with 500 ml of the cooled mixture.

In a second experiment, the fruits of *R. melanophloeos* were collected from the Nyiro Mountain in the Samburu district (Paper I). The fruits were then dried under the shade and transported to Nairobi. The collection was performed with the help of THs. For each sheep, 50 g of dried fruits were ground in a hammer mill and mixed with 250 ml of water, heated to 70 $^{\circ}$C and then allowed to cool before drenching once to each animal receiving the traditional dose (Paper I).

The mice preparation for *R. melanophloeos* was prepared in a similar manner to that of *M. africana*, with the resulting dose of 4 g kg^{-1} bwt (Paper III).

Fig. 3. A small holder farmer from the Machakos district harvesting strips of *Albizia anthelmintica* bark using a machete (top), and administration of a plant preparation using the methods and quantities used by traditional healers (bottom).

Proximate analysis

All of the plant materials evaluated were milled, and proximate analyses to determine the feed qualities of these samples were carried out (McDonald *et al.*, 1996). The analyses were done to determine the crude proteins, ether extracts (for fat), crude fibre and ash (mineral salts) and nitrogen–free extracts (soluble carbohydrate) content using the Weende analysis (Ranjhan, 1993).

Animals

Group size

Each plant treatment group had a minimum of 15 animals. It was considered, *a priori*, that efficacy of the plant preparations would be considered biologically significant if a reduction in FEC and TWC greater than, or equal to, 70% occurred. This cut-off value was chosen based on the 80% FECR that is described by WAAVP as "insufficiently active" for anthelmintic compounds (Wood *et al.*, 1995). The sample size of 15 animals was chosen based on residual variations observed in previous experiments, in order to detect such a reduction in FEC in the treatment group as statistically significant. The standard alpha error of 5% and power of 80% were used for calculating the sample size, which would then be sufficient to detect the reduction of 70%.

Within each plant treatment group, the group was further subdivided into three, with one sub-group of five animals each receiving half the traditional dose described above, and another subgroup of five receiving double the dose and five animals treated with the traditional dose. This was to test the variations observed amongst THs as to the dose to use per animal.

Sheep

The original aim was to use a known parasite-susceptible breed of sheep. Thus, in Experiments B – G (Table 6) the Dorper (D) breed of sheep was used. These sheep were acquired following weaning at the age of 3 - 4 months. The lambs were purchased from two neighbouring farms in the Laikipia district, 230 km north of Nairobi. After a prolonged drought in mid 2000 to late 2001, it became difficult to acquire lambs from this source. Subsequently, RM and D breeds of sheep and their crosses were obtained from ILRI's Kapiti plantation, Machakos district, 100 km to the east of Nairobi. These lambs were acquired after weaning when they were 4 - 5 months old. Accordingly, RM and D and their crosses were used in Experiments A and H, to determine the anthelmintic resistance status of the parasite isolate used in these studies, and to evaluate five plant preparations, respectively (Table 6).

After purchase, the lambs were transported to ILRI and housed in pens where they were fed on hay and commercial feed pellets formulated for young sheep. The lambs were treated with injectable ivermectin (Ivomec®, MSD) at a dose of 0.2 mg kg^{-1} bwt. After four weeks when the lambs were not shedding any nematode eggs, they were artificially infected with *H. contortus* L_3. Animals

infected with 5000 *H. contortus* L_3 (Table 6) displayed signs of haemonchosis. Therefore, the infecting inoculum was reduced to 3000 L_3, which was used in all other experiments (Table 6). The larval isolate originated from sheep managed at the ILRI Kapiti plantation, and was sustained in pen-raised, artificially infected sheep at the ILRI campus, Nairobi.

Four weeks after infection the lambs were divided into four blocks (within breed for Experiments using D x RM crosses). The lambs were first allocated into two blocks on the basis of high and low FEC. Then each block was further divided into two sub-blocks on the basis of high and low live weight (LWT) using measurements taken one day pre treatment. The lambs were then randomly allocated from within sub-blocks to treatment and control groups.

Table 6 *Experimental design showing the infecting dose, breed and number of lambs used per trial*

Experiment (inoculum x breed of sheep used)	Treatment	No. of animals	Plant part[a]
A	ivermectin	12	NA
(3000 x DxRM)	levamisole	11	NA
	albendazole	11	NA
	control	12	NA
B	*Hagenia abyssinica*	15	Fl
(5000 x D)	*Myrsine africana*	16	L
	control	15	NA
C	*Albizia anthelmintica*	15	B
(3000 x D)	*Rapanea melanophloeos*	17	Fr
	control	16	NA
D	*Myrsine africana*	16	Fr
(3000 x D)	*Rapanea melanophloeos*	16	Fr
	control	17	NA
E	*Albizia anthelmintica*	15	B
(3000 x D)	control	16	NA
F	commercial herbal preparation	16	NA
(3000 x D)	control	16	NA
G	*Dodonaea angustifolia*	15	L
(3000 x D)	*Hildebrandtia sepalosa*	14[b]	RB
	control	14[b]	NA
H	*Albizia anthelmintica*	15	RB
(3000 x DxRM)	*Ananas comosus*	15	L
	Annona squamosa	14[b]	L
	Azadirachta indica	16	L
	Olea europaea ssp. *africana*	16	B
	control	16	NA

[a] Plant part: B = bark, L = leaves, Fl = flowers, Fr = fruits, RB = root bark
[b] Groups with one animal that died (not due to haemonchosis) after infection
NA = not applicable

Mice

Swiss White mice (5 - 6 weeks old) were used to evaluate the various plant preparations. After selection, the mice were randomly allocated into cages of five and allowed to acclimatise for one week. In all studies, a dose of 200 one-week

old *H. polygyrus* infective larvae was used to infect the mice, suspended in 0.2 ml of water. The mice were infected orally into the stomach using a blunt ended needle. The isolate of *H. polygyrus* used in the study was obtained from University of Nottingham, UK, and thereafter maintained through passage in a colony of inbred CBA strain mice at ILRI, Kenya. In all trials there were 15 mice per treatment group and 10 mice for the control groups. Within each treatment group, a dose titration was carried out with five mice each receiving half the median dose, five receiving the median dose and another five each receiving double that dose. Treatment with the plant preparations was carried out 18 days post infection (Paper II and III).

