ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

STUDY ON OGADEN SMALL RUMINANT HAEMONCHOSIS: MORPHOLOGICAL CHARACTERIZATION AND SUSCEPTIBILITY TO ALBENDAZOLE AND TETRAMISOLE

BY
BERSISSA KUMSA ESETA

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DEBRE ZEIT, ETHIOPIA

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A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in partial fulfillment of Degree of Master of Science in Tropical Veterinary Medicine

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DEDICATION

This Paper is dedicated to the memory of my father Ato KUMSA ESETA DINGO, who was absolute, reasonable and genuine father. Whom I like than every thing but missed due to unexpected sudden death on December 19, 2003. But I am left with his wise remarkable ideas, sayings, suggestions and quotes that have special area and with me throughout my life

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXES

ABRRIVAITATIONS AND ACRONYMS

µm	Micrometer
ABZ	Albendazole
ANOVA	Analysis of variance
BHO	Black head Ogaden sheep
CI	Confidence interval
Cm	Centimeter
DF	Discriminant Function
DVM	Doctor of Veterinary Medicine
Epg	Eggs per gram of faeces
FECRT	Faecal egg count reduction test
FECRT	Fecal Egg Count Reduction Test
GDP	Gross Domestic Product
GI	Gastrointestinal
GIN	Gastrointestinal nematodes
GIT	Gastrointestinal tract
ILCA	International center for Africa
Km	Kilometer
Lb	Lower bound
m	Meters
m.a.s.l.	Meter above sea level
+	
ml	Milliliters
MRD	Manufacturers recommendations dosage
MRD	Manufacturers Recommendation Dosage
pcv	Packed cell Volume
PhD	Doctor of philosophy
ppp	prepatent period
Rpm	Revolution per minute
TTM	Tetraamisole
Ub	Upper bound

GABA	Gamma-amino butyric acid
DMSO	Dimethylsulphoied
WAAVP	World Association for the Advancement of Veterinary Prarsitology

ABSTRACT

The current study was conducted from August 2003 to March 2004 with an attempt to determine the prevalence, morphological characteristics and susceptibility of Ogaden isolate of *H. contortus* to Albendazole and Tetramisole. During the study period a total of 196 animals (114 sheep and 82 goats) of Ogaden origin were examined. The overall prevalence of *Haemonchus species* was 91.23% and 82.93 % in sheep and goats respectively. Where as 37.72% and 40.24% prevalence of *Trichostrongylus. axei* was recorded in sheep and goats respectively. Statistically significant difference ($p < 0.05$) was observed between different months of the study period for both abomasal parasites. Out of 3187 female Haemonchus worms recovered from sheep for vulvar morph study, 49.49%linguiform, 28.51% knobbed and 23% smooth were recorded. Similarly from goats out of 2386 female Haemonchus, 53.83%linguiform, 18.45% knobbed and 27.61% smooth were recorded. Statistically significant difference ($p < 0.05$) was observed among the three major vulvar flaps between different months of the study period in both sheep and goats. A total of 1580 linguiform female Haemonchus from sheep were further classified and differentiated into 27.16%A, 14.80% B, and 5.34%C and 2.18%I. Similarly from goat a total of 1285 linguiform female Haemonchus were identified as 27.35 % A, 17.54% B, and 6.63% C and 2.31% I. With in the linguiform morphotypes, the A type linguiform was noted to exhibit monthly fluctuation ($p < 0.05$) during the study period. In the current study from a total of 76 sheep a total of 1159 adult male Haemonchus recovered and identified into 95.08% *H. contortus*, 3.45% *H. placei* and 1.47%*H. longistipes*. Similarly from a total of 55goats, 841 male Haemonchus were collected and identified as 96.55%*H. contortus*, 2.97%*H. placei* and 0.48 %*H. longistipes*. With regard to the distribution of mono and/or poly specific *Haemonchus species,* out of the 76 sheep examined 57.89% were harbouring *H. contortus* only, 22.37% *H. contortus* and *H. placei,* 7.89% *H. longistipes* and *H. placei* and the rest 11.84% were having *H. contortus*, *H. placei* and *H. longistipes*. As for goats, out of the 55 animals examined, 58.18% were found to harbour *H. contortus* mono-species, 38.18% *H. contortus* and *H. placei,* 3.64% *H. longistipes* and *H. placei* and unlike sheep none was found to harbour triple *Haemonchus species*. This result unveiled the coexistence and sympatry of two or three *Haemonchus species* in a single small ruminant host thus requiring the consideration of such heterologous hosts in the control strategy of the parasite. On the other hand, a total of 30 lambs were used for efficacy evaluation of Albendazole and Tetramisole in the controlled experimental study.

The lambs were divided into four treatment groups of five lambs in each group, one positive and one negative control groups also consisting of five in each group. Exiptol Greece, Albendazole Pakistan, Tetramsole Greece and Duxamintic Pakistan anthelmintics were evaluated by FECRT, controlled anthelmintic efficacy test and egg hatch assay test. Epg was observed to be strong indicator of induced infection in all the infected groups of animals showing statistically very significant difference ($p < 0.05$) between pre infection, post infection, pretreatment and post-treatment during the entire experimental study period. All the drugs were found to possess a 100% efficacy value up on evaluation by aforementioned efficacy detection techniques indicating also the susceptibility of Ogaden isolate *H. contortus* to all tested drugs irrespective of their origin. In view of the fact where helminthosis dominated by haemonchosis is confronting animal productivity of the study area, due attention to the disease and control interventions should be launched to minimize any economic loses and increase small ruminant productivity. Any control option that needs to be conducted in the study area should consider the coexistence of two or three species of *Haemonchus spp* in single host and involvement of heterologous hosts. The efficacy of the evaluated anthelmintics can only be maintained and conserved by wise and better utilization of the existing drugs to prevent the inevitable problem of anthelmintic resistance as the consequence of anthelmintic usage.

Key words: Sheep, goat, *Haemonchus spp*, Prevalence, Vulval morphs, Efficacy, Albendazole, Tetramisole, Ogaden, Ethiopia.

1 INTRODUCTION

Africa has a high population of 205 million sheep and 174 million goats representing approximately 17% and 31% of the world total respectively (FAO, 1990). Where as the population of sheep in sub-Saharan Africa is estimated at 127 million head, while that of goats is estimated at 147 million (Winrock, 1992). The arid and semiarid zones together hold the majority of sheep (57%) and goat (64%) population of Sub Saharan Africa. Indigenous sheep and goat breeds constitute over 95% of the small ruminant population of Africa. They are owned by the majority of smallholder rural farmers for whom this resource is critical for nutrition and income. Sheep and goats are highly adaptable to broad range of environments. They can utilize a wide variety of plant species and are thus complementary to cattle and camel production (Rege, 1992).

Ethiopia with its great variation in climate and topography possesses one of the largest livestock populations in the world, which is managed by smallholder farmer under extensive low input traditional management system and adjunct to crop production. The latest estimate gives 34 million cattle, 24 million sheep, 18 million goats, more than 8 million equines and 59 million poultry (ILCA, 1993). Of the total sheep population in Ethiopia, 75% are raised in the highlands with altitude above 1500 m.a.s.l. receiving more than 700mm of annual rainfall and sustaining 92% of the human population. The rest 25% are reared in the lowlands. Goats are widely distributed in all climatic zones but with a high concentration in dry areas. This is because they are well adapted to hot and dry conditions and mainly to the fact that in dry zones there is less opportunity for alternative land use (ILCA, 1988).

In spite of the huge small ruminant population in Ethiopia however, underdeveloped infrastructure coupled with poor management practices, low nutritional status and diseases considerably affects productivity. The share of helminthosis in this regard has been anticipated to tantamount the combined effects of other ill health. Available fragmented information revealed that infection due to *H. contortus*, *F. hepatica*, *O. columbianum*, *T. coulibriformis*, *D. filarial*, etc are responsible for the prevailing marginal productivity resulting from morbidities and mortalities in sheep and goats in different parts of the country (Bekele *et al.,* 1982; Tekelye *et al*., 1987). Losses from livestock production due to parasitic diseases especially helminths are very high both in developed and developing countries (Jovanovic *et al*., 1981). Parasites inflict harm to animals due to their pathogenecity, sharing nutrients and by predisposing them to other diseases (Ageymang *et al.,* 1981).

In Ethiopia helminth infections in ruminants are characteristically chronic and insidious in nature and have attracted very little attention, including research funds, when compared with viral, bacterial and some protozoal diseases. This is in spite of the fact that they undoubtedly exert a heavy toll on the heath and productivity of this vitally important livestock resource, with obvious implications for the rural and national economies of the country (Bekele *et al.,* 1982; Tekelye *et al*., 1987).

In livestock production, the use of antiparasitic drugs to control internal and external parasites is a widespread practice throughout the world. The number of domestically available broad-spectrum anthelmintic drugs has increased since the introduction of thiabendazole in the early 1960's. The main objective of using various chemotherapeutic agents is to control the adverse effects of parasites on the productivity of the host before the worm burden reaches a high level and the resistance of the host is compromised (Waller, 1993). Several anthelmintics with different modes of action are available in the market for the control of Helminthosis. However, the intensive use of these drugs to suppress infestation has resulted in rapid selection for resistance (Waller, 1993).

Currently, failure of anthelmintic efficacy due to anthelmintic resistance in sheep and goat nematodes is becoming a wide spread threat in Europe, Australia, South America and is of increasing importance in certain African countries like South Africa and Kenya (Bjorn *et al.,* 1991).

In Ethiopia, the use of anthelmintics in helminth control is known and has been going on for a quite long time, taking a considerable share in drug costs. Smuggling and improper use of veterinary drugs including anthelmintics is a widespread practice in the country. Although, anthelmintics are very commonly used in Ethiopia, and despite the great variety of drugs circulating in the market legally or illegally, almost no efficacy trials are conducted in proper and regular manner and no report is available as to the susceptibility or resistance of economically important parasites such as *Haemonchus contortus* to different chemical groups.

Hence the main objectives of the present study are:

- To establish the prevalence of abomasal parasites in small ruminants of Ogaden region of Ethiopia and study some morphological characteristics of *Haemonchus species* isolated from the study animals.
- To evaluate the susceptibility of Ogaden isolate *H. contortus* to Albendazole and Tetramisole drugs originating from different countries
- To underline the global emerging problem of anthelmintic resistance and forward appropriate recommendations.

2. LITERATURE REVIEW

2.1. Role of Sheep and Goat Production

Small ruminants have a great potential to affect the socioeconomic development of the majority of African rural communities. Increasing small ruminant production can boost farm income by generating cash income that can be used to purchase inputs for other production activities hence improves the quality of life of the people of the sub-Saharan Africa. Tropical Africa has a high population of small ruminants but productivity is generally low with low contribution to the total GDP (ILCA, 1988).

Small ruminant production is an important component of livestock production in Africa. Human diets will not contain necessary amounts and kinds of amino acids unless they include protein from either animal products or an unususally well designed combination of foods from plants (FAO, 1983; ILCA, 1988).

It is difficult to determine accurately the contribution of small ruminants to human food supply and general welfare. Sheep and Goats fulfill important functions in the life of the small mixed farmer. They provide meat, some milk, skin, and hair and wool, generate cash income and play traditional social and religious roles (Devendera and Mcleroy, 1982; Jahnke, 1982).

Estimates indicate that ruminants contribute 80% of the total food production from livestock in tropical Africa. Of this, small ruminants account for about 22%. It is estimated that ruminants supply over 3.2 million tones of meat per year, representing over 72% of the total meat production (Jahnke, 1982).

Small ruminant meat account for about 30% of the total red meat production and over 20% of the total meat out of Sub Saharan Africa (FAO, 1990). Small ruminants account for about 21% of the total milk produced in Sub-Saharan Africa. This is about 26%of the milk output from cattle in the region. On the basis of dollar values used by Winrock (1992) for meat monetary contributions of small ruminant to GDP (gross domestic product) were estimated to be 1286 million while milk output contributed about 1162million dollar.

Small ruminants are useful to humans during periods of cyclical and unpredictable food shortages. They also help balance the energy and protein supply during normal variations between seasons and years. The small size and early maturity of sheep and goats give them several distinct advantages in smallholder situations, such as they can efficiently utilize

marginal and small plots of land, the risk on investment is reduced by smaller individual size, allowing more production units per unit of investment; and there is a faster turnover of capital because they are sexually mature earlier and are younger at slaughter. Small ruminants appear to withstand drought better than cattle (Campbell, 1978).

Crop sales were limited to certain seasons, but the sale of small ruminant and milk was less season dependent. It therefore represents a dependable asset for emergencies. In general, livestock contribute to the stability of farm income because they can be bought following good crop performance and sold following crop failures. Livestock also serves as a currency in which social obligations are expressed (Ingawa, 1986).

Small ruminants contribute in soil fertility because their manure and urine is very essential components for maintaining agricultural products as only few farmers have access to commercial fertilizers (Rege, 1992).

Small ruminants facilitate utilization of marginal lands in that crop production is a high-risk enterprise in Sub-Saharan Africa in which about 13-16 million km ^{2} or nearly half of the continent is desert or arid grassland and savanna. Livestock farming is the only way to support any human life at all in much of the pastoralist system (Brown, 1971).

In the semiarid areas of Ogaden, which are non-agricultural potential, livestock, production is the main and only possible activity of the nomadic pastoralists. Among the livestock sectors on which the pastoralists of the Ogaden region make their livelihood, sheep and goats are the main cash income and play an important role (CACC, 2003). The majority of Ethiopia's sheep mutton export comprise of BHO (black head Ogaden) sheep that has a high demand in the world market. So BHO sheep plays great role both in domestic and foreign market trade.

2.2. Helminthosis as Constraints of Sheep and Goat Production

Health disorders in all classes of small ruminants represent a factor that greatly affects the economics of sheep and goat production. GIT parasitism together with an inadequate husbandry system is the main constraints to small ruminant production. Parasites impose severe economic constraints and are the major important causes of diseases and productivity

losses, particularly helminths infections are among the most prevalent and widely distributed ones (ILCA, 1992; Barger, 1982).

The most serious problem confronting sheep and goats production worldwide is infection with GIT nematodes. Parasitism ranks high among the factors that limit the productivity of small ruminants although its effect is often underestimated. Economic losses related to these parasites are caused by reduced performance which includes reduced weight gain, decreased production in meat and milk, poor wool growth, poor feed utilization, treatment and prophylaxis costs and mortality (Edwards *et al.,* 1976).

In the US alone, parasites cost the American livestock industry an estimated 2 billion dollar per year in lost productivity and increased operating expenses (Sonstegard and Gasbarre, 2001).

There are few reliable estimates of the economic importance of GIT helminths in Africa and Ethiopia. Herlich (1978) reported a loss of more than 30 million sheep and goats throughout the world because of helminths. Akerejola *et al.* (1979) have estimated that the losses from parasitic diseases in Nigeria's sub humid zone are higher than those attributed to PPR and he reported Helminthosis was the most encountered and prevalent disease in the area and he estimated the loss because of helminth infection in sheep and goats in Nigeria to be NGN69 million per year. Also Rey (1991) indicated 11% of the marketable animals were lost because of endoparasitism in Nigeria. Assoku (1980) studied the helminths of sheep and goats on the Accra plains of Ghana and he found that 80% of the sheep and 83% of the goats were infected. In Kenya in most areas Haemonchus is identified as the predominant nematode species followed by Trichostrongylus species and in 1976, Haemonchosis alone was estimated to cause an annual loss of approximately 26 million dollar in sheep and goat production (Allonby, 1976).

Accurate and up to date estimates of its socioeconomic impact are lacking in Africa and Ethiopia but economic losses are believed to arise through poor growth rate and feed conversion, reduced meat and milk yield, carcass and offal condemnation, impaired reproductive efficiency and, in some localities loss of draught power (Teklye *et al.,* 1982).

Although quantitative data on direct and indirect losses due to helminth parasites in ruminants in Ethiopia is meager, available information indicates that the parasites occur in all ecological zones and production and economic losses may be high due to both clinical and chronic sub

clinical infections. Graber (1975) indicated that helminths are responsible for reduced productivity of animals by imposing losses every year that is difficult to number but probably very high. Mulugeta *et al.,* (1989) reported yearly losses of 700 million Birr due to endoparasites in Ethiopia. In highland Ethiopian sheep body weight losses due to endoparasitism ranged from 3-8% (Teklye *et al.,* 1982) and caused 28% mortality. The problem of parasitism is compounded by the fact that under the traditional system, livestock are usually reared extensively, which increases infestation and makes control measures difficult. Young animals are the most affected and parasitism could aggravate other conditions such as nutritional stress and susceptibility to other diseases.