Efficacy of commercial anthelmintics

To validate the test system used for the EVM preparations, the efficacy of commercial anthelmintics against the infecting parasites was evaluated in both the ruminant and monogastric test models. The AR status of *H. contortus* used to infect sheep was evaluated at the same time by using drugs from the three major classes of anthelmintics.

Sheep

In this trial, 46 8-month-old lambs artificially infected with 3000 *H. contortus* L_3 were weighed, and randomly allocated into four groups. Lambs from three groups were treated with ivermectin (Ivomec ®, Merial), levamisole (Nilzan ®, Coopers) and BZ (Valbazen ®, Pfizer) according to the manufacturers' instructions based on the individual live weight (LWT) of each sheep. The lambs were observed for 14 days, with FEC and PCV being determined three times a week. Two weeks post treatment the lambs were slaughtered and *H. contortus* recovered from the abomasa for enumeration.

Mice

Ivermectin (Ivomec®, Merial), piperazine citrate (Piperazine, Sanofi) and pyrantel (Banmith®, Pfizer) were tested in an experiment to determine their effectiveness on the strain of *H. polygyrus* used to infect mice. For each anthelmintic, a dose-response trial was carried out whereby five mice received a dose previously proven to be effective (Cook, 1969; Wahid, Behnke & Conway, 1989; Fakae, Harrison & Sewell, 2000) to remove the parasite, five received half that dose, and another five received double the dose. Suspensions of the different drugs were prepared in a volume of 0.2 ml per mouse. All mice were weighed prior to treatment and given the drugs based on the individual mouse body weight.

Diagnostic methods

Sheep faecal samples

Presence of nematode eggs in faeces was determined based on 3 g of faeces using the modified McMaster method with a sensitivity of 50 eggs per gram (EPG) of

faeces (Hansen & Perry, 1994). The faecal samples were collected three times in the first week, two times in the second week and once weekly thereafter.

Sheep live weight measurements

Animals were weighed weekly from the time of infection to the end of the trial two to three weeks later. The effect of plants on daily LWT gains was determined for the three weeks post treatment period in Experiments B – H (Table 6).

Sheep blood samples

Packed red cell volume (PCV), reflecting the degree of anaemia, was determined as described above for FEC. The microhaematocrit method was used to determine the PCV (Hansen & Perry, 1994).

Sheep worm recovery

The animals were humanely slaughtered and the abomasum was ligated at the junctions of the abomasum to the omentum and the abomasum to the small intestine. The abomasum was removed, and opened up with a blunt-tipped pair of scissors and the contents emptied into a bucket. The abomasal mucosa was washed gently with running tap water and the parasites washed off into the bucket. The contents of the bucket were adjusted to make two litres, and the mixture thoroughly mixed, and two aliquots of 200 ml each collected. The numbers of adult *H. contortus* in the aliquots were counted and the total number of parasites in the abomasum calculated.

Mice faecal samples

The collection of individual mouse faecal samples was done on the day of treatment, 2 and 4 days before treatment, and 3, 5 and 7 days post treatment. Mice were individually placed in labelled cages without bedding, or feed, for two hours. The faecal pellets produced were collected with a spatula, weighed and placed into labelled polythene bags and stored at 4 $^{\circ}$C and processed within a day. One gram of faeces was suspended in 60 ml saturated salt solution, and since in most cases the faecal material weighed less than 1 g, the amount of saturated solution was proportionately reduced. The suspension was homogenised using a tissue homogeniser and the fluid filtered into a beaker, and aliquots were examined under a microscope using McMaster egg counting slides. The presence of nematode eggs in faeces was analysed based on 1 g of faeces using a modification of the McMaster method adopted from a technique used at the University of Nottingham, UK (J. Behnke, personal communication), with a sensitivity of 400 EPG.

Mice worm recovery

Mice were humanely euthanized using carbon dioxide, and the body cavity was opened to remove the small intestine. The intestine was placed on a piece of gauze

and opened longitudinally with small round tipped scissors. The intestine was spread on the gauze with the mucosal surface facing downwards, then suspended in 30 ml of Hanks saline in a labelled 50 ml beaker. The beakers were incubated in a water bath at 37 °C for four hours. After this period, the intestines and gauzes were carefully removed from the beakers and the tissues discarded, and the temperature of the water bath was increased to 42 °C for 30 min and thereafter increased by 2 °C every five min. The increase in temperature helped to disentangle the parasites for ease of counting. After the water bath temperature had reached the 52 °C, the beakers were removed and stored at 4 °C. The gauzes were removed after four hours and scanned under a dissecting microscope to search for nematodes that may have been trapped in the mesh, and these were counted before discarding the mesh. The recovered parasites were counted within 36 hours under a dissecting microscope.

Statistical analysis

Mean FEC and PCVs were calculated over each week (days 0 - 7, 8 - 14, 15 - 21), as well as weekly weight measurements from week 0 - 3 post treatment for Experiments B – H (Table 5). The mean FEC were transformed to log (y + 25) based on the sensitivity of McMaster technique, while TWCs were transformed to log (y + 1) prior to statistical analysis for both mice and sheep experiments to stabilise variances. FEC, PCV and LWT gain were analysed using repeated measures analysis of variance using SAS software, in order to compare control and treatment means over time. TWC was analysed using a generalised linear model. Terms for breed and block within breed for Experiments A and H (Table 6) and for block in Experiments B – G (Table 6) were included in the model. Block was found to be non-significant, and so initial FEC and PCV were included instead as covariates in the analysis of FEC and PCV, respectively. Thus, the statistical model eventually used was:

$$y_{ijkl} = \mu + b_i + g_j + t_k + \beta_{xijkl} + e_{ijkl}.$$

where y_{ijkl} is the response variable (weight gain, PCV, log (FEC+25)), with terms b_i for breed in experiments A and H, g_j (j=1,…,ng where ng = number of groups) for treatment groups, t_k (k=1,…,nk, where nk is number of measurement times), and a covariate term β_{xijkl} for initial weight, PCV and log (FEC + 25), respectively, depending on the response variable.

Least squares linear regression was applied within groups to test for dose titration effects. The faecal egg count reduction (FECR) was determined by the method described by Coles *et al.* (1992) using the formula FECR% = 100 x (1 - T/C), where T and C are the geometric means of FEC in the treated and control groups, respectively, at week two for Experiments A and B and at week three post treatment for the other experiments. A similar formula was used for percentage TWC reduction (TWCR).

PROC GLM and PROC MIXED were used for all statistical analyses. All statistical analyses were performed using SAS system for Windows version 8.2 (SAS Institute Inc., Cary, NC, 1999). Pair-wise comparison between treatment and

control groups was done by the LSMEANS / TDIFF option in PROC GLM. The standard error of difference (SED) calculation was based on the square root of the residual variance multiplied by ($1/n_1+1/n_2$) where n_1 and n_2 are the number of observations per treatment group, respectively, for the two means to be compared, and was calculated using the LSMEANS / DIFF option in PROC MIXED. Similar analyses were carried out for mice with repeated measures ANOVA used for log (FEC + 25) and PROC GLM for TWC.

Results and discussion

In vivo evaluation in sheep

Commercial anthelmintics

All the synthetic anthelmintics reduced FEC and TWC by >90 and >95%, respectively, by day 14 post treatment in sheep. Interestingly, ivermectin caused a somewhat smaller FECR than albendazole and levamisole. These results indicated that the strain of *H. contortus* used was highly responsive to the three classes of anthelmintics tested (Paper IV).

Plant preparations

No abnormal behavioural change was observed after treatment of the animals with the different plant preparations (Paper I, II & IV). However, two animals amongst those treated with *A. anthelmintica* suffered from transient bloat. In addition, animals offered *A. indica* leaves ate poorly even after addition of molasses to increase the palatability of the leaves. Lambs treated with neem leaves *(A. indica)* had very high FEC, and as such the mean group FEC (104000 EPG) was more than twice that of the control group (50000 EPG).

Differences in FECR were observed in three groups of animals administered with the different preparations of *A. anthelmintica* (Table 7). In lambs treated with a CE preparation from bark of *A. anthelmintica* collected from the Machakos district, no significant effect on FEC was observed. In contrast, a HE preparation from the bark of *A. anthelmintica* collected from the Kajiado district and a CE preparation from root bark collected from the Samburu district, had significantly lower FEC than untreated lambs (Paper II). Similarly, preparations of *R. melanophloeos* exhibited differences in FECR based on where the fruits were collected. In one group treated with fruits collected from the Aberdares forest, a nearly significant FECR was observed, while the group treated with preparations from the Samburu district had higher FEC than the untreated controls (Table 7). This was not observed for preparations made from leaves and fruits of *M. africana* collected from the Aberdares forest and the Samburu district, respectively, which produced more consistent results (Table 7, Paper I).

Animals treated with plant preparations made from flowers of *H. abyssinica*, leaves, fruits of *M. africana*, fruits of *R. melanophloeos* collected from the Samburu district, and leaves of *A. indica*, respectively, had higher FEC than their

respective control lambs (Table 7). This could have been caused by reduced faecal output, and consequently faecal concentration of nematode eggs in these groups of treated animals.

Likewise, no significant effect on FEC was observed in sheep treated with a preparation of *A. squamosa* (Table 7). This was in accordance with observations made in an investigation of naturally infected goats treated for four days with juice from the leaves of *A. squamosa*, in which no reduction was established either in FEC, or adult *H. contortus* recovered from the abomasa (Vieira *et al.*, 1999). In contrast, Jovellanos (1997) reported a significant reduction on FEC in cattle dosed with an oral preparation of *A. squamosa* leaves. Plant preparations made from *A. comosus*, *D. angustifolia*, *H. sepalosa* and *O. europaea* ssp. *africana*, resulted in FECR ranging from 8 – 31%, however none of the reductions were significant (Paper IV).

Table 7. *Percentage faecal egg count reductions (FECR) of the different plant preparations two or three weeks post treatment when tested in sheep and FECR and total worm count reductions (TWCR) in mice seven days after treatment*

No [a]	Plant preparation and active principles	Sheep FECR (%)	Mice FECR (%)	Mice TWCR (%)
1	*Aframomum sanguineum*	NT	-10 [b]	4
2.1	*Albizia anthelmintica*	19	NT	NT
2.2	*A. anthelmintica*	28	-5	17
2.3	*A. anthelmintica*	34	-45	1
3	*Ananas comosus*	14	NT	NT
4	*Annona squamosa*	28	NT	NT
5.1	*Azadirachta indica*	-108	25	15
5.2	*A. indica*	NT	26	-11
6	*Dodonaea angustifolia*	31	12	-4
7	*Hagenia abyssinica* [c]	-23	NT	NT
8	*Hildebrandtia sepalosa*	22	1	1
9.1	*Myrsine africana* [c]	-10	NT	NT
9.2	*M. africana*	-10	16	10
10	*Olea europaea* ssp. *africana*	8	NT	NT
11.1	*Rapanea melanophloeos*	19	NT	NT
11.2	*R. melanophloeos*	-23	36	5
12	Embelin [d]	NT	49	7
13	Commercial herbal preparation	32	NT	NT

[a] See Table 5 for description of numbers based on place of collection
[b] A negative value indicate that FEC values were higher in treated than in untreated control animals
[c] Sheep observed for two weeks post treatment, other groups observed for three weeks
[d] Benzoquinone chemical found in plant species in the family Myrsinaceae
NT = Plant preparation or chemical not tested in that animal model

Most plant preparations did not have a significant effect on the degree of anaemia. However, animals treated with preparations made from the leaves of *A. squamosa* had significantly higher PCV three weeks after treatment than in the untreated controls. This suggests that the plant had a stimulatory effect on the hemopoietic system, or caused reduced water intake in the animals. However, it is unlikely that this observation was related to water intake, as all treatment groups in Experiment H (Table 6), with the exception of the *A. indica* treated group, were

managed under similar conditions and no behavioral changes were noted in any group (Paper IV).

No significant LWT gains were observed between treated and control groups in animals treated with any of the plant preparations tested. Nonetheless, no major conclusions on the long term effects of the plant preparations could be drawn for LWT gain, due to the short period of observation. However, all animals infected with 5000 L_3 *H. contortus* in Experiment B lost more weight than sheep infected with 3000 L_3 larvae, indicating that the size of the infecting dose had an effect on weight gains.