2.3. General Overview on Helminths

Helmins or *helminthos* is the Greek word for worm, and host is derived from the Latin *hospes*, a house or household. Helminths of veterinary importance belong to four Phyla: Nemathelminths, Platyhelminths, Acanthocephala and Annelida, of which the most important ones belong to the first two phyla. The important classes of helminths belong to the class of *Nematoda*, *Cestoda* and *Trematoda* (Reinecke, 1983).

Nematodes (round worms) are unsegmented, hair like, tubular worms ranging in size from a few millimeters to several centimeters. Nematodes are a group of worms, which are responsible for most of the helminth diseases of veterinary importance, and tissues or organs of every class of vertebrates and even some invertebrates are vulnerable to invasion by them (Brander *et al*., 1991; Dunn, 1978; Reinecke, 1983).

Trematodes (flukes) are non-segmented flat worms, nevermore than a few centimeters in length, which parasitize the organs and tissues of vertebrates. Their life cycles always involve more than one host in which diverse larval forms are capable of multiplying asexually (Brander *et al*., 1991; Dunn, 1978; Reinecke, 1983).

Cestodes (tapeworms) are flat, segmented, ribbon like worms, the adults of which may be a few millimeters or several meters in length and live exclusively in the small intestine, with few exceptions completion of the life cycle requires that their larval forms develop in intermediate hosts (Reinecke, 1983; Soulsby, 1986; Brander *et al*., 1991).

Gastrointestinal nematodes of the order Strongylida are the most common cause of clinical Helminthosis, which is caused by the infection of digestive tract due to the presence and development of nematodes in the wall or the lumen of the abomasums, the small intestine, and /or large intestine (Brander, *et al.* 1991; Bowman, 1995). The infestations are the single most important cause of losses, in both death and poor growth, in countries where ruminants are kept on pasture the whole year and the disease they cause is characterized by persistent diarrhea and wasting (Radostits *et al.,* 1994).

The trichostrongyoid nematodes are small, often hairlike worms in the bursate group, which are especially common and pathogenic in grazing ruminants, but horses, swine, cats, and birds also host important species. The abomasums and small intestine are the usual locations in ruminants, but Dictyocaulus reaches maturity in aberrant locations in the air passages (Dunn, 1978, Soulsby 1986; Bowman, 1995). Structurally they have few cuticular appendages and the buccal capsule is vestigial. The males have a well-developed bursa and two spicules, the configuration of which is used for species identification. The life cycle is direct and usually non migratory and the ensheased L_3 is the infective stage (Soulsby, 1986; Radostits *et al.,* 1994).

The trichostrongyloids including Dictyocaulus are responsible for considerable mortality and widespread morbidity, especially in ruminants. The most common gastrointestinal nematodes of small ruminants, which are very pathogenic and economically important ones belong to the following genera: *Haemonchus*, *Trichostrongylus, Ostertagia, Oesophagostomum, Bunostomum, Cooperia, Nematodirus, Marshallagia, Mecistocirrus, Hyostroyngylus, Strongyloides* and *Trichuris* (Dunn*,* 1978; Soulsby, 1986; Brander *et al.,* 1991; Aeillo and Mays, 1998*).*

In all of the genera the life cycle is direct with out requiring an intermediate host. The eggs are passed in the faeces and under suitable environmental conditions hatch, producing two successive non-parasitic larval stages and the 3rd infective larvae. Ingesting the 3rd larvae while grazing leads to infection in ruminants. The L_3 of the trichostrongyle group penetrate the mucus membrane (in the case of *Haemonchus* and *Trichostrongylus*) or enter the gastric glands (in the case of *Ostertagia*). During the next few days the L_3 moult to the 4th stage L_4 and remain in the mucous membrane or in the gastric gland, for about 10-14 days. Then they emerge and moult into the young adult stage L_5. Most trichostrongyle mature and start egg production at about 3 weeks after infection. The infective larval stage of L_3 of the

Oesophagostomum penetrate lumina properia of the intestinal wall and the host response to the infection which surrounds the L_3 result in the formation of fibrous nodule (Soulsby, 1986; Radostits *et al.,* 1994; Urquhart *et al*., 1996).

The transmission, incidence and intensity of infections are determined by several environmental, host and parasite dependent influences and phenomena; the most dominant of which are, meteorological factors, methods of husbandry and systems of livestock production; host age, nutrition and acquired immunity; larval hypobiosis and concurrent infections (Ogunsusi, 1979).

In most natural infestations a mixture of genera and a species is found but in most districts one species is of greater importance. The worms are predominantly parasites of young animals but in small ruminants the most commonly affected age groups are weaned lambs and yearlings. Important predisposing factors are high pasture contamination, malnutrition and overcrowding. In all animal species the disease is of most importance when the plane of nutrition is low but massive infestations can overwhelm well-fed animals. Moderate infestations can be borne by animals on good feed while poorly nourished animals may succumb to death (Radostits, *et al.,* 1994).

The effect of infestation will depend on the species present, their location and numbers; anorexia is important feature of all of the diseases. Some of the effects includes local effects on digestion, absorption and protein loss will depend on the organ affected and will interact with loss of appetite. Villous atrophy, plasma loss in to the intestine from increased vascular permeability, discontinuity of the epithelium, intestinal inflammation resulting in secretion of biogenic amines and loss of plasma, increase in catabolism of muscle protein and synthesis of the plasma protein, reduction in absorption of phosphorous and increase the loss of endogenous phosphorous leading to phosphorous deficiency, reduction in acid production and rise in abomasal P^H to 4, loss of copper from the bowel which exacerbate a marginal copper deficiency, superficial erosion of mucosa, hyperemia, edema, and diarrhea (Radostits *et al.,* 1994; Urquhart *et al*., 1996).

In Ethiopia wide surveys have been conducted in area of small ruminant gastrointestinal Helminthosis. All reported high prevalence as indicated in Annex 1.

2.3.1. Haemonchus

The genus is among the largest in the super family ranging from 10-30mm in length. In fresh specimens the worms can be easily seen due to their bright red colour and considerable size. In both sexes there are a pair of wedge shaped cervical papillae in the esophageal region and a tiny lancet inside the buccal capsule used for piercing small blood vessels (Dunn, 1978; Bomann, 1995; Kaufmann, 1996).

In fresh specimens the most obvious feature in females is that the white egg filled uterus winding spirally around the blood filled intestine-giving rise to the so-called barber's pole effect. The vulva is located about a quarter body length from the tail and may or may not be guarded by variously shaped cuticular inflations (vulvar flaps). The form of the vulvar flap may range from an extreme Linguiform shape to a knob shape or a complete absence (Linguiform, knobbed or smooth). The prevalence of these various vulvar flap configuration varies among species and subspecies (Soulsby, 1986; Bowman, 1995; Urquhart *et al.*, 1996).

In males the bursa is large enough to appear to the naked eye as a little eyelet on the end of the worm. The most important diagnostic features are the barbed short and wedge shaped spicules and asymmetrically placed small dorsal lobe and small lateral lobes (Dunn, 1978; Reinecke, 1983; Soulsby, 1986).

The species of Haemonchus that are so far known to infect ruminant animals are: *H. contortus* is the species most commonly found in sheep and goats and it can also be found in cattle when these animals graze the same pasture. *H. placei* is the usual *haemonchus species* in cattle and it can also develop well in sheep and goat and causes clinical disease but causes less severity than that caused by *H. contortus*. *H. longistipes* is the species that usually affects camels and dromedaries and it can also develop in other animals. *H.similis* is the one that usually affects cattle and deer in some countries and it can also affect other animals (Soulsby, 1986; Radostits *et al.,* 1994; Urquhart *et al*., 1996).

Infections with *Haemonchus Species* are a major constraint on domestic ruminant production throughout the world. *H. placei* and *H. contortus* are the most important species in cattle and sheep respectively (Lichtenfels *et al*., 1986).These parasites mainly affect the abomasal mucosal of their hosts. Adults and the late L_4 stage larvae ingest blood which causes severe anaemia. Heavy infections can result in the death of the host (Eysker and Ogunsusi, 1980).

Haemonchus is a voracious blood sucking abomasal nematode and is responsible for extensive losses in sheep, goat and cattle especially in the tropics. Haemonchosis is for the most part a primary parasitosis, predisposing causes for infestation including overcrowding lush pasture and hot humid climatic conditions. However, development of clinical illness favored by a fall in plane of nutrition particularly in young animals (Radostits *et al.,* 1994). Haemonchus is the most common in tropical and sub tropical areas or in those areas with summer rainfall, while *T.axei* and Ostertagia are more common in winter rainfall areas.

Haemonchosis is characterized by cardinal signs like anemia, which causes pallor of the skin and mucous membranes and a haematocrit reading of less than 15%, generalized edema due to loss of plasma proteins and progressive weight loss. But diarrhea is not a common feature of haemonchosis; the lesions are those associated with anemia. The abomasa become edematous and, in the chronic phase the p^H increase causing gastric dysfunction (Bowman, 1995; Urquhart *et al*., 1996).

At peak infection, naturally acquired populations of Haemonchus contortus can remove one fifth of the circulating erythrocyte volume per day from lambs and may average one tenth of the circulating erythrocyte volume per day over the course of nonfatal infections lasting two months. Thus each worm removes about 0.05ml of blood per day by ingestion and seepage from lesions so that a sheep with 5000 *H. contortus* may lose about 250ml of blood daily. The pathogenic effect of *H. contortus* result from the inability of the host to compensate for blood loss (Radostits *et al.,* 1994; Urquhart *et al*., 1996). The spectacular depression of HB level, accompanied by weakness and death, are the classical features of Haemonchosis. The anaemia of Haemonchosis is generally considered to be moderately macrocytic normochromic in nature (Allonby and Urquhart, 1975).

Observation of a phenomenon called self-cure is found to be the characteristic feature of Haemonchosis in endemic areas in which the major part of the adult worm burden is expelled resulting in sharp drop in epg to near zero after the advent of a period of heavy rain (Urquhart, 1996).

2.3.2. Trichostrongylus

The adults are small and hairlike, slender, usually less than 7mm in length and difficult to see with the naked eye. They are pale reddish-brown worms without a specially developed head end and no buccal capsule. A most useful generic character is the excretory pore is usually situated in a distinct conspicuous ventral notch in the esophageal region (Reinecke, 1983).
In females the most useful characteristics are their small size, double ovijectors, and the absence of accessory structures at the head and vulvar region. The vulva opens short distance behind middle of the body, and has amphidelphic uteri (Bowman, 1995).

In males the bursa has long lateral lobes, while the dorsal lobe is not well defined. The ventroventral ray of the male bursa is separated widely from the others and is conspicuously thinner than the lateroventral, which runs parallel with the lateral rays. The dorsal ray is slender and cleft near its tip in to two branches, which have short digitations. The spicules are usually pointed, short, stout, ridged or twisted and pigmented brown, a spindle shaped gubernaculum is present (Reinecke, 1983, Soulsby, 1986; Urquhart *et al.*, 1996).

T. axei is the smallest member of the genus and parasitizes the simple stomach of ruminants or abomasums of a wide range of hosts including ruminants, horses, and leporids where as the other species of trichostrongyles are parasites of the small intestine of ruminants and display a higher order of host specificity (Bowman, 1995).

In *T.axei* the spicules are unequal in length, the left being longer than the right. Both spicules end bluntly, are rounded at the tip and have a tongue like semitransparent membrane projecting from the tip. In female *T.axei* vulval flap is not present, gravid worms contains only up to 5 eggs arranged pole to pole and the cone shaped tail ends bluntly (MAFF, 1979; Soulsby, 1986; Urquhart *et al.*, 1996).

2.3.3. Effects of parasites on their hosts

Parasites exert their effect on the host through various ways. Some of the effects to be cited include:

- Competition with host for food, which result in decreased utilization by host (Peter, 1989).

- Suck blood often causing heavy blood loss, anemia and death e.g. Hookworm, *Haemonchus* (Brander *et al.,* 1991).
- Destruction of tissues during larval migration e.g. Liver fluke, *Ascaris, hydatid* cysts (Brander *et al.,* 1991; Peter, 1989).
- Transmission of infectious diseases e.g. *Histomonas meleagridis* transmitted by *Heterakis* (Peter, 1989).
- Embolism of blood vessels resulting in infraction, necrosis, colic and death e.g.Strongyles in horses (Urquhart *et al.,* 1996).
- Mechanically obstruct different organs e.g. flukes*, ascardis*, lungworms, adult *cestodes* (Brander *et al.,* 1991; Peter, 1989).
- Cause hypersensitivity and immune suppression e.g. *Oesophagostomum* (Urquhart *et al.,* 1996).

2.4. Control of Helminths

The evolution of the means and methods to control nematode parasites of livestock is known to have a very long history, centralizing itself around chemotherapy (Waller, 1993). However, the threats of resistance, residue and ecotoxicity have lead some to believe that sun is now setting on the chemotherapeutic era, as far as parasite control of livestock is concerned (Waller, 1991; Barger, 1996). Therefore, today the strategy of helminth control has shifted to the notion of integrated parasitic control scheme, which encompasses the interrelated approaches of grazing management, utilization of natural or artificially induced immunity and use of anthelmintics

2.4.1. Grazing Management

Grazing management that involves epidemiological control of nematodes by preventing the build up of large number of larvae on the pasture is useful to reduce the number of anthelmintic treatment. In this sort of grazing management (clean grazing), treated animals

are moved to pastures of lower infectivity, pasture not used the previous year by farm animals. This is usually based on the knowledge of population dynamics of nematode parasites whilst in host or pasture (Taylor *et al.,* 1991). Alternative grazing of livestock species e.g. sheep and cattle is also one way of reducing infective larvae from pasture. This is mainly due to the little consequence of cross infection between these two species.

2.4.2. Utilization of Natural or Artificially Induced Immunity

Infection with GIT nematodes and exposure to their numerous antigens generates a complex immune response in the host both against ingested larvae and the resident adult (Miller, 1984).

The genetic resistance of host which is also the ultimate sustainable parasitic control, particularly for resource poor farmers is the most important merit which have to be exploited This is because the need to reduce dependence on chemotherapy is now urgent; but most approaches still require sustained research effort (Donald, 1994).

Genetic resistance: Genetic resistance of hosts to endoparasites can influence the epidemiology by reducing the number of eggs shed and also reducing the frequency of anthelmintic treatment (Graber, 1978; Gruner, 1991). Host genetic resistance is a potential helminths control method. The immune response of some individual animals to infestation by a given parasite is better than that of others within the same population. Also, certain population and breeds tolerate a given parasitosis better than others. Under normal condition this is due to the result of natural selection in endemic areas, but recent observations indicate that there exist genes responsible for resistance to parasites. A relative tolerance to helminth infestation exists, for instance, helminth and trypanotolerant N'Dama cattle of West Africa, helminth resistant Red Masai of East Africa and the St. Coroix and Barbados Black Belly sheep of the Caribbean are well studied (Uilenberg, 1996). The introduction of known resistant genotypes is the fastest route of success. Research works in the selection of nematode resistant hosts have demonstrated that the heritability of resistance is mostly moderate to high (Windon, 1990).

Vaccination: Substantial progress toward successful vaccination against animal parasites has been less readily forthcoming than prophylaxis against bacterial and viral infections of veterinary importance (Emery, 1996). There are only few examples of vaccines used against parasitic diseases. As far as helminths are concerned, there is a commercial vaccine against

lungworm of cattle *(Dictyocaulus viviparus) and Ancylostoma caninum dogs* using irradiated infective larvae (Radostis, 1994). Immunization trials using brood capsules of *Ecchnicocus granulosus,* and preparations from *Haemonchus contortus* and *Trichostrongylus coulbriformis* confer different degree of protection against challenge infestation. Irradiated forms of *Schistosoma bovi,* S*chistosoma mattheei* and *Schistosoma mansoni* proved to confer resistance in experimentally infected animals (Sheelagh, 1981*)*

2.4.3. Use of Anthelmintics.

Anthelmintic treatment is the favorite and very common method of keeping worm burdens at a minimum level in ruminants in most countries of the world. The effect of anthelmintic treatment is manifested in many ways, such as enhanced growth rate, reproductive performance and wool production (Darvil *et al*., 1978).

Protective dosing with anthelmintics has become an important part of most preventive program against clinical or sub-clinical parasitic disease. Two usual classes of treatment programmes are strategic treatments carried out at the same time each year, or at the same stage in the management program, with the specific purpose of reducing contamination; and tactical treatments which are added to the strategic particularly in pastured animals, to abort outbreaks when abnormal climatic or nutritional conditions arise (Radostis *et al.,* 1994).