Most of the plants evaluated, had been identified by various sources to have anthelmintic properties. Some plants, like *A. anthelmintica*, had been evaluated against other helminth species such as the trematode *Fasciola gigantica* in artificially infected goats, where an aqueous extract of the plant was observed to reduce the number of flukes recovered from the livers of treated goats (Koko, Galal & Khalid, 2000). Similarly, an aqueous preparation of this plant was found to be effective in reducing the number of cestodes in infected mice (Galal *et al.*, 1991a) and in humans infected with the cestode *Taenia saginata* (Desta, 1995). In addition, preparations of the same plant were evaluated in naturally infected sheep in Kenya and Uganda (Grade & Longok, 2000; Gathuma *et al.*, 2004). In both investigations, the infecting parasites were not characterized, despite claims of high efficacy (Grade & Longok, 2000; Gathuma *et al.*, 2004). No comparison could be made between the current study and the study in Uganda due to their low number of animals, and since they used an egg floatation technique that only demonstrated presence of eggs (Grade & Longok, 2000). In the Kenyan study where THs treated naturally infected sheep with preparations of *M. africana, H. sepalosa* and *A. anthelmintica*, the FEC range of *Moniezia* spp. eggs before treatment was 300 - 1500, while the pre treatment trichostrongyle nematode eggs counts ranged from 250 - 450 EPG (Gathuma *et al.*, 2004). In that study, FEC reductions of 77, 89 and 90% were reported for each plant species tested, respectively (Gathuma *et al.*, 2004). However, in the current study, sheep were infected with a monospecific infection of *H. contortus* and average FEC in both treated and control animals were sufficiently high to make sound estimations of treatment effects.

Studies in the Philippines and Malaysia reported the activity of *A. indica* against nematode parasites in ruminants (Baldo, 2001; Chandrawathani *et al.*, 2002). Similarly, cattle provided with feed blocks containing different levels of dried leaves of *A. indica* had significantly lower EPG than untreated control animals, 90 days post treatment (Pietrosemoli *et al.*, 1999). In both the Philippines and Malaysian studies, sheep were provided with fresh leaves daily and reportedly ate without addition of molasses to increase the palatability of leaves (Baldo, 2001; Chandrawathani *et al.*, 2002). However, in the current study, no significant decrease of FEC was observed in sheep fed on *A. indica* leaves daily for three weeks. Leaves were collected every three days, and this may have had an impact on the palatability as well as the efficacy in this study. Similarly, controlled studies in sheep artificially infected with *H. contortus* and *T. colubriformis*, and treated with seeds of *A. indica*, reported failure of this plant to reduce the number of eggs

shed in faeces, and there was no reduction in the population of adult parasites recovered from the infected animals (Hördegen *et al.*, 2003).

No effect was observed on FEC in my study from sheep treated with extracts from the two plants *M. africana* or *R. melanophloeos*, which belong to the same family Myrsinaceae (Paper I). Similar observation to this study of a lack of effect on FEC was made in goats treated with a preparation from *H. abyssinica* flowers (Abebe *et al.*, 2000). In contrast, *in vitro* activity of alcoholic extracts of *M. africana* fruits was reported against the cestode *Taenia solium*, and the nematodes *Bunostomum trigonocephalum* and *Oesophagostomum columbianum* (Kakrani & Kalyani, 1983). Animals dosed with flowers of *H. abyssinica* were also said to have expelled *Moniezia* spp. tapeworm after treatment (Abebe *et al*., 2000).

In vivo evaluations in mice

Commercial anthelmintics

Pyrantel and ivermectin were highly effective in reducing the numbers of *H. polygyrus* in mice. These drugs reduced FEC by > 99% at all doses administered. Pyrantel caused a highly significant ($p < 0.001$) reduction in TWC of 99% or more, at all dose rates used. Nevertheless, in mice treated with ivermectin, TWCR indicated a dose response to treatment with increasing concentration. Ivermectin at a dose rate of 20 mg kg^{-1} bwt eliminated *H. polygyrus* by day seven post treatment, but at 5 mg kg^{-1} bwt the reduction in TWC was only 87%. In contrast, piperazine citrate had no significant effect on TWC or FEC at doses of 1000 or 2000 mg kg^{-1} bwt by day seven post treatment, and only resulted in a 34% reduction of TWC at the highest dose rate of 4000 mg kg^{-1} bwt. However, when another group of mice was treated with piperazine citrate at a dose of 2000 mg kg^{-1} bwt, highly significant reductions ($p < 0.001$) of FEC (99%) and TWC (61%) were observed, which is similar to observations made by Nicolay *et al.* (2000). Mice treated with piperazine citrate at a dose of a 2000 mg kg^{-1} bwt in the earlier experiment had similar levels of reduction in FEC on day three, but by day seven post treatment the FEC were higher than in untreated controls (Paper III).

The high efficacy of ivermectin and levamisole indicated that the test system could be used to validate other products which had putative anthelmintic activity. Interestingly, the effects of piperazine were more varied, although the doses used were much higher than those recommended against roundworms of swine and companion animals.

Plant preparations

Due to difficulties in administration of preparations of the fruits of *M. africana* and *R. melanophloeos*, the milled form of these plants was mixed with peanut butter (Paper III). Peanut butter was used as a vehicle based on information from the University of Nottingham, where they use this medium when trapping wild rodents (J. Behnke, personal communication). No reduction of FEC was observed in mice fed on peanut butter but on the contrary EPG significantly increased by day 7 post treatment. This increase was probably due to reduced feed intake, as smaller amounts of faeces were shed in this group. *M. africana* administered to

mice in this form had no effect on FEC but had lower TWC compared to untreated controls. In contrast, mice treated with *R. melanophloeos* fruits in peanut butter had significantly lower FEC but no effect on TWC (Table 7). Mice treated with embelin, a chemical found in the two plants, had the highest FECR of 49%, although similar reductions of TWC were not observed suggesting that the chemical affected the egg laying capacity of the parasite (Paper III). Embelin was demonstrated to lack *in vivo* effect on TWC in mice infected with *H. polygyrus*, but the chemical had an *in vitro* effect at concentrations above 2.5 mg ml^{-1} (Bøgh, Andreassen & Lemmich, 1996). In contrast, they observed that embelin was highly effective in the removal of the cestode parasite, *Hymenolepis diminuta*, in rats, but not in mice, although destrobilation was observed in rats (Bøgh, Andreassen & Lemmich, 1996).