The control of gastrointestinal nematodes in general is at present dependent on the repeated use of anthelmintics and, where possible pasture management. However, clean pastures are not readily available under intensive grazing conditions and, perhaps more importantly, there is an increasing occurrence of parasites resistant to the action of anthelmintics (Borgsteede *et al*., 1996). Further more there are concerns regarding drug residues in animal by products like meat and milk, and the environment.

The increasing occurrence of anthelmintic resistance (Jakson, 1993) has promoted the need for the development of alternative methods of nematode control options like vaccine production, host genetic resistance, and biological control. Despite quite significant advances in some areas of non-chemotherapeutic control, non- is sufficiently advanced to offer any practical alternative until possibly the turn of the century (Waller, 1993). Therefore, chemotherapy will continue to be an important part of efficient systems well into the future (Donald, 1994).

In spite of the availability of the highly efficacious and safe anthelmintic drugs gastrointestinal nematode infections remain a major constraint to the efficient raising of ruminant livestock through out the world (Sonstegard and Gasbarre, 2001). The efficacy of anthelmintics can only be conserved and the effective field life of these drugs is extended by relying on better use of the available anthelmintics preferably in combination with management strategies, because new drugs with different mechanism of action or novel means of control are unlikely to appear soon on the market (Borgsteede *et al*., 1996).

2.5. General Description of Anthelmintics

An anthelmintic is a compound, which either destroys or eliminates helminth from the host (Reinecke, 1983). Modern anthelmintics belong to the following chemical groups: benzimidazole compounds, imidazothiazole derivatives, tetrahydropyrimidines, piperazine and its salts, macrocyclic lactones and phenothiazine and others (Aiello and Mays, 1998). The major groups of anthelmintics currently in use against nematodes, trematodes and cestodes are shown in the following table (Table 1).

Table 1: The major groups of anthelmintics

Parasites	Chemical Group	**Drugs**
Nematodes	Piperazine	Piperazine salts, Dietyhylcarbmazine
	Imidazothiazoles/Tetrahydropyrimidines	Tetramisole, Levamisole, Morantel, Pyrantel
	Benzimidazoles/ pro-benzimidazoles	Thiabendazole, Mebendazole, Parbendazole, Fenbendazole, Oxfendazole, Albendazole, Oxibendazole, Cambendazole, Flubendazole, Febantel, Thiophanate, Netobimin
	Avermectins/ Milbemycins	Ivermectin, Dormactin, Abamectin, Moxidectin, Milbemycin
	Organophosphates	Dichlorvos, Haloxon, Trichlorfon (Metriphonate)
	Salicylanilides/Substituted phenols	Nitroscanate, closantel
Trematodes	Salicylanilides/Substituted Phenols	Nitroxynil, Rafoxanide, oxyclozanide, Brotianide, Diamphenethide, Niclofolan, Colsantel
	Others	Clorsulon
	Benzimidazoles/ Probenzimidazoles	Triclabendazole, Albendazole, Netobimin
Cestodes	Salicylanilides/Substituted phenols	Niclosamide
	Others	Praziquantel, Bunamidine, Arecoline

Source: Urquhart *et al.,* 1996

2.5.1. Modes of Action of Anthelmintics

The mode of action of many anthelmintics is not known in detail, but basically depends on interference with essential biochemical process of the parasite like energy production and paralysizing the worms (Kapalan, 2002).

Benzimidazoles and pro-benzimidazoles: Drugs of this group cause degeneration of intestinal cells of helminths by binding to the structural protein tubulin and prevent its polymerization into microtubules, which are important for cellular transport. Susceptible parasites are unable to absorb and transport nutrients like glucose and amino acids and will eventually starve to death. Furthermore these drugs inhibit fumarate reductase in the parasites, which serves as terminal electron acceptor in the generation of ATP (Ngomu and Gryd-Hansen, 1986; Urquhart *et al.,* 1996).

Imidazothiazoles and tetrahydropyrimidines**:** These compounds act as depolarizing neuromuscular blocking agents in helminths by stimulating cholinergic receptors at the ganglionic level, leading to continuous muscular contraction (Ngomuo and Gryd Hansen 1986).

Salicylanilides and substituted phenols: Although details of the modes of action of drugs in these groups are not well understood, they appear to act by uncoupling the oxidative phosphorylation in the mitochondria thus blocking ATP-synthesis in the worms (Ngomuo and Gryd-Hansen, 1986; Urquhart *et al.,* 1996).

Avermectins: They act by potentiating the release and binding of gamma-aminobutyric acid (GABA), which increases the normal resting potential of postsynaptic cells making neurotransmission more difficult or impossible. Thus muscle cells cannot contract (Ngomuo, and Gyrd -Hansen 1986).

Piperazine: These drugs produce paralysis in helminths by an anticholinergic action at the neuromuscular junction (Urquhart *et al.,* 1996).

Organophosphates: They act by inhibiting cholinesterase resulting in a build up of acetylcholine, which leads to neuromuscular paralysis of parasites and their expulsion (Urquhart *et al.,* 1996).

2.5.2. Desirable Characteristics of Anthelmintics

An ideal anthelmintic should posses the following properties:

- A broad-spectrum activity against adult and larval helminth parasite: a number of factors influence the efficacy of an anthelmintic drug. Animals often harbor several different species of helminth, which may not have the same sensitivity to a given anthelmintic. In addition there is usually a difference in sensitivity between adults and larval stages, with immature stages being less sensitive than adult parasites. (Hazelby *et al.,* 1994).
- A rapid metabolism in the body and short-lived presence at low levels in the milk and/or tissues: animals shouldn't be slaughtered for human consumption and milk not distributed to consumers until the drug residues have reached acceptable low levels. The withdrawal period of the drug should be considered before its use (Hansen and Perry, 1994).
- A low toxicity in the target species. The ratio of the therapeutic dose to the maximum tolerated dose should be as large as possible. It is desirable that anthelmintic has a safety margin of at least six fold (Aiello and Mays, 1998).

- No unpleasant side effects to the animal or to the operator- drugs may cause vomiting, or pain at the injection site. (Hansen and Perry, 1994).
- Suitable for practical and economical integration into various management systems: the selected drug(s) should be competitively priced and ready to use in a simple way. They should be stable and not decompose on exposure to normal ranges of temperature light and humidity, and have a long shelf life. (Urquhart *et al.,* 1996).

2.5.3. Problems Associated in the use of Anthelmintics

It is now generally accepted that a total reliance on anthelmintics to control nematode parasites of livestock is no longer tenable. Despite the extremely high level of efficiency of modern broad-spectrum drugs, eradication (except in very few instances) has not been achieved. It is a sobering fact that the more effective the anthelmintic treatment, the greater the potential for development of resistance (Prichard *et al.,* 1980).

Other undesirable consequences of intensive treatment are the accumulation of drugs in animal products and possible untoward effect on non- target organisms in the environment (Waller, 1993). However, in the case of most anthelmintics, residues are not major problems since their mammalian toxicity is low and treatment close to slaughter is unlikely (Donald, 1994).

The benzimidazoles and their prodrugs are subjected to close scrutiny because some are known as teratogens at doses, which are not maternotoxic. The other and important effect associated with the use of anthelmintic is its effect on the environment, which has become in certain instances a disaster to the ecology (Donald, 1994).

Large amounts of phenothiazine fine or its metabolites were considered responsible for a decline in clover content, which in turn resulted in a reduction in pasture growth and animal productivity. Apart from the ivermectin, which was shown to have adverse effects on the primary dipteran decomposing fauna, the benzimidazoles, which are excreted largely, unchanged in feces (ex oxfendazole and fenbendazole) are likely to have residual effect on saprophytic fungi that invade feces and assist in nutrient recycling (Waller, 1993).

2.6. Anthelmintic Resistance

2.6.1. Over View on Anthelmintic Resistance

Resistance as defined by Prichard *et al* (1980): in (Coles *et al.,* 1992), is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and is heritable. Anthelmintic failure could be due to resistance or lack of potency. Resistance is considered to have been selected when previously effective drug ceases to be so, due to selected heritable changes in the exposed population (Jackson, 1993).

Resistance of worms to anthelmintic treatment was first reported from the USA in 1954 (Drudge *et al*) when evidence of phenothiazine resistant *Haemonchus contortus* was found. Ten years later strains of *Haemonchus contortus* resistant to thiabendazole were detected in sheep only three years after this product was launched. Since then thiabendazole resistance has been demonstrated with *Trichostrongylus colubriformis, Trichostrongylus vitrinu*s, *Oesophagostomum circumcincata* and species of *Nematodirus (*Brander, *et al.* 1991).

In spite of the availability of large number of effective drugs in the anthelmintic armamentarium, gastrointestinal nematodiasis still continues to be a menace in ruminants, particularly goats and sheep. Treatment failures and anthelmintic resistance is a growing problem in certain geographical areas and some host species (Sonstegard and Gasbarre, 2001). Waller (1987) pointed out that most of the nematodes of domestic animals posses the capacity to develop resistance to anthelmintics. Resistance to antiparasitic drugs in sheep and goats is rapidly increasing particularly in warm and humid climatic regions probably due to frequent dosing and adoption of common managemental, nutritional and therapeutic strategies. Furthermore the difference in anthelmintic metabolism and degree of rumen bypass between sheep and goats should be taken into account so that higher doses are required for goats compared to sheep. There fore, apart from other factors, sub-optimal dosing is considered to be one of the important factors for drug resistance in goats (Coles *et al., 1989*).

Resistance to anthelmintics is a widely described phenomenon in ruminants. Some nematode species and some ruminant species are more prone to develop resistance. An index for nematode resistance was built up and the following ranking of the host was goat > sheep > horses > cattle. A similar ranking of nematode was *H. contortus* > *Teladorsagia circumcincata* > Trichostrongylus species > Oesophagostomum species > Cooperia species,

among which only Cooperia was recorded mainly in cattle. In small ruminants resistance is mainly directed against benzimidazoles in 43% of the cases and then against each of levamisole or macrocyclic lactones in 23% of the cases (Coles, 2002).

Continued application of a highly effective anthelmintic selectively removes most susceptible genotypes, with the resultant progeny of succeeding generations being composed of resistant strains. Resistance to an anthelmintic is expressed by passage of increased numbers of parasite eggs, higher establishment rates of adults in the host, and greater numbers of larvae on the pasture after treatment than would occur if the parasites were susceptible to the drug (Aiello and Mays, 1998). The development of resistance to various chemical groups of anthelmintics by nematodes is recognized as a major problem. Until relatively recently, resistance to anthelmintics in nematodes had been slow to develop under field conditions in comparison with antibiotic resistance in bacteria. And more recently the problem of anthelmintics resistance is reported from different parts of the world to different chemical components (Aiello and Mays Asa, 1998). Resistance of *Haemonchus species* is becoming a global problem. Resistance of *Trichostrongylus* and *Ostertagia* species in sheep and goats is also becoming common in all parts of the world where small ruminants are treated frequently. Resistance of small *Strongyles* in horses has also been documented in many areas. Resistance to benzimidazole and levamisole has also been reported in nematodes of swine. Although resistance to benzimidazoles, levamisole, and recently to macrocyclic lactones have all been reported for nematodes of cattle, resistance is less of a problem in cattle than in sheep, goats, and horses (Aiello and Mays, 1998).

Cross-resistance is frequently seen between members of the benzimidazole group because of their similar mechanisms of action. Control of benzimidazole resistant parasites by levamisole can be expected because of its different mode of action (Aiello and Mays, 1998).

2.6.2. Factors Responsible for Anthelmintic Resistance

Several factors can be responsible for the lack of efficiency of a drug and development of anthelmintic resistance of which the following can be mentioned:

Underdosing: Most farmers usually estimate (guess) the weight of their animals and many surveys have shown that such estimates are often considerably below the actual weight. In addition farmers often use the average weight to establish the dose. This automatically results in under dosing (Aiello and Mays, 1998).

Prolonged use of same anthelmintic drug: Frequent and regular treatment using the same anthelmintic given at low dosages over a prolonged period of time will predispose to the development of drug resistance (Hansen and Perry, 1994).

High frequency of treatment: If treatment intervals approach the prepatent period of parasites the selection pressure increases dramatically and the entire parasite population will be screened for resistant individuals and eventually resulting in a highly resistant population (Waller, 1987).

Animal management practice: Such as drenching accompanied by movement of stock to clean pastures and using anthelmintic treatment at very high frequency can lead to resistance. Farming enterprises in countries such as Australia, South Africa, and Brazil, which have a high resistance problem, largely practice continuous grazing on permanent pastures (Waller, 1987).

2.6.3. Mechanisms of Anthelmintic Resistance

The mechanisms by which parasite resistance develops are not completely understood (Smith and Sherman, 1994). Most anthelmintics are non-persistent chemicals with peak concentration occurring from 30 minutes to 24 hours after treatment (Prichard 1978). The recommended dose rate of anthelmintics generally removes 95-99% of the susceptible population but there usually remain a small number of survivors. The survivors probably have the capacity for the detoxification of chemicals, which will form the basis of resistance and will be inherited by their progeny (Le Jambre, 1982). In the presence of continuous and frequent anthelmintic treatment, subsequent generations of worms will inherit more and more resistance alleles leading to a resistant population.

In the case of benzimidazole class of drugs the possible mechanism of anthelmintic resistance is the association with point mutations in the β-tubulin genes (Ouellette, 2001). Most cases of resistance against the benzimidazoles appears to be due to changes in the β-tubulin isotype pattern which results in the loss of high affinity receptors binding sites (Lacey and Gill, 1994). Studies using *H. contortus and T.colubriformis* have shown a common point mutation at aminoacid 200 in isotype 1β tubullin from resistant isolates. Resistant isolates have tyrosine at this locus where as susceptible have phenylalanine. It has been suggested that there may be two mechanisms that associate with resistance to the benzimidazoles, the selection of an

isotype 1β-tubulin with a reduced affinity for the benzimidazoles and the elimination of isotype 2 β –tubulin genes from highly resistant individuals (Conder *et al*., 1998).

In the case of Ivermectin the mechanism of resistance is due to the fact that these drugs opens worm's chloride channels that lead to starvation or paralysis. A p-glycoprotein homologue may be responsible for ivermectin resistance in a number of worm genuses, which is reasonable, as ivermectin is an excellent substrate of the mouse MDIA p-glycoprotein. In the nematode *Caenorhabditis elegans,* simultaneous mutation of three genes encoding glutamate-gated chloride channel α-type subunits confers high-level genes resistance to ivermectin suggesting that both target mutation and transport alternation can lead to ivermectin resistance in worms (Quellette, 2001).

Resistance against levamisole in *H. contortus* and *T.colubriformis* appears to be due to alterations in drug pharmacokinetics at the ACH receptor. Levamisole resistant *H. contortus* have more binding sites and different drug affinities at the low affinity binding sites than susceptible *H. contortus* and are thus desensitized against the action of levamisole (Sangster, Riley and Wiley, 1998). Levamisole acts as a cholinergic antagonist in nematodes however; the resistance mechanism to this class of drugs as described by Ouellette (2001) is that reduction in the number of receptors has also been proposed as one possible mechanism for resistance.

2.6.4. Detection of Anthelmintic Resistance

Helminth resistance should be differentiated from failure of treatment for other reasons such as rapid re-infection, use of inappropriate anthelmintic, use of too low does, presence of larval stages that are not affected by the anthelmintic, or reduced efficiency due to certain disease conditions (Prichard *et al.,* 1980).

Reliable methods are required in order to detect resistant helminths population in livestock, monitor changes in levels of resistance, and identify affected animals in order to restrict the dissemination of resistant parasites. Several in vivo and in vitro tests have been developed to assay for resistance in nematode populations (Jackson, 1993). There are a number of tests that can be used to evaluate the efficacy of anthelmintic treatment on a mixed population of gastrointestinal nematodes. The efficacy of most modern anthelmintics usually approaches 100% for the majority of pathogenic gastrointestinal parasite species (Taylor and Hunt, 1989).

Methods for detection of anthelmintic resistance are divided into in vivo and in vitro techniques. They are summarized in table 2. The in vivo methods are suitable for all types of anthelmintics in all species of animals. The in vitro techniques offer rapid and sensitive but suffer from certain limitations (Taylor and Hunt, 1989).

2.6.4.1. Faecal Egg Count Reduction Test (FECRT)

The fecal egg count reduction test was one of the first methods used to detect anthelmintic efficacy by comparing worm egg counts from a group of animals before and after treatment (Presidente, 1985).