The preparation from the bark of *A. anthelmintica*, described by THs as a "strong medicine", caused death of mice at doses of 1000 mg, although it was ineffective in removal of parasites from intestines of mice, and did not have any significant effect on FEC at all doses used. Signs of toxicity were observed within two days of treatment in this group of mice. Although death was observed to occur in mice administered with an aqueous preparation of 33 g kg^{-1} bwt, this dose was much lower than that administered to rats (50 g kg^{-1}) without lethal effects (Galal *et al.*, 1991b). Butanolic extracts of *A. anthelmintica* were found to be lethal to mice at concentrations above 25 g kg^{-1} bwt (Galal *et al.*, 1991b). Likewise, death occurred in all groups of mice infected with *H. polygyrus* and treated with doses of 5, 10 and 20 g kg^{-1} bwt of a methanol extract of *A. anthelmintica* (Gakuya, 2001). However, an aqueous preparation of *A. anthelmintica* eliminated the cestode *Hymenolepis diminuta* from artificially infected albino rats at doses above 50 g kg^{-1} bwt (Galal *et al.*, 1991a). Similarly, significant reductions in FEC and TWC were reported in mice treated with an aqueous extract. Nevertheless, the results from that study could not be compared to my study since they utilized mice which were more than three months old (Gakuya, 2001). In my studies, I demonstrated that outbred mice, older than two months, were able to spontaneously expel GI parasites from their intestines (Paper II).

Treatment of mice with preparations of *A. indica* leaves collected either in Kenya or Malaysia did not have any effect on FEC or TWC (Table 7). Plant preparations made from *A. sanguineum*, *D. angustifolia*, and *H. sepalosa* resulted in FECR ranging from -10 to 12%, and none of these changes were significant. Likewise, the same preparations had no significant effect on TWCR (Table 7). Similarly, no signs of toxicity were observed in mice treated with preparations made from any of the other plant species, except for animals treated with *A. anthelmintica* (Paper III).

Proximate analysis

Varying levels of feed components were observed for all plants (Table 8). It was observed that some plants, like the bark of *O. europaea* ssp. *Africana*, had very low levels of crude proteins. Since the plant preparations were administered once

in most cases, the effect of high crude proteins, as observed in the bark of *A. anthelmintica*, cannot be ascertained.

Table 8 *Proximate analysis of the plants used in the experiments*

Plant	DM% [a]	ASH% [b]	CP% [b]	EE% [b]	CF% [b]	NFE% [b]
H. abyssinica	94.2	9.0	12.1	9.2	29.3	40.3
A. anthelmintica	90.5	5.6	40.5	1.8	32.8	19.2
R. melanophloeos	90.7	10.3	8.3	16.5	25.8	39.2
M. africana	89.3	4.5	6.6	9.9	28.0	51.1
D. angustifolia [c]	NA	NA	NA	1.2	NA	NA
H. sepalosa	78.1	4.0	8.6	1.1	16.4	34.0
A. comosus	91.2	7.9	7.3	3.9	40.0	44.9
A. squamosa	89.8	10.0	17.3	5.8	22.4	44.4
A. indica	91.1	10.4	18.4	2.8	28.8	39.65
O. europaea ssp. *africana*	93.4	4.9	2.6	5.9	49.5	37.2
A. sanguineum	90.0	5.0	9.8	12.2	18.9	54.1

[a] DM = dry matter, ASH = ash content (mineral content), CP = crude protein (proteins), EE = esterified ethers (fats), CF = crude fibres (non soluble carbohydrates), NFE = nitrogen-free extractives (soluble carbohydrates)
[b] Percentage of the DM content
[c] NA = not applicable because sample was not adequate for all analyses

Summary and concluding remarks

None of the plant preparations evaluated reduced nematode FEC to the *a priori* cut-off level of 70%, either in sheep or mice. However, statistically significant FECR were observed in animals treated with preparations of *A. anthelmintica* root bark collected from the Samburu district. On the other hand, the geometric mean EPG in the *A. anthelmintica* treated group was 4500, while in the untreated animals, the average FEC was 6850, and it is not clear whether the FECR would be in a similar range in animals shedding fewer eggs.

Signs of toxicity were only observed in mice treated with the *A. anthelmintica* preparation. Although no specific toxicity assessment was undertaken in the current studies, and most of the plant preparations did not cause a change in the behaviour of animals, any further evaluations should consider this issue.

The number of animals in most other EVM evaluations is below the minimum that WAAVP recommends for *in vivo* evaluation of anthelmintic drugs. The number of animals in any study is determined by the power of the test as well as the size of the effects measured. In these studies, an *a priori* cut-off value of 70% reduction was used. This meant that a minimum of 15 animals was used per treatment group. The numbers of animals needs to be sufficient to be able to conclusively determine a treatment effect.

In several studies, *in vitro* investigations have been used and extrapolations are then made on the *in vivo* anthelmintic efficacy. For example, a dose dependent *in vitro* activity of embelin was demonstrated against adult stages of two cestodes, one trematode and one nematode (Bøgh, Andreassen & Lemmich, 1996).

However, *in vivo* evaluation of the same compound only revealed anthelmintic activity against the cestode in rats. No activity was observed against the cestode, trematode or nematode of infected mice. This highlights the importance of species differences in regard to host-parasite models under study.

It is imperative that parasitic infections should be well characterised, particularly as to whether the animals are artificially or naturally infected. Similarly, the methods of application of any plant needs to be ascertained. Obtaining accurate information from THs, and validating this data, is of paramount importance in these types of studies. It is equally important to gather information on the type of parasites being treated and their location within the host. In this study, it was noted that 'worms' treated by THs were mainly those that were macroscopically visible after application of the plant remedies. Similarly, farmers in South Africa had very poor understanding of GI parasite infections in their cattle, although traditional remedies against 'worms' were available (van der Merwe, 2000). In many instances, what are considered to be 'worms' by the THs and owners of livestock are likely to be destrobilated segments of tapeworms, although these may also be shed irrespective of treatment.

Apart from *in vivo* evaluation in sheep, a mouse-parasite model was used to evaluate the anthelmintic efficacy of some plants used in this study. The *in vivo* model was found to be a useful tool for rapid screening of anthelmintic activity of plant preparations against nematode parasites. This parasite-host model has been exploited by the pharmaceutical industry in anthelmintic screening procedures for many years because it is relatively easy to maintain (Wahid, Behnke & Conway, 1989). Since the lifecycle of *H. polygyrus* is similar to that of parasitic nematodes of veterinary importance, it is likely that this model can be used to draw inference to related parasites of other monogastric animals such as the horse, pig and humans. However, no major differences in the results obtained from evaluation of the few plant preparations, either in the sheep or mouse models, were determined (Table 7).