An untreated control group is also included to monitor any changes that occur during the treatment period. Percentage efficacy is corrected for changes that occur in the control groups by the equation: $$FECR\% = 1 - \frac{T2_{x}}{T1}\frac{C_{1}}{C2} \times 100$$

Where, T and C are geometric means for the treated and control groups and subscript 1 and 2 designate the counts before and after treatment (Presidente, 1985). The shortcoming of this test is that anthelmintics may cause temporary suppression of egg out put, and if the interval is less than ten days, it will give an over estimate of efficacy. It is also less sensitive when the egg counts are low and the level of resistance is low. Moreover, it doesn't detect the presence of immature parasites, which may survive the treatment and is unable to indicate the resistance genera in mild nematode infection. This demands the use of larvae culture to identify the resistance species of nematode involved. Nevertheless, the test is the best initial screening method for detection of anthelmintic resistance in the field (Johnsen, 1989).

The main advantage of the FECRT is its relative low cost as there is no requirement for highly skilled personnel, expensive resources or sophisticated equipment and facilities. It can be carried out on the farm and doesn't require the movement or slaughter of livestock (Presidente, 1985) and can be used to detect resistance in all groups of anthelmintics in any type of animal.

2.6.4.2. Controlled Anthelmintic Efficacy Test

The test involves artificially infecting groups of worm-free animals with single species third stage infective larvae and treating them at specific time intervals, usually after 21 days (when the parasite have reached the adult stage). The animals are then slaughtered, including the control group, 10 to 14 days after treatment and their worm burdens counted. The worm burdens are recorded and dose response parameters ED 50 and ED 90 (concentration of drug which kills 50% and 90% of the nematode population may be (Taylor and Hunt, 1989) Calculated if a range of dose employed (Hazelby *et al.,* 1994.

The percentage efficacy of the treatment can be calculated using:

$$\% \text{ Efficacy} = \frac{\text{Worm in control} - \text{Worm in treated}}{\text{Worm in control}} \times 100$$

This is the most reliable method of assessing anthelmintic efficacy against mixed nematode infection, but it is costly in terms of animal usage, lab our and time required. Therefore, it has only a limited use in screening for resistance (Taylor and Hunt, 1989).

2.6.4.3. In Vitro Egg-Hatch Assay

The egg hatch assay is the most widely used in vitro test for benzimidazoles. There are several variations of the assay but they are all based on the ovicidal properties of the benzimidazole drugs and the ability of eggs from resistant strains to develop and hatch at higher concentrations of the anthelmintics than their counterparts (Hunt and Taylor, 1989).

This test is based on the principle of determining of the percentage of eggs that hatch or die following incubation with serial concentration of anthelmintic known to prevent embryonation and hatching as in the case of benzimidazole drugs (Le. Jambre, 1976; Coles Simpkin, 1977) or causing paralysis of the first stage larvae as in the case of levamisole and morantel (Presidents, 1985). In detecting benzimidazole resistance, eggs recovered from a group of animals are incubated in serial concentration of benzimidazole. The percentage of eggs that hatch (or die) at each concentration is determined and percentage egg hatch/death corrected for the natural mortality of untreated eggs using probit analysis (Finney, 1971; Dobson *et al.,* 1986). The corrected values are then used to plot a dose response curve, against log concentration of drug and LD 50 (concentration of drug which prevent 50% of eggs from hatching) values calculated (Coles *et al.,* 1992). Fresh eggs or anaerobically stored eggs should be used for this test. It is cheaper, more accurate and less time consuming (1-3 days) to perform than the fecal egg count reduction test. However, it requires more skilled personnel

than in vivo techniques and, therefore, is better suited for research purpose than primary screening (Hazelby *et al*, 1994).

Known susceptible and resistant reference strains of parasites are maintained to compare with the response of the test isolate. Eggs from susceptible nematodes rarely hatch at concentrations of more than 0.1µg/ml of thiabendazole (Whitelock *et al.,* 1980). This concentration often used as the discriminating dose (cutoff value) for determining whether a parasite is susceptible or resistant to the bezimidazole group of anthelmintics.

Paralysis of first stage larvae with in the egg that prevents hatching is the basis of the development egg hatch assay in detecting resistance to levamisole and morantel (Presidente, 1985). The test compares the difference between resistant and susceptible strains of nematodes in the rate of recovery of unhatched larvae in serial dilutions of levamisole. This test is complex to perform, comparisons of results between different laboratories are often difficult and therefore not recommended for field screening for resistance (Johansen, 1989).

2.6.4.4. Larval Development Assay

This assay is an adaptation technique used by Georgi and Le Jambre (1983) for testing both ovicidal and larvicidal effects of various anthelmintics. Eggs are incubated for 7 days at 26^{o}c in serial dilution of a range of anthelmintics incorporated into Agar in wells of flat-bottomed microtitration plates. After incubation the number of eggs, first, second and third stage larvae are counted and percentage of eggs that fail to hatch or to reach the third stage are estimated This assay is simple, several anthelmintics may be tested simultaneously and differentiation of species in mixed infection is easier than other in vitro tests. However, species identification requires expertise and is time consuming. Nevertheless the assay is suitable for both field screening and research work (Johansen, 1989).

The following table reveals the different methods detecting anthelmintic resistance in nematodes of animals:

Table 2: In vivo and in vitro assays used in the detection of anthelmintic resistance

Assay	Spectrum	Type
Controlled Test	All Drugs	In vivo BA
Egg Count Reduction	All Drugs	In vivo BA
Egg Hatch Assay	BZ	In vitro BA
Egg Hatch Assay (larval paralysis)	LEV	In vitro BA
Larval paralysis	LEV, IV,	In vitro BA
Larval Development	BZ, IV, LEV	In vitro BA
Tubulin Binding	BZ	In vitro BA
Esterase Activity	BZ	In vitro BA
Tubulin Probe	BZ	In vitro G

BA-Bioassay; BC-Biochemical assay; G-Genetic assay; BZ-Benzimidazole; LEV- Levamizle; IV-Ivermectin

Source: Jackson, and Coop, 2000

2.6.5. Strategies to Prevent Anthelmintic Resistance

It is doubtful that control programmes which have anthelmintic treatment as a component can avoid selecting for resistance. However by careful use of anthelmintic in easily understood, well presented, and properly serviced control programs, we should at least be able to delay selection for resistance and so extend the effective field life of these drugs. This will allow more time to explore the possibilities of other methods of control (Waller, 1987).

There is an urgent need for the development and adoption of strategies to prevent the spread of anthelmintic resistance, particularly in nematodes of sheep and goats and prevent it from becoming a problem in cattle (Waller, 1997). The following strategies will help conserve anthelmintic efficacy and limit the drug resistance problem.

Using full anthelmintic dosage: It is better to set the dosage for the heaviest animal rather than for the average animal of the group, in order to avoid under dosing of some animals. Reduced dosages are likely to allow survival of worms with partial resistance (heterozygotes).

They may then mate with similar worms, producing offspring that are highly resistant (homozygotes) (Hazelby *et al*, 1994).

Rotation of anthelmintics: An annual rotation of drugs of different chemical families (ex ivermectin, levamisole, benzimidazoles) is recommended because frequent rotation of anthelmintic types has led to the selection of multiple drug resistance in the past (Hansen and Perry, 1994; Howard, 1993).

Avoiding high frequency of anthelmintic use: Suppressive treatment program dosing sheep every 2-4 weeks will eliminate susceptible worms but leave only resistant worms to contaminate pastures (Howard, 1993).

Taking care in selecting the anthelmintics: It is a waste of time and money to use a drug if worms have already developed resistance to it. This is where the fecal egg reduction test is useful. It is useful to remember there may also be side-resistance to a string of related drugs that share a common mode of action (Howard, 1993).

Developing strategic treatment programs: fewer treatments, epidemiologically based, will be just as effective for worm control, be more economic than continuous treatments, and have reduced selection for drug resistance (Radostis *et al.,* 1994).

Synergism of anthelmintics: Combination of drugs sometimes results in synergistic increases in efficacy. For example, the experimental administration of both levamisole and mebendazole in sheep resulted in improved efficacy against benzimidazole resistant worms in Australia. Chemical modification of existing drugs and novel delivery systems may also be used to enhance drug efficacy in the future (Cabaret, 2000). .

Genetically resistant hosts: New techniques of embryo splitting and transfer introduce the possibility of accelerating the selection of flocks genetically resistant to parasites. Heritability of resistance to worms appears to be high, and associations have been found between acquired resistance and certain lymphocyte antigen markers (Eady*, et al.,* 1998).

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Treating all introductions: sheep from an outside farm with a resistance problem may introduce resistant worms to a clean farm. Double dosages or a combination of two anthelmintics may be a useful safeguard (Howard, 1993).

Avoiding prolonged drug encounter: This can occur with licks, block, or small dose sustained release rumen retention devices that have a gradual "taileng off" of drug concentration. It may also occur with ivermectin because of its persistence at low concentrations for several weeks after treatment (Aiello and Mays, 1998).

In addition to the aforementioned strategies, integrated control approaches employing effective management and husbandry practices, alternative use of immunization and biological control approaches can be instituted for effective control of helminth infestation and therefore minimization of the anthelmintic use and development of anthelmintic resistance (Waller, 1997).

2.6.6. Current Status of Anthelmintic Resistance

The first report on resistance to an anthelmintic reported by Drudge and his collogues when a field strain of *Haemonchus contortus* in USA was shown to be resistant to phenothiazine. It took only three years after the introduction of thiabendazole before the first strains of sheep nematodes had developed resistance (Waller, 1987). Since then resistance problems have been reported from most parts of the world including Africa. The extent of the problem of anthelmintic resistance on a regional/ continental basis is presented as follows:

Europe: The recent meeting of the European Union in Brussels disclosed the existence of the problem of anthelmintic resistance in almost all countries except Portugal and Greece, where the very low usage of anthelmintics and animal management practices of herding over extensive areas would preclude this from occurring (Waller, 1997). Resistance was mainly associated with the benzimidazole anthelmintics and most reports were for parasites of sheep and goats and the small *Strongyles* of horses. The levamisole/ morantel group still seems to be generally efficacious, although parasites of another animals species, namely the pig have been found resistant to this group in Denmark and Germany. Of particular concern were the reports of ivermectin resistance in ovine or caprine parasites, not only in Scotland, which was reported in 1992, but also in Denmark (Waller, 1997).

Australia: A compilation of regional surveys in the high rainfall zone in Australia has shown that approximately 80% of the sheep farms have resistance to both the benzimidazole and the levamisole/ morantel groups of anthelmintics (Waller, 1997). The combination product was

also failed to control worms on approximately 50% of sheep farms and resistance to the salicylanilide and closantel now causes serious parasite control problems in the *Haemonchus contortus* endemic regions. Individual farm reports are becoming more frequent in resistance to the macrocyclic lactones. Goat producers face a much greater problem, with multiple high level resistance (involving the benzimidazole and levamisole/morantel groups) together with macrocyclic lactone resistance, resulting in re-structuring and in some cases, abandonment of goat farming operations in the high rain fall, costal areas (Waller, 1997).

Similar to surveys in western in Europe, benzimidazole resistance has been found to be widespread in the *Cyathostome* parasites of horses and case reports have been made of resistance in cattle parasites in both Australia and New Zealand, which include the first reports of resistance to ivermectin in bovine *Cooperia* species (Waller, 1997).

Southeast Asia/South Pacific: Anthelmintic resistance is considered to be one of the greatest threats to the future of small ruminants in the South Pacific. The humid tropical/subtropical weather conditions that prevail across these regions have necessitated the intensive use of anthelmintics in sheep and goats flocks to control nematode parasitism. Consequently, with the increased number of stock there has been a concomitant increase in the prevalence and the level of resistance. Different surveys showed combined resistance to benzimidazoles and levamisole on approximately one third of commercial farms in Fiji, with resistance to ivermectin emerging. Another survey conducted in Malaysia has shown high levels of resistance, particularly to the benzimidazoles, in parasites of small ruminants and resistance is also on the increase on the sub-continent of India (Sani, and Chandrawathani, 1998).

North America: Limited surveys have shown the predictable pattern of high-level resistance to the benzimidazoles. In the Southern Sates of Louisiana and Florida, anthelmintic resistance is now so prevalent and at such a high level for all anthelmintics (including ivermectin) that sheep farming using the British breeds is becoming unmanageable, largely due to the failure to control *Haemonchus contortus*. The parasite-resistant native sheep breeds are seriously being considered for breed substitution and in some cases cross breeding programs are underway to help reduce losses due to haemonchosis (Williams, 1997).

South America: Southern Latin America has the dubious distinction of having the highest and most widespread levels of anthelmintic resistance in the world. The situation ranges from alarming (Uruguay) to critical (Paraguay). The two most commonly groups, the

benzimidazoles and levamisole / morantel have virtually reached the end of their chemotherapeutic usefulness in Brazil, Paraguay and Uruguay. The combination product benzimidazole + levamisole is virtually ineffective in Brazil. Four surveys were conducted to detect resistance problems in Uruguay, Paraguay, Brazil and Argentina and all reported the existence of resistance to ivermectin. No where in the world has ivermectin resistance been detected in such randomly based surveys with increasing prevalence of ivermectin resistance, coupled with virtual failure of the benzimidazoles and levamisole/ morantel groups, the ultimate disastrous scenario of complete exhaustion of all the chemotherapeutic options for nematode control in sheep and goat flocks will be reached. Unfortunately, this is already the case of many Paraguayan sheep farmers (Echevarria *et al.,* 1996). The following tables reveal the situation in the region.

Table 3: Anthelmintic resistance in nematode parasites of sheep flocks in southern Brazil and Uruguay

Nematode	Country	Percentage resistance				
		BZ	LEV	COM	IVM	CLOS
Ostertagia	S. Brazil	8.7	72	81	5	-
	Uruguay	18.4	12.8	-	Nill	
Haemonchus	S. Brazil	68	19	15	7	20
	Uruguay	61.3	28.5	-	1.2	
Trichostrongylus	S. Brazil	7	5	4	2	-
	Uruguay	91.7	95.0	-	Nill	-
Others	S. Brazil	35	16	-	-	

BZ, benzimidazole; **LEV**-levamisole; **ClOS-**closantel, BZ+LEV; **IVM,** ivermectin**: CLOS**: closantel, COM-comination.

Source : Nari *et al.,*1996 and Echevarria *et al.,* 1996

Table 4: Prevalence of resistance of Haemonchus against various anthelmintics in South Africa and South America.

Country	No of Farm	Percentage of resistant farms for given anthelmintics					
		BZ	IV	LEV	BZ + LEV	Rfx	Clos
S. Africa	80	79	73	23	-	89	-
Brazil	182	68	7	29	-	-	-
Paraguay	37	70	67	47	-	-	-
Uruguay	242	61	1	29	-	-	-
Argentina	65	37	2	8	5	-	-

BZ- benzimidozole group, **IVM**-macrocyclic lactonne, **LEV**- levamisole/Morantel group;, Rfx-rafoxanide **Clos-**closantel, + - anthelmintic not tested,

Source: Jackson and Coop, 2000.

Africa: Anthelmintic resistance to all of the three major classes of broad-spectrum anthelmintics has been recorded in a number of countries on the African continent. Studies in Kenya by Waruiru, (1997) showed resistance occurring on half of 42 farms to at least one anthelmintic group with multiple high level resistance to benzimidazoles, ivermectin and salicylanilides recorded on a large sheep and goat farm used as a source of breeding stock for small holders. South Africa has generally been regarded as an anthelmintic resistance "hot spot", largely through the reporting of Van Wyk (1997) and colleagues. Here, some of the first cases of benzimidazole, levamisole, salicylanilide, ivermectin and multiple resistances were reported and this country is where farmers first abandoned sheep farming because of chemotherapeutic failure to control worms. A recent survey made in South Africa on approximately 60 farms showed that 90% have parasite strains resistant to compounds from at least one anthelmintic group and 40% of farms had resistance to three or more of the five groups that were tested (Maingi *et al*., 1998).