The parasites used in the sheep and mice models responded to broad spectrum anthelmintics. Breed of animals, and previous exposure of lambs to parasite infections, did not seem to have an effect on the establishment of artificial infections. The plant preparations at doses expected to span those used by THs had no adverse effects on the sheep. The mouse-parasite model was found to be suitable for quick and initial evaluation of anthelmintic properties of plants within a relatively shorter time than in ruminants. Thus, the mouse model can be useful for screening large number of plants, such as gene banks of plants with putative anthelmintic properties. However, conclusions on the efficacy of such plants in ruminants must be based on validation in the definitive host.

In conclusion, none of the plants tested in this study had the hypothesized reductions, for recommendations to be made that they could be used as an alternative form of nematode parasite control in sheep.

Future perspectives

Although most of the plants tested had no effect on FEC, or the number of nematode parasites in the abomasa of sheep or intestines of mice, this study only assessed a very small number of plants with putative anthelmintic properties. Thus, it is proposed that future investigations into plant derived deworming preparations should consider a number of issues. These include:

- Coordinated approaches to validation of EVM preparations, need to be put in place. Towards this objective, a set of guidelines established by an authoritative, independent body (*e.g.* the WAAVP) should be developed. The *in vivo* tests should be standardised, with adequate numbers of animals and characterisation of the target parasite.
- Involvement of THs in future validation processes is critical, especially when accurate information needs to be obtained from them. To enhance free flow of information, building confidence with the THs is vital. This must include the safeguarding of their 'intellectual property', as well as establishing a platform for reciprocal exchange of information and results from any research carried out.
- Definitive proof of anthelmintic efficacy of any EVM preparations against GI parasites of ruminant livestock must be based on *in vivo* testing of such products against parasites species in the normal host (*e.g. H. contortus* in sheep, or goats). Even though this approach will be constrained by expertise, costs, labour, time and facilities, anything less would yield ambiguous results.
- A rapid *in vivo* screening test using the nematode *H. polygyrus* in mice can be used as an alternative model, especially to evaluate plant substances that are used both in humans and livestock by THs. However, it should be recognised that the effects of plant preparations in the mouse may be different in ruminant hosts. Young mice (< 2 months) should preferably be used in all occasions since spontaneous expulsion of parasites invariably occurs in older mice.
- A number of trials have evaluated the *in vitro* anthelmintic activity of EVM preparations and extrapolated these results to the host. No possible extrapolations can be made without first ascertaining anthelmintic activity *in vivo*, and ovicidal and larvicidal activity should only be claimed for plant preparations that show activity *in vivo.*
- There is a need to determine whether some of the EVM plant preparations have a beneficial effect by directly contributing to the nutrition of the parasitized animals. Those are the so-called "nutraceutical" plants, to which it is claimed that some plants containing CTs belong. Methodology to test this will differ from testing anthelmintic effects, as these nutraceutical plants need to be provided for much longer periods of time before any beneficial effects can be expected.
- Since the THs understanding of 'worms' refers to macroscopic GI parasites, it would be worthwhile to test the effects of these plants against cestodes and trematodes, and especially the effects of plant preparations on zoonotic parasites also found in livestock.

- Illiteracy does not equate with inadequacy or inefficiency, and it is only reasonable to assume that traditional owners of livestock are no better – but certainly no worse – in livestock management than their contemporaries in the Developed world. Therefore, there is an urgent need for technology transfer, similar to that which has been an increasingly important part of veterinary parasitologists' responsibilities in the Developed world. Significant funding needs to be specifically allocated for this purpose, to enable this to be undertaken and increasingly promoted amongst the pastoralist and SHF communities.
- It is also essential to transfer the results of scientifically based research on the anthelmintic efficacy of herbal dewormers to the 'end-users' particularly, pastoralists and SHF. This would help to address the issue of conserving such plants by preventing harmful, destructive and excess harvesting.

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Acknowledgements

The present study was carried out at the International Livestock Research Institute (ILRI), Nairobi, Kenya in collaboration with the Department of Parasitology (SWEPAR) of the Swedish University of Agricultural Sciences (SLU) and the National Veterinary Institute (SVA).
Financial support was provided by the Swedish International Development Agency, Department for Research Cooperation (SIDA-SAREC).

This study would not have succeeded without the role played by **Traditional Healers** from the Samburu, Mbeere, Kajiado and Machakos districts. The traditional healers provided information on plant species they used as anthelmintics and demonstrated the methodologies for preparation. I particularly would like to mention **Albert Leleshep** from South Horr and his team of healers, **Ambrose** for interpreting the Turkana language, **Alice Lorot** for your time and demonstrations not to forget all the other THs in Baragoi, Tuum, Marti, Nachola, Ekwam, Masikita, Morulem and other locations in the Samburu district. **Simon Murangiri** from Mbeere for, providing me with information, welcoming me to your home and the keen interest in the results obtained after the validation in sheep. **Mr Mwangangi**, formerly of the East African Herbarium, for introducing me to the THs from the Machakos district, and from Kajiado district.

In addition, I wish to express my appreciation and gratitude to the many people who have in one way or another helped me over the course of study. In particular I would like to thank the following:

Associate Professor Johan Höglund, my main supervisor, for your untiring efforts to make sure that an excellent piece of work was done and for always making sure that I managed to hit deadlines and for making me comfortable while in you country. Thanks for the encouragements, allowing me to develop at my own pace and for inviting me to your home.

Peter Waller, my assistant supervisor, for your zeal for a perfect job. I felt honoured by your encouragement, the excellent dinners, the humour and the advice offered that always raised my spirits when I was away from my family. I also learnt how important it was to look at the nitty gritty in any publication. In my next visit to Sweden during winter, I will take up you challenge and ski downhill.

Leyden Baker, my assistant supervisor at ILRI, for always making my stay at ILRI comfortable and for the advice and encouragement as well as supporting my ideas.

Professor Arvid Uggla, for always making sure that I was comfortable while in Sweden. I appreciated your help, patience, advice and you always listened to my innumerable questions, in addition to the wonderful dinners offered.

The staff of Helminthology laboratory at ILRI. **John Mugambi**, for the help, advice and discussions on some of the parasitological techniques, the friendship and the lifts when I did not have means to get to ILRI.

Samuel Muthee for your help and thirst for knowledge. I can only urge you to continue with your quest until your goal is achieved. **Fredrick Moseti** and **Sarah Kanyingi** for helping in the laboratory despite the amount of work that was always in the 'kinyesi factory'.