Ethiopia: Very few and fragmented reports are available with regard to the status of anthelmintic resistance in farm animals. Though the scope of the study is narrow and limited, the available reports indicate that there is a development of anthelmintic resistance to tetramisole in *Oesophagostomum, Bunostomum and Trichuris* parasites of goats in Adami Tullu (Nessru *et al.,* 1997). Kasshun, 1997 reported suspicion of resistance in nematodes of

small ruminants in a study made in the Southern part of Ethiopia. Daniel, 1998 has also reported the presence of resistance to albendazole in nematodes of Stella state farm cross breed and moderate resistance in indigenous zebu cattle under extensive production around Sebeta towns. Anthelmintics are widely used all over the country for the treatment and control of helminth parasites of farm animals. Drug smuggling and improper use of anthelmintics is very common all over the country because of the presence of great number of illegal traders and anthelmintics are sold in open market as ordinary drugs by non professionals (Nessru *et al.,* 1997).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted on Ogaden small ruminants slaughtered at Debre Zeit Elfora Export Abattoir from August 2003 to March 2004. Ogaden, the origin of the study animals, is located at 9^0 $20^/$ N in the eastern part of Ethiopia and is one of the semiarid parts of the country. The area has two rainy seasons of which the first short rainy season occurs in the spring from March to May and the second long rainy season is from July to September. The annual rainfall of the two seasons varies from 250-600mm. The mean temperature of the region ranges from 25-35^{oc} and the average altitude of the region is 1200 m above sea level (CACC, 2003).

The vegetation of the study area is composed of very little forest and woodland that is only about less than 0.3% of Ethiopia. The grassland part of the area is considerable covering about 38% of the region. However 54% of part of the region consists of bush, shrubs and Afro plane (IAR, 1993). The map of the study area is indicated in Figure 1.

3.2. Study Animals

From August 2003 to March 2004 a total of 114 sheep and 82 goat abomasums were examined and used to determine prevalence of abomasal parasites and monthly worm burden study. The average sample size per month was thus, about 14 sheep and 13 goat abomasums. Out of the total 114 sheep and 82 goat abomasums examined 32 sheep and 24 goats were used to study the vulvar morphology and mucosal larvae of Haemonchus species. On the other hand 76 sheep and 55 goats were used for identification of Haemonchus species based on spicule morphometrics. All the slaughtered animals were male and their age varies from 1-4 years, the majority being 1-2 years of age.

For the purpose of experimental infection and evaluation of the efficacy of anthelmitics, 30 lambs aged four to six months were bought from Sagure town of Arsi zone and transported to FVM of AAU at Debre Zeit. These experimental lambs were infected experimentally with the Ogaden isolate of *Haemonchus contortus* that was collected from Ogaden sheep slaughtered at Elfora export abattoir.

Map of Administrative Ethiopia

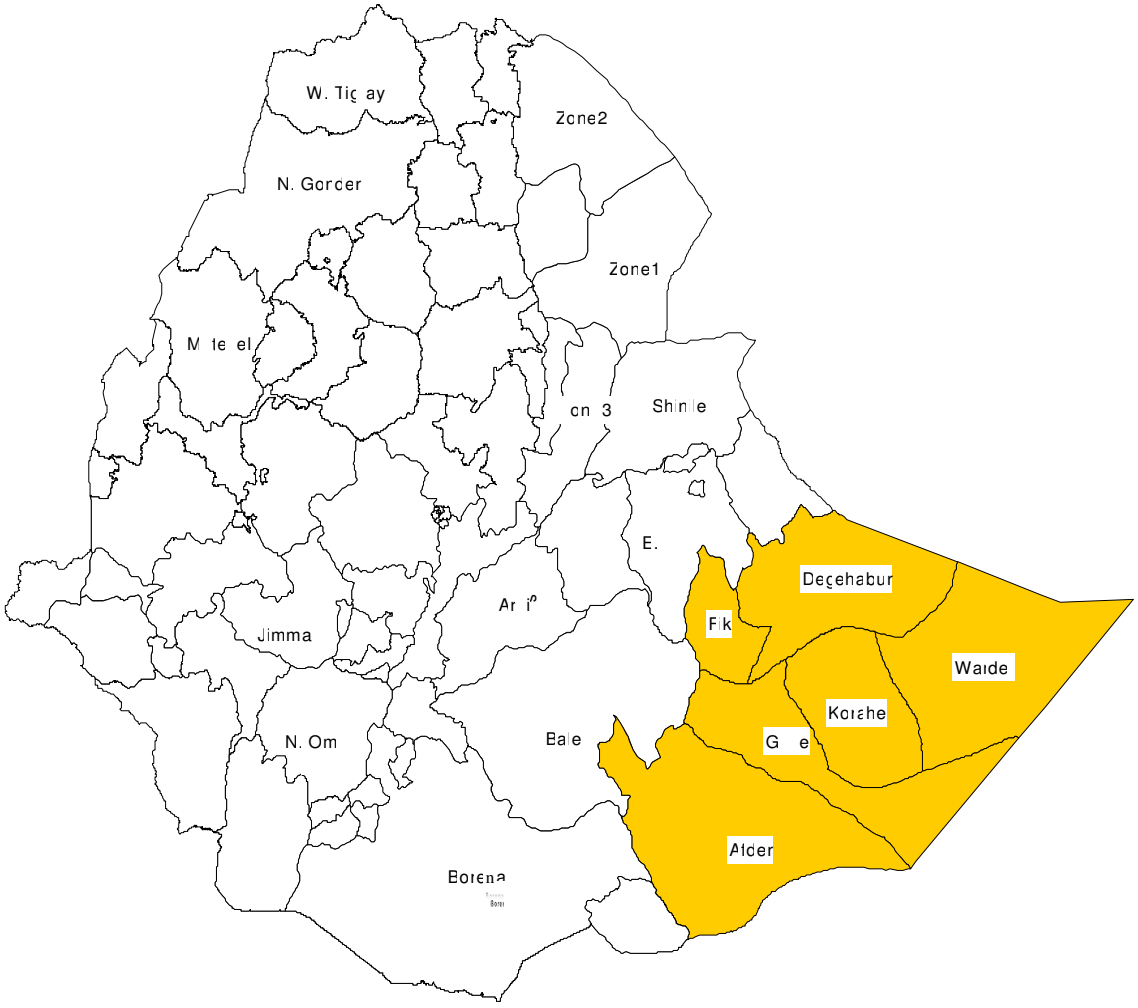


Figure 1: Map of Ethiopia showing the study area (the origin of study animals) in the shaded part.

3.3. Study Design

3.3.1. Abattoir Survey

3.3.1.1. Prevalence and Worm burden Study

Weekly regular visit was made to the Elfora export abattoir and an average of 13 abomasums per month was brought to the Faculty of Veterinary Medicine for recovery of any abomasal parasites. Recovery, counting and identification of abomasal parasites were made using the procedure described in Jorgen and Perry (1994) and MAFF (1977) as indicated in Annex 2.

Isolation and identification of mucosal larvae (L_4) was performed using Williams incubation method in saline solution as indicated in MAFF (1979) and Wood *et al* (1995) and the detail of the technique is presented in annex 3

3.3.1.2. Vulvar Morphology

The vulvar morphology of female Haemonchus worms from 32 sheep and 24 goats were studied on monthly basis by taking 100 worms from each abomasums and categorized to Linguiform, Knobbed or Smooth as described in Rose, 1966 and LeJambre and Whitelock, 1968.

A total of 3187 female worms from sheep and 2386 worms from goats were subjected to vulvar morphology identification. All the linguiform morphs of the female worms found in each abomasums of each sheep and goat were further classified into linguiform A (with one cuticular inflation), linguiform B (with out cuticular inflation), linguiform C (with two cuticular inflation) and linguiform I (the cuticular inflation arises from the linguiform process) as described in Le Jambre and Whitlock (1968). Based on this classification the proportion and distribution of each type of linguiform morphs encountered in each abomasums of sheep and goats was studied on monthly basis.

3.3.1.3. Spicule Morphometry

Fifteen male Haemonchus worms were chosen at random from each abomasums and then tails of male worms were cut before the bursa and stained with lactophenol blue for clear observation of the spicules under a microscope as indicated in Jacquiet *et al.* (1997). For each worm three morphometric parameters of spicules namely, total length (TL), distance from the hook to the tip of the right spicule (THr) and distance from the hook to the tip of the left spicule (THl) were measured using an ocular micrometer. The discriminant function (DF) that combines these three measures of male spicules was used to identify the species of each individual male worm (Jacquiet *et al.,* 1997). The discriminate function (DF) applied was:

DF = 0.0016TL + 0.128THr + 0.152THl – 9.97

Species identification was established based on the following criteria:

If DF < 0.63: *Haemonchus contortus*

If 0.63 < DF < 2.5: *Haemonchus placei*

If DF > 2.5: *Haemonchus longistipes*

The method permits identification of all *Haemonchus species* and is very useful in the study of natural populations especially when two or three *Haemonchus species* are sympatric.

3.3.2. Experimental Study

A total of 30 male Arsi breed lambs, aged four to six months were purchased and used as experimental animas to determine the susceptibility of *H. contortus* of Ogaden isolate to Albendazole and Tetramisole. On the date of arrival all the lambs were treated with ivermectin at 0.2mg/kg live weight to remove any burden of parasites and then checked for three-consecutive feacal egg count performed on the 1st, 2nd, and 3rd weeks during the 21-adaptation period. All animals were housed in an isolation area in concrete based units, fed on concentrates and locally dried straw throughout the adaptation period to preclude any accidental parasitic infections. At the end of the adaptation period the animals were ear tagged and allocated randomly into six groups of five animals each, viz; Albendazole (Exipitol) Greece, Albendazole Pakistan, Tetramisole Greece, Duxamintic (Tetramisole) Pakistan, Positive and Negative control groups.

The anthelmintics that were used for evaluating the susceptibility of the Ogaden isolate of Haemonchus were selected by considering the results of my personal observation and communication conducted with senior veterinarians, veterinary drug importers and livestock owners. All of them indicated that albendazole and tetramisole are much more available on the market and are widely throughout the country. From the communication, it was also observed that drugs form European countries despite their relatively high cost are more preferred than drugs from other developing countries such as India, Pakistan etc which are usually claimed as less efficacious. Therefore albendazole and tetramisole originating from European countries and developing countries were used for comparison purposes. The dose, cost and names of the drugs that were used in the controlled experimental study is indicated in Table 5 below.

Table 5 Anthelmintics used for Susceptibility Test

No	Trade name of the drug	Manufacturer Country	Cost per animal	Active ingredient	Dose mg/kg body weight (MRD)
1	Exiptol	ERFAR s.a. (Greece)	0.55 cents	Albendazole	3.8
2	Albendazole300mg bolus	STAR Laboratories (PVT) LTD (Pakistan)	0.40 cents	Albendazole	5
3	Tetramisol(dl-tetramisole)	ERFAR s.a. (Greece)	0.40 cents	Tetramisole Hcl	15
4	Duxamintic 600mg bolus	STAR Laboratories (PVT) LTD (Pakistan)	0.30 cents	TetramisoleHcl (B. P.Vet.)	15

MRD= manufacturers recommendations dosage.

Each animal in all the treatment groups were orally infected with 4000 infective 3rd stage larvae of Ogaden isolate of *Haemonchus contortus*. On the other hand all animals in the positive control group are infected with 4000 infective 3rd stage but not treated where as all animals in the negative control group were kept as non-infected and non-treated animals.

Except the negative control group (non-infected and non-treated control groups) all the other animals were treated on day 35-post infection with Albendazole (Exipitol) Greece, Albendazole Pakistan, Tetramisole Greece and Duxamintic (Tetramisole) Pakistan according to the dose recommended by the manufacturer (Table 6).

Faecal samples were collected and examined using modified Macmaster technique on weekly basis starting from day 0 of experimental infection up to 10 days after treatment with the anthelmintics mentioned above. PCV and body weights of the animals were also recorded on weekly basis through out the experimental period (Wood *et al.,* 1995). Thirty-five days after inoculation all the lambs in the treatment groups were dosed orally according to the experimental design (Table 6). All the drugs were administered by means of bolling gun for each animal. The manufacturers dose for each animal was calculated according to the lambs live weights.

On day 42-post experimental infection all the animals were slaughtered. The abomasums were separately ligated and the contents examined to count for the number of adult and immature parasites using classical counting procedures indicated in (MAFF, 1979; Hansen and Perry, 1994; Wood *et al*., 1995). Immature worms were collected by soaking the abomasal mucosa in warm saline solution overnight and counted under stereomicroscope (Urquhart *et al,* 1996). The overall experimental design used in the current study is presented below in the Table 6.

Table 6: Design of experimental study to test the susceptibility of Ogaden isolate *H. contortus* to Albendazole and Tetramisole drugs.

Group	Isolate of Haemonchus	No of L_3 administered on day 0	No of lambs	Acclimatization days	Infection day	Treatment day	Drugs used for Treatment	Slaughter day & post mortem exam.	Oral dose mg/kg body weight (MRD)
1	Ogaden	4000	5	30 days	Day 0	35 days post infection	Exiptol Greece	42 days post infection	3.8
2	Ogaden	4000	5	30 days	Day 0	35 days post infection	Albendazole Pakistan	42 days post infection	5
3	Ogaden	4000	5	30 days	Day 0	35 days post infection	Tetramisol Greece	42 days post infection	15
4	Ogaden	4000	5	30 days	Day 0	35 days post infection	Duxamintic Pakistan	42 days post infection	15
5	Ogaden	4000	5	30 days	Day 0	35 days post infection	Positive control	42 days post infection	0
6	Not infected	0	5	30 days	Non infected	Not treated	Negative control	42 days after acclimatization	0

3.3.2.1. Faecal Egg Count Reduction Test (FECR %)

The efficacy of the different anthelmintic used in this study was determined by comparing the fecal egg count reduction test from a group of animals before and after treatment as described in Presidente, 1985. The faecal egg count of each experimentally infected as well as control positive groups was made using modified MacMaster egg counting techniques (Soulsby, 1986) on the day of treatment and on the 10^{th} day post treatment.

Using the arithmetic means of faecal egg count, the efficacy of each drug was calculated using the following formula.

$$\text{FECR \%} = \frac{T_1 - T_2}{T_1} \times 100$$

Where T_1 pre treatment and T_2 is post treatment arithmetic mean of egg per gram of feces

An efficacy of less than 90% and a 95% confidence level of less than 90% is taken as indicative of the presence of anthelmintic resistance (Coles *et al.,* 1992). If the value of FECR % is less than 90%, it can be used as a criterion to indicate the presence of resistance to anthelmintics (Wood *et al.,* 1995).

3.3.2.2. Controlled Anthelmintic Efficacy Test

All lambs were slaughtered 10 days after treatment and the number of worms present in the lumen and mucosa of the abomasums recovered and counted. Efficacy percentage was calculated as the difference between the geometric mean worm counts in the untreated control and the treated groups expressed as a percentage of the geometric mean worm counts in the control group (Presidente, 1985). The worm burdens were determined according to standard worm counting procedures described in (MAAF, 1979; Jorgen and Perry, 1994; Wood *et al* 1995).

$$\text{Percent efficacy} = \frac{\text{Worm in control} - \text{Worm in treated}}{\text{Worm in control animals}} \times 100.$$

The geometric mean can accurately represent the distribution of nematode populations within a group of animals and would give a more accurate indication of the degree of a product (Wood *et al.,* 1995).

3.3.2.3. In Vitro Egg Hatch Assay

The egg hatch assay was used as outlined in the WAAVP recommendations (Coles *et al.,* 1992; Wood *et al.,* 1995). Pretreatment samples were pooled for all lambs and undeveloped eggs were recovered using Magnesium sulphate as a flotation fluid from freshly collected faeces in less than two hours before processing. Magnesium sulphate was removed from eggs with excess tape water. The recovered eggs were adjusted at 50-100 eggs in 100 µl of water and were incubated for 48 hours at 23^{oc} in serial concentrations of albendazole Greece dissolved in 1% DMSO ranging from 0-8.96µg/ml (17-20 different concentrations) (Dorny *et al*., 1994). The control was prepared in 5 replicates where as each of the different concentrations were prepared in triplicates. Lugol's iodine was used to stop further hatching and all eggs and larvae at each Albendazole concentration counted as dead, embryonated or hatched to L_1.

Then the percentages of eggs that hatch, embryonate or die at each concentration was determined by counting the whole content of each labeled tube under a microscope. Natural mortality was corrected from the percentage of eggs that hatch in the controls and the percentage death value of each tube was plotted against each different concentrations of the anthelmintic under evaluation (Presidente, 1985). The LD_{50} was calculated for each sample using probit analysis (Finney, 1971) and then used to plot a curve against log concentration of drug. In addition to the Ogaden isolate of *H. contortus*, known susceptible and resistant reference strains were used to compare with the response of the test isolate.

3.4. Statistical Analysis

Microsoft excel soft ware was used to store the all the data of abomasal parasites and experimental studies. Soft ware programmes known as Stata (Intercooled Stata 7.5) and SPSS11.5 for windows (2) were used for data analysis.