John Rowlands, for helping in the study design, statistical advice and for reading through my manuscripts whenever you could. Thanks for helping open my eyes to the need for details.

Doug Gray, for the discussions and advice during the study design and for sending the material I needed from the Philippines.

Jerzy Behnke, for the advice on mouse evaluation studies

The staff of the large animal unit at ILRI, for helping in handling of sheep. I particularly wish to thank **David Ndolo** for the very keen eye on animal observation after treatment.

The staff of the Small Animal Unit at ILRI, for helping with the mice evaluation studies. I appreciate that you did not have weekends off during that period. G**ideon Ndambuki, Jane Ikanyi** and **Dominic Masenge**, thanks for the extra help that you offered.

Bob King, the Animals Units Manger at ILRI, for your help with the experiments both in the large and small animal units. I appreciated the extra help that I got with the mice and animal handling.

Tammi Krecek, for the encouragement and the parasites sent from South Africa. I cannot forget to thank you for hosting us in your country and for the advice you have continued to offer.

David Morrison for your valuable comments not only on the botanical descriptions of the plant species, and the weekends that I kept you occupied reading, but also for your cheerful approach to issues.

Gunilla Lindgren, for your organisational acumen that helped to provide timely and efficient travel plans and a comfortable stay in Sweden.

Jens, Janne and **Erland,** it was nice to introduce me to the Swedish way of cheering ice hockey.

Karin, for your role in organising events that kept the SWEPAR family busy and always having the time to listen to my stories.

Anna Larsson, for the stories shared. I still wait for what you promised to fulfil and best of luck.

Chandrawathani for sending neem samples from Ipoh, Malaysia.

Eva Osterman-Lind my former officemate for having to put up to the noise and clutter I created during my visits. My fellow PhD students, **Jackie** and **Sissay**, for the friendship offered.

Malin, my 'Swedish half', for interesting stories and opinions shared.

Jacob Wanyama and other staff of ITDG for helping in organising THs workshops in the Samburu district.

Leina Mpoke of the Community Livestock Initiative Program East Africa, for translating the responses of the Samburu THs during the workshops and for hosting me in your home at Tuum.

Jamleck Ndambiri and your wife **Josephine**, for introducing me to Mr Murangiri from the Mbeere district and the help in collection of information, both in the Kiambu and Mbeere districts, and for your friendship.

Joseph Machua, of the Kenya Forestry Research Institute, for the help with the collection of plant species from the Kitui district.

Norman Gachathi for identification and collection of plants from the Aberdares forest, and for sharing your knowledge on traditional plants used in Central Kenya.

The librarians at the SVA, Sweden and ILRI, Kenya for providing me with some of the literature that was quite hard to get.

Dr Gichuki for the encouragement and ideas

George Ogoti and **Sylvester** in the IT department at ILRI, and **Katrin** and **Göran** at SWEPAR for always helping with the computer problems.

Stella Massawe and **Russ Kruska**, for providing me with the maps.

Mr Kaniaru for your timely advice over official matters.

Phil and your family, AHSANTE Sana for the proof reading.

All the staff of SWEPAR, for making me feel at home whenever I visited the Department.

To all my other friends not mentioned; accept my gratitude for your support.

Stene my friend at the SVA. We might come from two different continents and backgrounds, but I appreciated the time, the stories, and opinions shared, and for making my stay Sweden pleasant. The evenings watching chef Stene going through his expertise were always full of fun. Thanks for the hockey game that was lovely, although football game took too long. I am waiting for that visit to Kenya. I hope by that time the big question will have been answered.

My brothers **Dominic** and **Simon**, and my sister **Ann**, and their families, for the constant support and the encouragement offered.

Fred and **Melanie**, my children, thanks for the patience and love. **Lois**, my wife, your patience, love, support and constant encouragement helped me to push through the good and rough times. AHSANTE SANA.

Appendix A

In vitro evaluation of plant preparations in Tables 1, 2, and 3 in non-parasitic and different parasitic nematode species respectively.

Table 1. In vitro *assay of plant preparations evaluated against the free-living nematode* Caenorhabditis elegans

Plant species	Active principles[1]	Parts used[1a]	Target[1]	Reference
Butea monosperma	sterols, palasonin	S	A	Prashanth *et al.*, 2001
Combretum spp.	phenantherenes,	L	A	McGaw *et al.*, 2001
Cymbogon martini	geraniol [2]	W	A	McGaw, Jager & van Staden, 2000
Evodia ruteacarpa	atanine [2]	Fr	A	Perrett & Whitfield, 1995
Ocimum sanctum	eugenol	L	A	Asha *et al.*, 2001
Taverniera abyssinica	phytoalexins	R	A	Stadler, Dagne & Anke, 1994
Terminalia macroptera	triterpenes	W	A	Conrad *et al.*, 1998

[1] Where specified
[2] Active principles evaluated
[a] Parts used (if specified): Fr = fruits, L = leaves, R = root, S = seeds, W = whole plant, Target (if specified): A = adult parasites

Table 2. In vitro *assay of plant preparations evaluated against various stages of ascarids (parasites of humans, pigs and chicken)*

Plant species	Active principles[1]	Parts used[1a]	Target [1]	Reference
Against *Ascaris lumbricoides*				
Acacia auriculiformis		F	E	El Garhy & Mahmoud, 2002
Albizia lebbek		B	E	El Garhy & Mahmoud, 2002
Apium graveolens		Sh	E	El Garhy & Mahmoud, 2002
Artemesia santonica	santonin	Sh	E	El Garhy & Mahmoud, 2002
Cassia obtusifolia		Sh	E	El Garhy & Mahmoud, 2002
Inula helenium	alantalactone	Sh	E	El Garhy & Mahmoud, 2002
Against *Ascaris suum*				
Carica papaya	benzyl isothiocyanate[2]	S	A	Kermanshai *et al.*, 2001
Mentha cordifolia	β-sitosterols[2], glucosides	L	A	Villasenor *et al.*, 2002
Against *Ascaridia galli*				
Carica papaya	benzyl isothiocyanate	S	A	Singh & Nagaich, 1999

[1] Where specified
[2] Active principles evaluated
[a] Parts used (if specified): B = bark, F = funicles, L = leaves, S = seeds, Sh = shoots
Target (if specified): A = adult parasites, E = eggs

Table 3. In vitro *evaluation of plant preparations against the nematode parasite* Heligmosomoides polygyrus