The prevalence of abomasal parasites, average worm burdens, total worm burdens, the monthly value of different stages of abomasal parasites and the percentage values of the different vulvar morphs of female Haemonchus between different months of the study period and between the two hosts were all compared by ANOVA. When p value is less than 0.05 the presence of significant difference is considered (Coles *et al.,* 1992). Mean, confidence interval, percentage value, standard deviation and standard error were all used where they are found to be necessary to compare and describe abomasal parasites in both sheep and goats.

As for the second part of the study, which is the controlled experimental study, the statistical analysis applied was percentage reductions in the nematode and eggs, compared with the control group were calculated by using the arithmetic and geometric mean counts for each group. Pre- and post treatment egg and nematode counts were analyzed by an analysis of variance between the groups by the repeated measures of analysis of variance (ANOVA) using the general linear models (GLM) procedure with pair wise comparisons between the groups. Worm burdens and epg were compared between groups by ANOVA when a p value is less than 0.05 it is considered significant (Coles *et al.,* 1992).

4. RESULTS

4.1. Study on Abomasal Parasites

4.1.1. Prevalence and Burden of Abomasal Parasites

Of the total 114 sheep abomasums examined for the presence of abomasal parasites an overall prevalence rate of 92.9% was recorded during the study period from August 2003 to March 2004. The abomasal parasites recovered and identified from sheep were *Haemonchus* with the prevalence of 91.2% and *T. axei* with the prevalence rate of 37.7 %(Figure 2). On the other hand out of the total 82 goat abomasums examined an overall prevalence rate of 90.2% of abomasal parasites was recorded, with specific prevalence of 82.9 % *Haemonchus* and 40.24% *T. axei*. Both sheep and goats of the study area remained infected with different parasite stages throughout the study period (Figure 3).

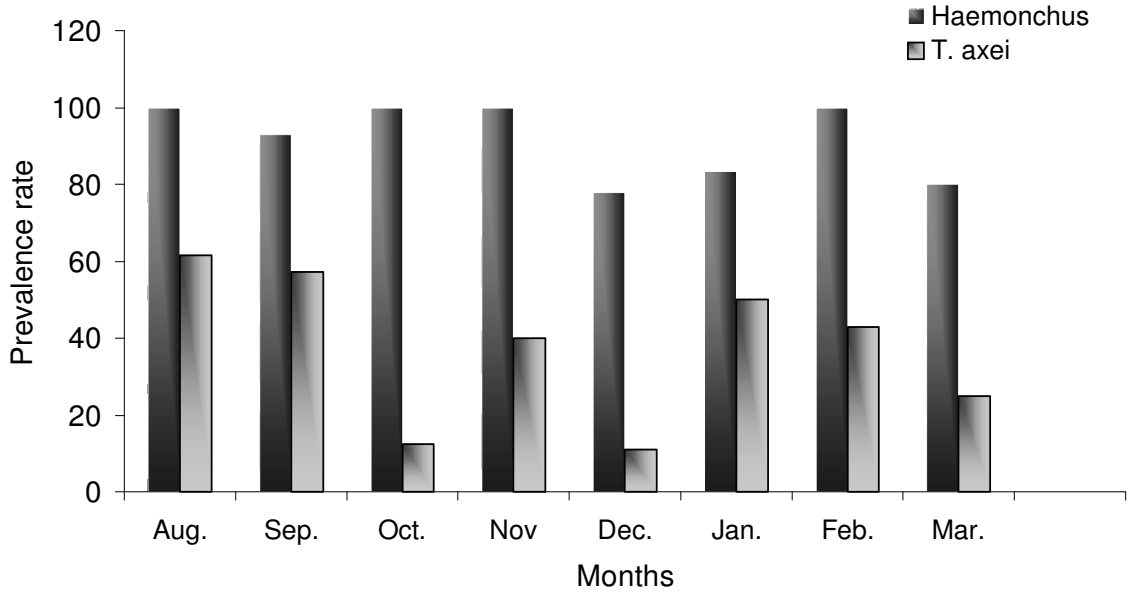


Figure 2: Monthly Prevalence of Haemonchus and *T. axei* in Sheep

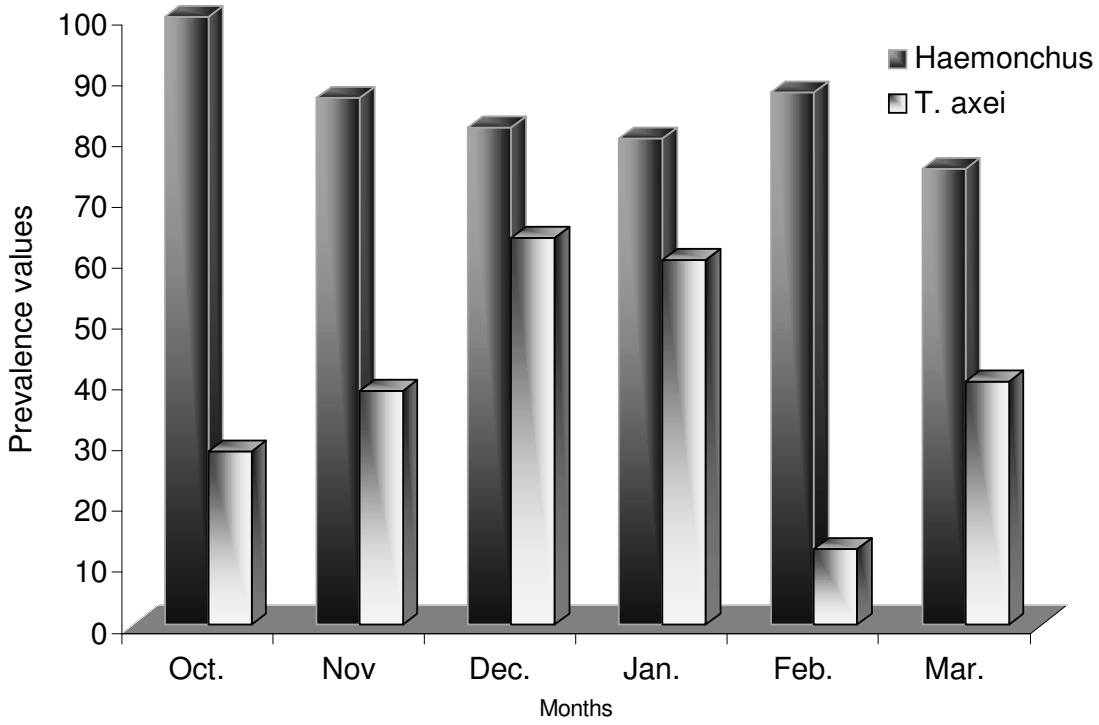


Figure 3: Monthly Prevalence of Haemonchus and *T. axei* in goats

In the current study the monthly average worm burden of Haemonchus in sheep was found to be highest in August (1433.1) and lowest in December (388.9) and falls between these values for other months as indicated in Table 7 below. Similarly the highest *T. axei* burden in sheep was recorded in August (638.5) and the least in December (44.4). Similarly the monthly average worm burden of Haemonchus in goats is highest in December (914.5) and lowest in March (450.0) and lies between theses values for the other months. Where as in *T. axei* the highest value is recorded in December (572.7) and lowest in March (210.0) as presented in Table 8 below. Generally sheep were more heavily infected than goats as it can be seen from the above Figures 1 and 2. The worm burden of *Haemonchus species* was found to be significantly different among different months of the year ($P < 0.05$ at $F=7.37$) in both sheep and goats.

Table 7: Monthly mean worm burden and 95% confidence interval of Haemonchus and T. axei in sheep (n=114)

Month	No of sheep examined	Haemonchus			*T. axei*		
			95% CI			95% CI	
		Mean	UB	LB	Mean	UB	LB
August	13	1433.1	1067.2	1798.9	638.5	299.7	977.2
September	14	1020.0	598.3	1441.7	328.6	139.9	517.3
October	16	867.5	625.8	1109.2	106.3	-81.9	294.4
November	10	934.0	497.6	1370.4	490.0	-262.8	1242.8
December	9	388.9	168.5	609.3	44.4	-58.0	146.9
January	18	495.7	320.6	670.6	138.9	66.2	211.5
February	14	522.9	335.8	709.9	192.9	-63.8	449.5
March	20	416.0	264.6	567.4	115.0	-2.9	232.9

CI: confidence interval, UB: upper bound, LB: lower bound

Table 8: Monthly mean worm burden and 95% confidence interval of in Haemonchus and *T. axei* goat (n=82).

Month	No of goat examined	Haemonchus			*T. axei*		
			95% CI			95% CI	
		Mean	UB	LB	Mean	UB	LB
October	7	751.4	598.2	904.7	414.3	-326.4	1154.9
November	13	572.3	325.1	819.5	292.3	-77.3	661.9
December	11	914.5	185.6	1643.5	572.7	146.7	998.8
January	15	574.7	303.3	846.1	320.0	31.3	608.7
February	16	528.8	295.9	761.7	318.8	-319.3	956.8
March	20	450.0	263.2	636.9	210.0	67.6	352.4

The different stages of *Haemonchus species* viz; adult (male and female), Juvenile (L_5), L_4 in the digesta and L_4 in the mucosa were identified and counted by standard procedures from 114 sheep and 82 goats. Accordingly the average percentage distribution of each of the different stages of *Haemonchus species* in sheep were 79.5 % *adults*, 14.1 %L_5, 6.0 % L_4 in digesta and 5.0% L_4 in the mucosa Where as in goats 81.5 % adults, 11.6 %L_5, 6.9 % L_4 in the digesta and 5.3% L_4 in the mucosa were detected (Table 9). The monthly worm burden of *Haemonchus species* was found to be significantly different among different months of the year ($P< 0.05$ at $F=7.4$) in both sheep and goats.

Sex ratio of male to female was about 0.5 and 0.6 in both Haemonchus and *T.axei* in sheep and goats respectively (Annex 11 and 12).

Table 9: Average values of the different stages of *Haemonchus species* in sheep and goat on monthly basis.

Host Spp	Worm stage	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Percentage
Sheep (n=114)	Adults (M+F)	1176.9	778.6	612.5	820.0	311.1	355.5	400.0	305.0	79.5
	Juvenile (L_5)	207.7	207.1	212.5	60	0.0	77.8	42.9	35	14.1
	L_4 in the digesta	30.8	14.3	25.0	30.0	66.7	50.0	71.4	70	6.0
	L_4 in the mucosa (n=32)	57.5	70.5	70.0	60.0	25.0	55.0	30.0	30.0	5.0
Goat (n=82)	Adults (M+F)	ND	ND	628.6	407.7	781.8	440.0	412.5	345	81.5

Juvenile (L_5)	ND	ND	85.7	107.7	90.9	67.3	37.5	40	11.6
L_4 in the digesta	ND	ND	14.3	38.5	27.3	53.3	68.7	55	6.9
L_4 in the mucosa (n=24)	ND	ND	40.0	60.0	45.0	35.0	40.0	50.0	5.3

ND: Not done

L_4D: L_4 in the digesta, L4M: L_4 in the mucosal

Mucosal L_4 stages of *Haemonchus* were isolated using the William's Method, cited in Soulsby (1986) and MAFF (1979) incubating the abomasal mucosal surface in saline solution of 32 sheep and 24 goat abomasums in order to observe the contribution of mucosal larvae to the overall worm burden. Based on this the contribution of the mucosal L_4 stage to the over all total worm burdens of *Haemonchus species* in sheep and goats were 5.0% and 5.3% respectively (Figure4) No significant difference was observed between different months in both sheep and goats ($p > 0.05$).

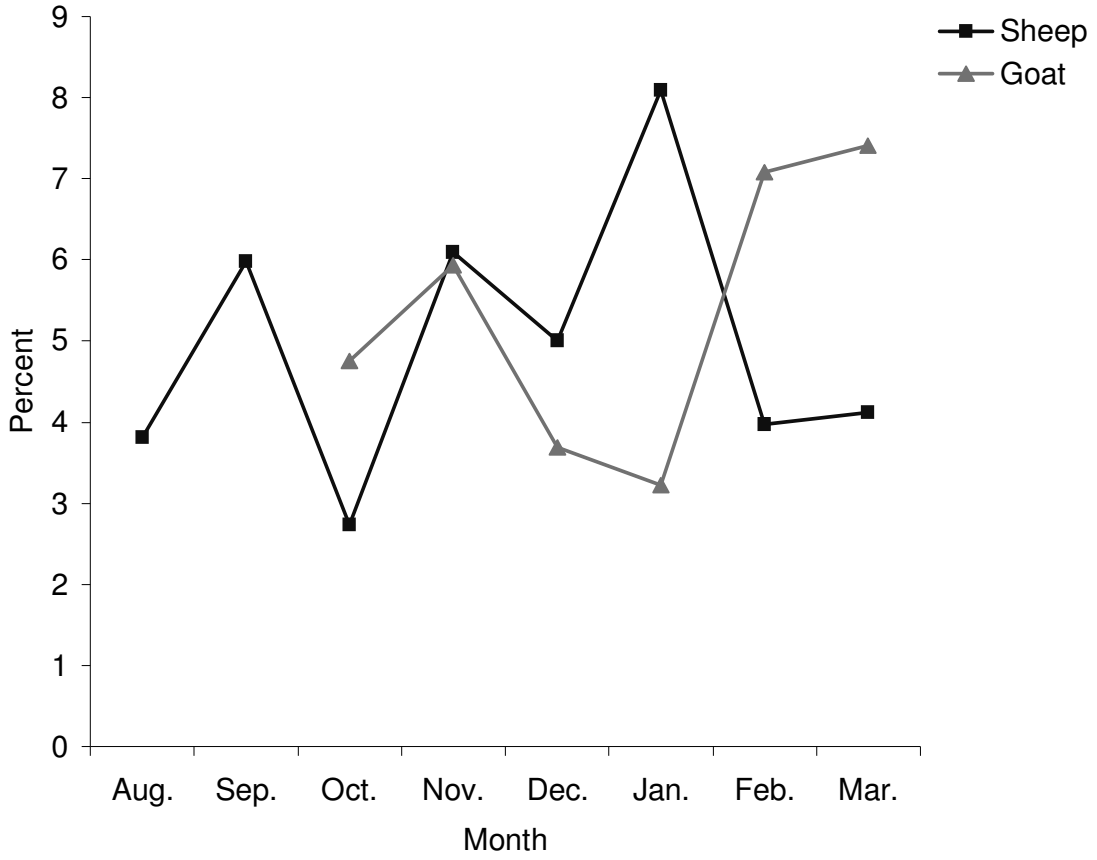


Figure 4: Monthly Percentage value of Mucosal L_4 Haemonchus in sheep and goats

4.2. Morphological Characteristics of *Haemonchus species*

4.2.1. Types of Vulvar Morphs

Out of the 3187 female worms randomly selected from 32 sheep, 49. 9% were having linguiform vulval morphs, 28.5% knobbed and 23.0% were having smooth vulval morphs (Table 10)

Similarly from 24 goats, a total of 2386 female worms were studied, and 53.8% linguiform, 18.4% knobbed and 27.6% smooth vulvar flap category were recorded (Table 10) Morphological photograph to show the important features is presented in Annex 17

In both host species a significant difference ($p < 0.05$) was observed in the proportion of the three major vulvar morph types; but there was no significant statistical difference ($p > 0.05$) for each vulvar morphs in different months of the study period.

Table 10: Monthly distribution of the different vulvar morphs of female *Haemonchus species* in percentage value

Host species	Vulvar morphs	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	**Total**
	Linguiform	63.1	60.0	69.2	40.0	60.0	41.5	29.4	32.7	49.5
Sheep	Knobbed	19.5	27.2	22.2	43.1	14.5	18.1	35.7	39.7	28.5
(n=3187)	Smooth	17.3	12.7	8.5	16.8	25.5	40.4	34.9	27.7	23.0
Goat	Linguiform	ND	ND	59.2	66.5	53.8	59.0	37.6	46.7	53.8
(n=2386)	Knobbed	ND	ND	20.7	20.7	15.1	20.2	20.1	13.7	18.4
	Smooth	ND	ND	20.0	12.7	31.0	20.7	41.4	39.7	27.6

A total of 1580 linguiform Haemonchus collected from sheep were further identified and the following percentage was obtained: 27.2% A type, 14.8% B type, 5.3% C type and 2.2% I type linguiforms. In goats out of the1285 Linguiform morphotypes 27.3 % were A type, 17.5% B type, 6.6% C type and 2.3% I type (Table11). Morphological photograph to show the important features is presented in Annex 17

Table 11: Linguiform Morphotype Haemonchus Distribution in Percentage value on Monthly basis.

MONTH	Sheep (n=1580)				Goat (n=1285)			
	LA%	LB%	LC%	LI%	LA%	LB%	LC%	LI%
Aug.	33.2	15.1	5.4	9.4	ND	ND	ND	ND
Sep.	29.7	20.2	10.0	0.0	ND	ND	ND	ND
Oct.	35.7	23.0	9.7	0.7	45.0	10.7	2.2	1.2
Nov.	20.9	18.4	0.8	0.0	41.5	17.2	5.2	2.5
Dec.	37.5	11.2	9.5	1.7	25.4	15.9	8.7	3.8
Jan.	21.4	14.8	2.8	2.5	19.5	27.0	10.7	1.7
Feb.	18.5	7.3	2.3	1.3	20.2	11.6	4.5	1.2
Mar.	20.3	8.3	2.3	1.8	12.5	22.7	8.2	3.2
Total	27.2	14.8	5.3	2.2	27.3	17.5	6.6	2.3

ND: Not done;

LA: with only one cuticular inflations

LB: with out cuticular inflation,

LC: with two cuticular inflations,

LI: the cuticular inflation arises from the linguiform processes

4.2.2. Identification of *Haemonchus Species*

Based on spicule measurements and the discriminant function (Jacquiet *et al*., 1997), a total of 1159 adult Haemonchus males collected from sheep on monthly basis were identified to their respective species. Accordingly, 1102 (95.1%) were *H. contortus*, 46 (3.4%) were *H. placei* and the rest 17 (1.5%) were identified as *H. longistipes* (Table 13). Similarly out of 841 adult Haemonchus males collected from goat, 812 (96.5) *H. contortus*, 25 (3.0%) *H. placei* and 4 (0.5%) *H. longistipes* was recorded. The maximum and minimum measured values of parameters on spicules that were used in determining Haemonchus species are presented in Table 12. The proportions of the three *Haemonchus species* were not significantly different between sheep and goats ($p > 0.05$).

The average measurement values on spicules used in determining Haemonchus species are presented in Annex 13.

Table 12: Minimum and maximum morphomertric values of spicules in µm in sheep and goats on monthly basis

Animal species	Parameters measured		Month							
			Aug.	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Sheep	TL	Min.	416.8	416.8	416.8	406.2	416.8	366.3	416.8	404.2
		Max.	530.5	568.3	606.2	555.7	543.1	530.5	543.1	530.5
	THr	Min.	37. 9	37.9	37.9	31.7	31.6	31.6	31.6	37.9
		Max.	88.4	88.4	101.0	56.8	63.1	56.8	56.8	56.8
	THl	Min.	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6
		Max.	37.9	37.9	37.9	44.2	31.6	25.3	31.6	25.3
	DF	Min.	-2.5	-2.5	-3.4	-3.1	-2.4	-3.4	-3.1	-2.5
		Max.	7.9	8.0	9.65	4.9	3.8	1.2	3.0	1.2
Goat	TL	Min.	ND	ND	429.4	404.2	391.5	353.6	404.2	416.8
		Max.	ND	ND	530.5	530.5	505.2	511.5	530.5	530.5
	THr	Min.	ND	ND	37.9	25.3	31.6	31.6	31.6	31.7
		Max.	ND	ND	63.1	50.5	50.5	56.8	56.8	56.8
	THl	Min.	ND	ND	12.6	12.6	12.6	12.6	12.6	12.6
		Max.	ND	ND	25.5	25.3	25.3	25.3	25.26	25.26
	DF	Min.	ND	ND	-2.5	-3.2	-2.5	-2.5	-3.29	-3.29
		Max.	ND	ND	2.8	1.2	0.3	2.0	1.99	1.99

Even though the percentage of the two species of Haemonchus (3.4% and 3.0% *H. placei*, and 1.5% and 0.5% *H. longistipes* in sheep and goats respectively) identified in the current study seems small when compared to *H. contortus*, these two species were observed to infect considerable proportion of animals 28.9% sheep and 34.5% goats by *H. placei* and 14.5% sheep and 5.4% goats by *H. longistipes* examined during the study period as indicated in Table 13 below.

Table 13: Occurrence of *Haemonchus* species compared to the proportion of hosts infected in Sheep and goats

		Species of Animals examined	
		Sheep	Goat
No of animals examined		76	55
No of worms measured		1159	841
Mean No of worms measured in each animal		15.3	15.3
H. contortus	Percentage of worms	95.1	96.5
	Percentage of animals concerned	100	100
H. placei	Percentage of worms	3.4	3.0
	Percentage of animals concerned	28.9	34.5
H. longistipes	Percentage of worms	1.5	0.5
	Percentage of animals concerned	14.5	5.4

4.2.2.1. Mono and Poly-Specific Haemonchus infections in Small Ruminants

Mono-specific *H. contortus* infections were found to be very abundant in both sheep and goats of the study area, 57.9% in sheep and 58.2% in goats. 22.4% of sheep and 38.2% of goats were having both *H.contortus* and *H. placei* in their abomasums. While 11.8% of sheep were having mixed infection with *H.contortus, H. placei* and *H. longistipes* and no triple Haemonchus species infection was recorded in goats (Figure 5). On the other hand the association *H. contortus* + *H. longistipes* in sheep is 7.9% and 3.6% in goats as indicated in (Figure 5). No association was observed between month and type of *Haemonchus species* combination during the study period.

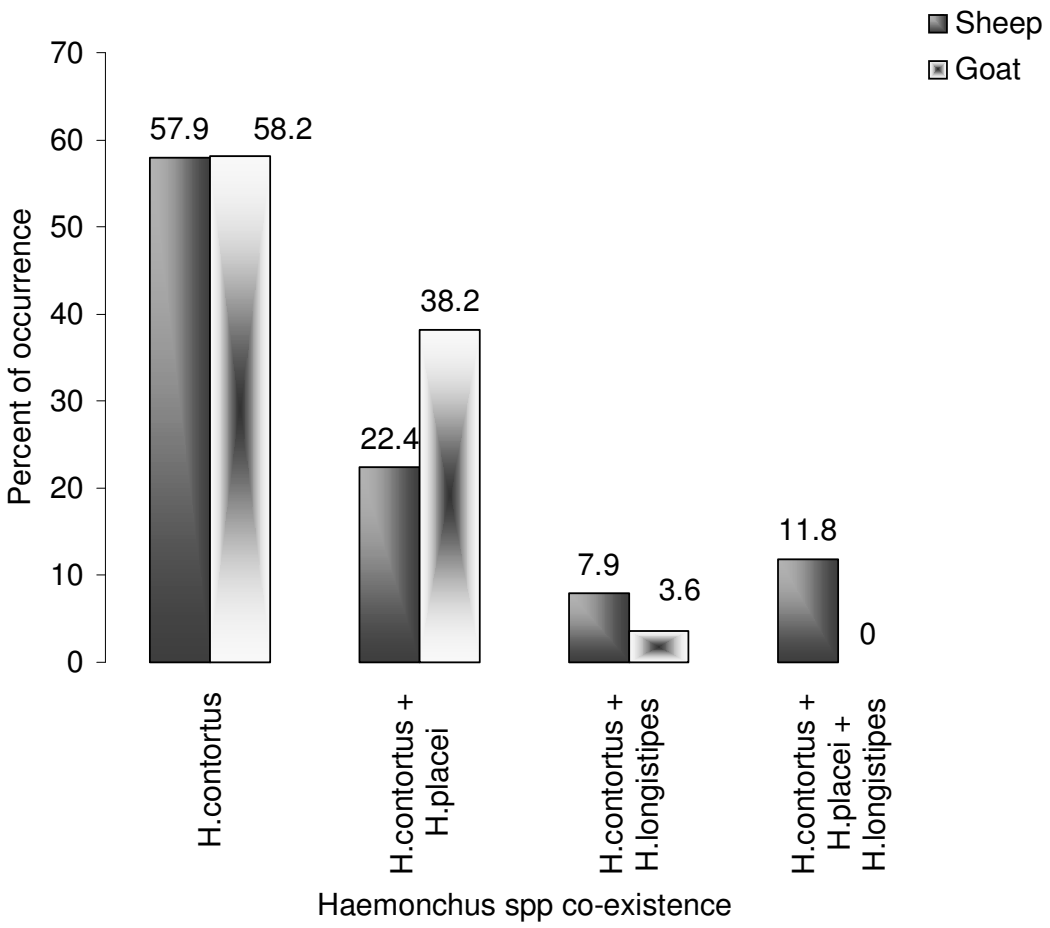
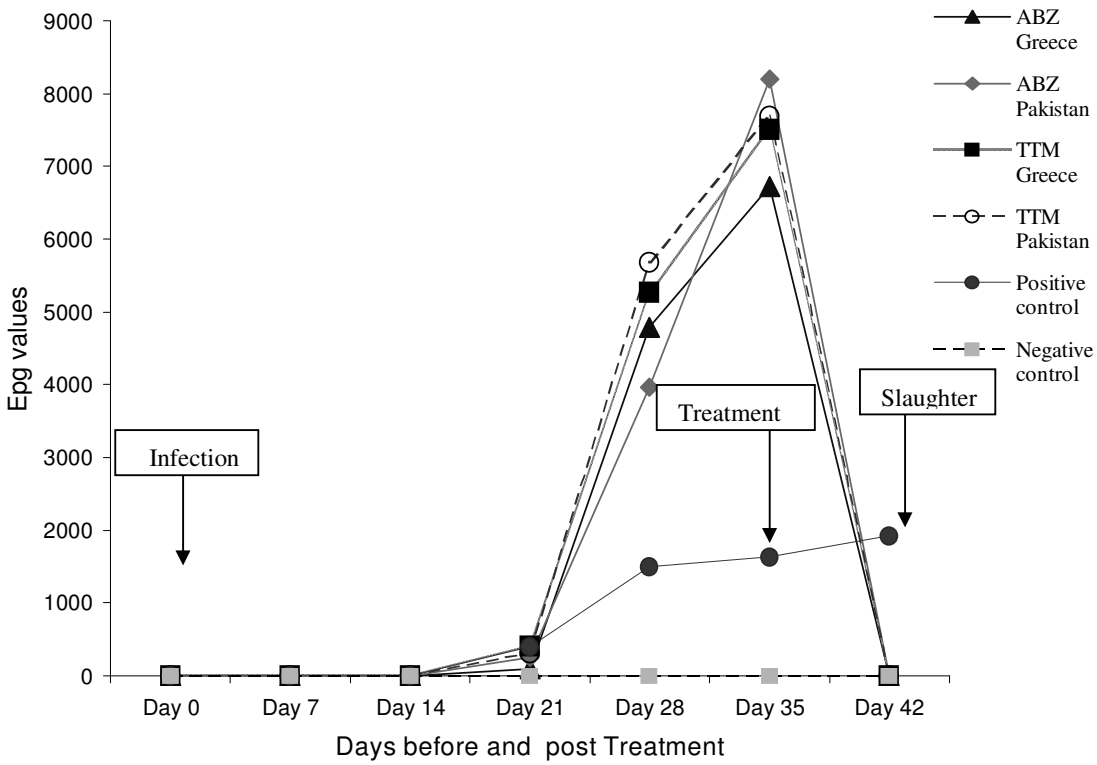


Figure 5: Percentage value of Mono and Poly specific Haemonchus infections in sheep (= 76) and goats (n = 55).

4.4. Efficacy of Albendazole and Tetramisole

4.4.1. Fecal Egg Count Reduction Test (FECR %)

Results of faecal egg count reduction after treatment with all the tested drugs viz; Exiptol Greece, Albendazole Pakistan, Tetramisole Greece and Duxamintic Pakistan are given in Figure 6. Compared to the control group all the four anthelmintics reduced the faecal egg counts by 100% suggesting that the *Haemonchus contortus* Ogaden isolate is susceptible to the four tested anthelmintics irrespective of their origin.



Day 0, 35 and 42 were days of infection, treatment and slaughtering, respectively.

FECRT%: reductions percentage of feacal egg counts

Figure 6: Epg values before and after treatment up to the day of slaughtering of experimental lambs (n=30) compared to the control groups.

4.4.2. Controlled Anthelmintic Efficacy Test

The average worm burden counted on day 42 was 630 for the positive control group while the four treatment groups that have received either albendazole or tertamisole of Greece and Pakistan origin had no worm count following the standard worm counting procedure. Therefore the controlled anthelmintic efficacy test which is based on worm count reduction compared to positive control groups confirmed that Ogaden isolates of *H. contortus* remained fully susceptible to Exiptol Greece, Albendazole Pakistan, Tetramisole Greece and Duxamintic Pakistan. The percentage efficacy values calculated for all types of the drug tested is therefore 100%, further confirming the result obtained by FECRT and EHA techniques.

4.4.3. In Vitro Egg Hatch Assay

The LD50 values of albendazole Greece evaluated on Ogaden isolate, known susceptible and resistant strains of *H. contortus* are indicated in Figure 7. The LD_{50} values of albendazole Greece obtained after correcting for natural mortality was about 0.06µg/ml for Ogaden isolate while that of the known susceptible and resistant reference strains was found to be 0.08µg/ml and 1.28µg/ml respectively.

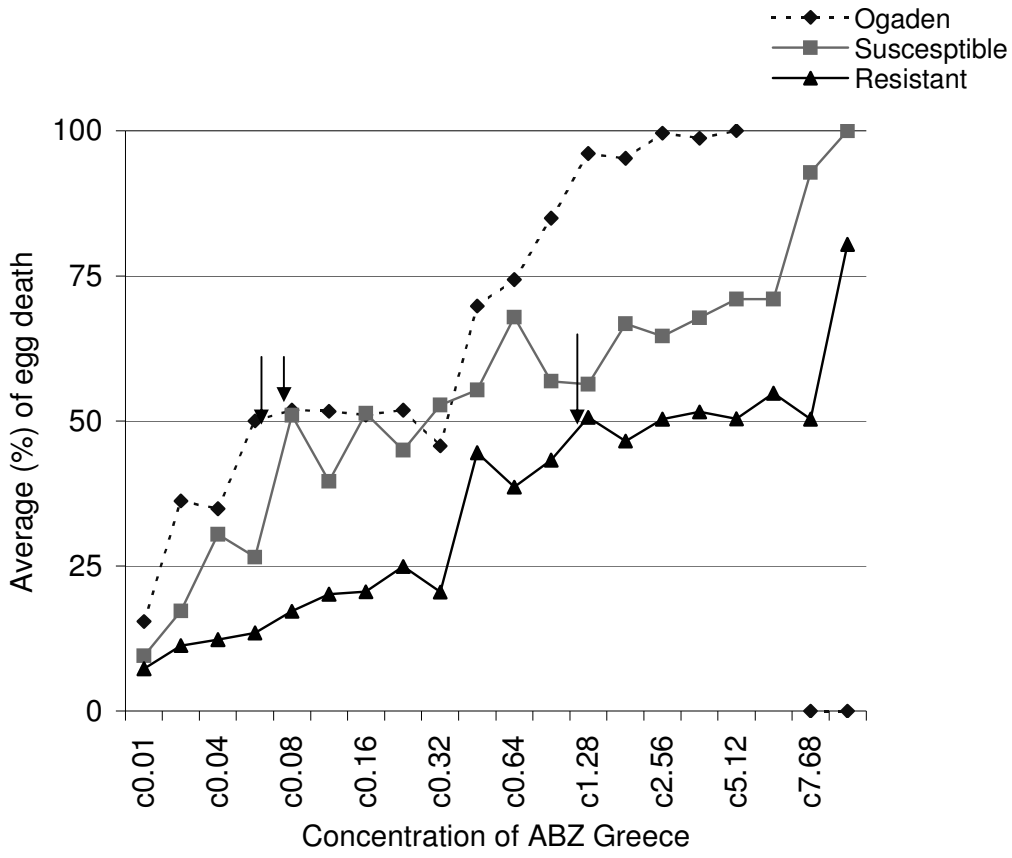


Figure 7: Result of Egg Hatch Assay of Albendazole Greece on Ogaden Isolate Compared to known Susceptible and Resistant Reference Strains (the concentration of the drug is in µg/ml).