Plant species	Active principles[1]	Parts used[1a]	Target [1]	Reference
Albizia anthelmintica	embelin[2]	B	E A	Gakuya, 2001 Bøgh, Andreassen & Lemmich, 1996
Alstonia boonei		B	L_3	Fakae, Harrison & Sewell, 2000
Nauclea latifolia	alkaloids saponin	L	L_3	Fakae, Harrison & Sewell, 2000
Ocimum gratissimum	oleanolic acid	L	L_3	Njoku & Asuzu, 1998; Fakae, Harrison & Sewell, 2000
Piliostigma thonningii	tannins, alkaloids	B	L_3	Asuzu & Onu, 1993; Fakae, *et al.*, 2000

[1] Where specified
[2] Active principles evaluated
[a] Parts used (if specified): B = bark, L = leaves,
Target (if specified): A = adult parasites, E = eggs, L_3 = infective larvae

Appendix B

In vivo evaluation of plant preparations against cestodes and trematodes, ascarids of humans and animals, and nematode infections of rodents in Tables 4, 5 and 6, respectively

Table 4. In vivo *evaluation of plant preparations against cestode parasites in different host species*

Plant species	Parts used[a]	Active principles[1]	Parasite[b]	Host[c]	Reference
Tested against cestodes					
Albizia anthelmintica	RB	kosotoxin sesquiterpene	C	S	Gathuma *et al.*, 2004
Embelia schimperi	Fr S, R	embelin [2]	Hd, Hm Ts	R, M H	Desta, 1995; Bøgh, Andreassen & Lemmich, 1996
Ficus insipida *Ficus carica*	Lx	ficin	C	M	de Amorin *et al.*, 1999
Hagenia abyssinica	Fr	kosotoxin	C	H	Desta, 1995
Hildebrandtia sepalosa	B		C	S	Gathuma *et al.*, 2004
Mallotus philippinensis	Fr	rottlerin	C	G	Akhtar & Ahmad, 1992
Myrsine africana	Fr	benzoquinone	C	S	Gathuma *et al.*, 2004
Peganum harmala	S	tetra-hydro-harmine [2]	C	G	Akhtar & Riffat, 1986
Tested against trematodes					
Albizia anthelmintica	B		Fg	G	Koko, Galal & Khalid, 2000
Embelia schimperi	Fr	benzoquinone	Ec	M	Bøgh, Andreassen & Lemmich, 1996

[1] Where specified
[2] Active principles evaluated
[a] Parts used (if specified): B = bark, Fr = fruits, Lx = latex, S = seeds, R = root, RB = root bark
[b] Parasite (if specified): C = unspecified cestodes, Ec = *Echinostoma caproni*, F = *Fasciola gigantica*, Hd = *Hymenolepis diminuta*, Hm = *Hymenolepis microstoma*, Ts = *Taenia saginata*
[c] Host (if specified): G = goats, H = humans, M = mice, R = rats, S = sheep

Table 5. In vivo *evaluation of plant preparations against ascarid parasites in different host species*

Plant species	Parts used[a]	Active principles[1]	Parasite[b]	Host[c]	Reference
Artemisia maritima		santonin	Tv	B	Akhtar, Chattha & Chaudry, 1982
Caesalpinia crista	S		Tv	B	Akhtar *et al.*, 1985
Calliandra portoricensis	R		Tc	D	Adewunmi & Akubue, 1981
Carica papaya	S Lx		Ag As	Ch P	Satrija *et al.*, 1994; Singh & Nagaich, 1999
Cassia alata	S		Ag	Ch	Fernandez, 1991
Clitorea ternatea	S		Ag	Ch	Fernandez, 1991
Lansium domesticum	S		Ag As	Ch P	Fernandez, 1991
Leucaena leucocephala	S		Ag As	Ch P	Fernandez, 1991
Melia azederach	Fr		Ag	Ch	Akhtar & Riffat, 1985
Momordica charantia	St S		As Ag	P Ch	Fernandez, 1991
Moringa oleifera	S		Ag As	Ch P	Fernandez, 1991
Ocimum gratissmum	L	oleanolic acid	Ag	Ch	Njoku & Asuzu, 1998
Piliostigma thonningii	B		Ag	Ch	Asuzu & Onu, 1994
Quisqualis indica	St		As Ag	P Ch	Fernandez, 1991

[1] Where specified

[a] Parts used (if specified); B = bark, Fr = fruits, Lx = latex, L = leaves, R = root, S = seeds, St = stem

[b] Parasite (if specified): Ag = *Ascaridia galli*, As = *Ascaris suum*, Tc = *Toxocara canis*, Tv = *Toxocara (Neoascaris) vitulorum*

[c] Host (if specified): B = bovids, Ch = chickens, D = dogs, P = pigs

Table 6. In vivo *evaluation of plant preparations against nematode parasites in different rodent host species*

Plant species	Parts used[a]	Active principles[1]	Parasite[b]	Host[c]	Reference
Aloe barteri	L	anthraquinone	Nb	R	Ibrahim *et al.*, 1984
Annona senegalensis	L, B, R	anthraquinone	Nb	R	Ibrahim *et al.*, 1984
Anogeissus leiocarpa	B, S	anthraquinone	Nb	R	Ibrahim *et al.*, 1984
Cassia occidentalis	L	anthraquinone	Nb	R	Ibrahim *et al.*, 1984
Diospyros mollis		diospyrol [2]	Na Hp	GH	Sen *et al.*, 1974
Embelia schimperi	S, R Fr	embelin [2]	Hp	M	Bøgh, Andreassen & Lemmich, 1996
Ficus insipida	Lx	ficin	Ox	M	de Amorin *et al.*, 1999
Ficus carica					
Maerua edulis	Tb		Hp	M	Gakuya, 2001
Maerua subcordata					
Mangifera indica	B	mangiferin [2]	Ts	M	Garcia *et al.*, 2003
Terminalia avicennoides	L, R	anthraquinone	Nb	R	Ibrahim *et al.*, 1984

[1] Where specified
[2] Active principles evaluated
[a] Parts used: B = bark, L = leaves, Lx = latex, R = root, S = seeds, Tb = tuber
[b] Parasite (if specified): Hp = *Heligmosomoides polygyrus*, Na = *Necator americana*, Nb = *Nippostrongylus brasiliensis*, Ox = oxyurids, Ts = *Trichinella spiralis*
[c] Host (if specified): GH = golden hamster, M = mice, R = rats