5. DISCUSION

Prevalence and Worm burden

The current study revealed an overall prevalence of 93.0% and 90.2% of abomasal parasites in sheep and goats respectively, which is very significant in view of the worldwide interest in Haemonchosis, which is regarded as one of the most prevalent, pathogenic and economically important nematodes of small ruminants. The findings of the current study confirmed the wide abundance of abomasal parasites in the study area supporting previous studies conducted by Solomon (1987) who reported 90 %, Donald (1999), and Abebe and Esayas (2001) reported more than 90% prevalences of abomasal parasites in the Eastern part of Ethiopia. Many other studies conducted elsewhere in Ethiopia also support the present finding thus indicating the rampant nature of gastrointestinal helminthosis in general and Haemonchosis in particular. Gebreyesus (1986) reported 94 % in Gondar, Tesfalem (1989) 59.2 % in Bale, Bayou (1992) 90.2% in Illubabor, Derege(1992) 91.0% in Wolliata Sodo and Gennene (1994) 91.4% in Kombolcha pointed out the significance of GIT parasites Helminthosis is very considerable all over the country.

There was no significant difference ($p > 0.05$) in prevalence of abomasal parasites between sheep and goats indicating that both species are almost equally susceptible to both *Haemonchus spp* and *T. axei.* The current study also clearly indicated the existence of year round infection of small ruminants of the study area by abomasal parasites which is in line with Teklye (1987) finding that pointed out severe contribution of ovine nematode morbidity throughout the year. Sheep and goats of the study area are affected by a very high prevalence rate of 91.2% and 82.9% Haemonchus species respectively. Where as the overall mean worm burden of Haemonchus was found to be 759.5 and 472.4 in sheep and goats respectively. This finding suggest that Haemonchus to be the most dominant abomasal parasite and owing to its known pathogenic significance it will undoubtedly contribute to production losses, ill thrift, morbidity and mortality of small ruminants of the study area. Both late L_4 and L_5 stages of Haemonchus are known to be a voracious blood suckers (Soulsby, 1986; Bowman, 1995 and Urquahrt *et al.*, 1996). This finding on Haemonchosis agrees with other studies mainly conducted by 6 th year students of FVM in other parts of Ethiopia, Solomon (1987) 93.6 in the Ogaden region, Ahmed (1988) 88.2% in Wellega, Derege (1992) 80% in Wolloita Sodo, Genene (1994) 83.9% in Kombolcha, Getachew (1998) 95.5% in Mekele and Abebe and

Esayas(2001) 96.5% in Eastern part of Ethiopia. . The result of the present study is also in agreement with the findings of Maingi (1997) who regarded Haemonchus as the most important parasite against which worm control is primarily targeted in Kenya and Fakae (1990) who reported 77.8-100% prevalence rate in Nigeria.

The different stages of Haemonchus that were recovered, isolated and identified from the abomasums of small ruminants are indicated in Table 9 and Figure 4. A great proportion of the abomasal parasites of sheep and goats constitutes high number of adults with an overall percentage of 79.8% and 81.5% of the total worm burden, followed by 14.1% and 11.6 % juvenile, 6.0% and 6.9% L_4 in the digesta and in sheep and goats respectively. This finding is inline with the previous works conducted in Mauritania by Jacquiet *et al*., (1992), Jacquiet *et al*., (1995) and Abebe and Esayas (2001) in Eastern part of Ethiopia in that both of them reported predominance of the adults and followed by other stages depending the season of the year.

In both sheep and goats females were more abundant in number than males contributing 28.6% and 49.9% respectively. In sheep female's share is 51.1% and male's share is 28.1% where as in goats males and females contribute 28.6% and 49.3% to the total worm burden respectively and the variations in months in both host species were significant throughout the study period ($P < 0.05$). This finding is in agreement with previous study conducted in Eastern part of Ethiopia by Abebe and Esayas (2001).

The overall contribution of the mucosal larvae to the total worm burden was found to be 5.0% and 5.3% in sheep and goats respectively. Up on statistical analysis ($p=0.4826$ at $F=0.94$) of the result by ANOVA significant difference between months was not observed. This finding doesn't agree with the previous study conducted in Eastern part of Ethiopia by Abebe and Esayas (2001), which is due to the difference in study area in Afar and Somali and months of the study period, in which the previous study was conducted in dry periods of the year from November to April.

The total prevalence rate of Haemonchus was not significantly different between sheep and goats ($p=0.0002$ at $F=4.29$) which is because of the two host species are sharing the same environment and have similar susceptibility to this parasite. But the monthly worm burden of the two host animals was found to be statistically significant ($p < 0.05$) between different months of the study period, which was due to the effect of the difference in climatic

conditions among different months of the study period. This finding also agrees with the previous study conducted in Mauritania by Jacquiet *et al*., (1992) and Abebe and Esayas (2001) in Eastern part of Ethiopia in that both of them indicated similar findings.

Vulvar Morphs and Species

During the study period from a total of 32 sheep a total of 3091 and from a total of 24 goats a total of 2842 female Haemonchus worms were collected and categorized in to their respective vulvar morphs.

Out of the 3091 female Haemonchus worms collected from sheep, 50.8% of them were having linguiform type of vulval flap, 29.4 were knobbed and 19.8 were smooth type with no vulval process (Table 10). While vulval identification of 2842 worms collected from goats revealed an overall percentage value of 53.8 linguiform, 18.6 knobbed and 27 smooth. The linguiform morphs predominate and out numbered the other morph types in both sheep and goats and this finding is found to be in line with the previous studies of Jacquiet *et al*., (1992), Jacquiet *et al*. (1995), Jacquiet *et al*. (1998) and Esayas and Abebe (2003). In both host species statistical analysis of the result indicated the absence of significant difference ($p > 0.05$) in the proportions of the three major morph types; linguiform, knobbed and smooth among the different months of the study period. The absence of monthly dependant fluctuations in the proportion of vulval morphs is also indicated by several authors (LeJambre and Whitelock, 1968; Jacquiet *et al*., 1995; Esayas and Abebe, 2003). The study revealed the morph distribution in sheep and goats was not very different. The linguiform females were found to be the most common and the knobbed and the smooth females were not rare.

In the present study, within the linguiform morphotypes, an overall relative percentage values of 27.2% L_A, 14.8% L_B, 5.3% L_C and 2.2% L_I in sheep and 27.3% L_A, 17.5% L_B, 6.6% L_C and 2.3% L_I in goats were recorded during the entire study period (Table 11). A clearly defined seasonal variation was observed in which the A type linguiform showed statistically very significant ($p < 0.05$) fluctuations in different months of the study period while others such as B type didn't show significant fluctuation during the study period. This is most probably suggestive of the lower ability of A type linguiform as compared to the B type linguiform in coping up dry months of the study period which is in agreement with the previous findings of LeJambre and Whitelock (1968). The A type linguiform was also found to be the one which holds the greatest proportion contributing the largest share to the linguiform complex.

Species identification of individual male worms in natural populations indicated that all types of combinations of Haemonchinae infections occurred in the study area. Out of the 1159 male Haemonchus collected from 76 sheep, 1102 (95.1%) were identified as *H. contortus*, 40 (3.4%) *H. placei* and 17 (1.5%) *H. longistipes* based on the values of measurements of the morphometric parameters of the spicules as outlined in Jacqueit *et. al,* (1997). Similarly from a total of 55 goats a total of 841 male Haemonchus were isolated and identified in to 812 (96.5 %) *H. contortus*, 25 (3.0%) *H. placei* and 4 (0.5%) *H. longistipes*. Both finding in goats and sheep suggest the coexistence and circulation of *Haemonchus species* among different animal species of the study area particularly that of cattle and camels. The different ruminant host species of Ogaden region of Ethiopia normally share the same grazing pasture favoring the transmission of infection among hosts. Even though mono-specific infections with *H. contortus* were common in the majority of the cases, 57.9% in sheep and 58.2% in goats, it seems that other *Haemonchus species* associations infections are also very important. The sympatric existence of two or three *Haemonchus species* in one host is promoted by extensive breeding on mixed pastures, which is very evident in the context of the study area.

The presence of non-specific *Haemonchus species* in both sheep and goats is comparatively lower than that of *H. contortus*, which is specific to small ruminants. *H. placei* was found in 28.9% of sheep and 34.5% goat populations studied but the proportion of these species to the total number of worms subjected for identification account only 3.4% in sheep and 3.0% in goats (Figure 5). On the other hand *H. longistipes* was found in 14.5% sheep and 5.4% goat populations but the proportion of these species within the total number of worms identified in sheep is 1.5% in sheep and only 0.48% in goats. This finding is inline with the study conducted in Northern Ivory Coast by Achi *et al* (2003). These findings suggest the possible appearance of a wide range of susceptible hosts could be one of the means of survival strategies of these species in the study area.

Efficacy of Albendazole and Tetramisole

Although the situation, degree and extent of susceptibility or resistance of gastrointestinal parasites of small and large ruminants in Ethiopia is not known, the results of the present study support the idea that in the current context of the study area anthelmintic resistance never pose serious economic losses in rural small holder farming system. Since anthelmintic resistance is an inevitable fate of anthelmintic usage it is essential to formulate strategic

programmes for the control of gastrointestinal nematodes, which do not rely entirely on the use of anthelmintics. The efficacy of commercially available anthelmintics on sheep and goat farms should also be regularly monitored to avoid losses due to anthelmintic failure. In order to preserve the full potential of all the evaluated anthelmintics the drugs should be used strategically in alteration with other broad-spectrum anthelmintics.

The results of the FECRT carried out and interpreted according to the WAAVP recommendations (Coles *et al*., 1992 and Wood *et al*., 1995) provided evidence of susceptibility of Ogaden isolate *H. contortus* to Albendazole Greece, Albendazole Pakistan, Tetramisole Greece and Duxamintic anthelmintics. According to Coles *et al* (1992), FECRT results below 90 % strongly suggest the presence of anthelmintic resistance.

In spite of the considerable variation in cost and preferences by the professionals and user society based on the country of origin of drugs, the results obtained in this study clearly confirmed equal susceptibility of Ogaden isolate to Exiptol Greece, Albendazole Pakistan, Tetramisole Greece and Duxamintic Pakistan. As indicated in Figure 7, the feacal egg counts (Epg) was reduced by 100% by all four anthelmintics according to the manufactures dose, in lambs infected with Ogaden isolate of *Haemonchus contortus,* which is one of the well known and important gastrointestinal parasites of small ruminants in tropics. Thus it was very interesting to note that all the evaluated anthelmintics irrespective of the country of origin appeared to retain a high level of efficacy of 100% against Ogaden isolate of *H. contortus*. It was generally believed that drugs from European countries are truly effective while those coming from developing countries are considered as fake and ineffective. In our present study, the two drugs were taken to accommodate the concern of most professionals whereby drugs coming from countries like Pakistan are considered as inefficacious despite the low cost of the drugs in open market. This piece of work generally disproved the irrational bias we have towards the drugs coming from developing countries. However, it is always advisable to undertake regular quality checkup for all drugs circulating in open markets to protect any undesirable effects.

The present FECRT finding is also supported by necropsy data from animals treated by the anthelmintics and by in vitro egg hatch assay results. The results of the controlled anthelmintic efficacy tests for each group of all experimental lambs and the calculated efficacy percentages of all the four anthelmintics were 100%. All the evaluated anthelmintics viz; Albendazole Greece, Albendazole Pakistan, Tetramisole Greece and Duxamintic Pakistan

at the manufacturers recommended dose rate completely removed the Ogaden isolate Haemonchus from all the experimental lambs further validating the 100% efficacy of all tested drugs. All the uninfected (negative) control group lambs were found to be negative for Haemonchus worms confirming that these lambs were kept in conditions that precluded any accidental infection during the whole experimental period.

Similarly the results of the egg hatch assay made on the Ogaden isolate in comparison with a known susceptible and resistant reference strains indicated the susceptibility of the Ogaden isolate of *Haemonchus contortus* to benzimidazole drugs (Figure 7). The LD_{50} values obtained for a known susceptible and resistant reference strains were 0.08µg/ml and 1.28µg/ml respectively while for that of Ogaden isolate, the LD_{50} value of concentration of Albendazole was 0.06µg/ml. The LD_{50} of 0.06 µg/ml albendazole for the Ogaden isolate *H. contortus* is with in the range of values indicative of susceptibility reported elsewhere for benzimidazole susceptible strains (LeJambre, 1976). LeJambre (1976) reported that nematode strains resistant to benzimidazoles generally had an LD_{50} of more than 0.12µg/ml.

The results of the present study clearly indicate that the three methods of susceptibility measurement techniques were observed to match in indicating the susceptibility of the Ogaden isolate *H. contortus* to Albendazole Greece. This is in accordance with the recommendations of WAAVPA that requires validation of the result of FECRT by egg hatch assay (Coles *et al*., 1992). Johansen (1989) also indicated that the egg hatch assay validated very well and can distinguish benzimidazole susceptible and resistant nematodes reliably.

Some of the previous studies made at field level in Ethiopia suggest the susceptibility of GIT parasites to benzimidazole and imidazothiazole compounds. Kasahun (1997) reported susceptibility of GIT parasites to Albendazole Tetramisole in Woliata sodo, Nessiru *et al.* (1997) stated the good efficacy of albendazole and Daniel (1998) also reported susceptibility of different species of parasites to Oxfendazole and Tetramisole at Sebeta farm.

The susceptibility of Ogaden isolate *H. contortus* to all the tested drugs observed in this study is indicative of the absence of development of anthelmintic resistance in the area of study. One of the most probable reasons is due to the very low frequency of anthelmintic treatment practiced in the area. In most cases, animals are treated only when they get sick and as a result there are large numbers of parasites as refugia in the study area.

The high efficacy of all the studied drugs observed in the experimental study can only be conserved and the effective field life of these drugs be extended by relying on better use of the available anthelmintics that avoids all the factors that selects resistant strains of parasites. Hence the tested anthelmintics of the different chemical groups can alternatively be used to control this isolate of parasite in the study area, provided these drugs are used appropriately and wisely to conserve their susceptibility by avoiding all the advantages that select resistant strain to these drugs. It should be noted that resistance genes are present in unexposed populations, albeit at very low frequency, and serve as a primer in the evolution of resistance when effective selection pressure is applied. This means that resistant individuals are already present in a population of parasites and resistance can be brought about when the selection pressure of resistant genes is increase (Martin, 1985). Moreover in order to maintain the effectiveness of the few broad-spectrum anthelmintic groups the mechanism of anthelmintic resistance should be clearly understood very well.

6. CONCLUSION AND RECCOMMENDATIONS

The results of the present study clearly indicated that the productivity of small ruminants of the study area is confronted by amazingly very high prevalence rate of 91.2% and 82.9% Haemonchus and 37.7% and 40.2% *T.axei* in sheep and goats respectively through out the whole year. This high frequency of occurrence of abomasal parasites coupled with very significant pathogenic effect and the losses it impose on productivity of the small ruminant sector of agriculture which is very important for the livelihood of pastoralists of the study area should receive special attention.

Traits of vulvar morphology are considered as markers of ecological adaptation in which linguiform vulval flaps were found to predominate throughout the study period both in sheep and goats. However, the absence of fluctuations in the proportions of the major vulvar flaps (linguiform, knobbed and smooth) among different months of the study period is indicative of the non-seasonal variation in proportion of vulval types except in the linguiform type A where a clear seasonal trend is observed.

The current study also clearly indicated the sympatric existence of two or three species of Haemonchus in a single small ruminant host, which may be one of the very important factors in the survival strategy of the parasite in which a wide host range is considered to be very useful mechanism. This suggests that control strategies of the parasites in the study area should consider the decisive role exhibited by all other possible hosts of the parasite with out which the control methods suffer from ineffectiveness.

The result of the controlled experimental study of the susceptibility of Ogaden isolate by egg hatch assay, FECRT and controlled anthelmintic efficacy test revealed that all the evaluated anthelmintics were observed to be very effective with the efficacy percentage of 100% for the tested batch of each drug on Ogaden isolate *H. contortus* of the study area. In spite of the general fact in Ethiopia and particularly of the pastoral farming situation of the study area that lack professional guidance and supervision of anthelmintic usage, coupled with the ineffective and, in some cases, nonexistent official control of the licensing and sale of these drugs, fortunately and perhaps surprisingly, anthelmintic resistance doesn't appear to be as common nor to pose such serious practical problems in the study area.

It will not be surprising to detect anthelmintic resistance in the near future this is so because anthelmintic resistance is indicated to be an inevitable consequence of anthelmintic usage due to the fact that genes that are responsible for anthelmintic resistance are naturally found in most important parasites of farm animals. Hence a highly effective anthelmintic can be converted to ineffective one within a year or less in the presence of an effective factor that selects resistant genes of the parasite via drug misuse. Moreover the dramatic and rapid spread of resistance to all the major classes of anthelmintics in many countries of the world should worn Ethiopia to use wisely and properly the existing drugs.

The high efficacy of all the four drugs detected in the study can be conserved if and only if consistently wise drug use and appropriate application of anthelmintics is applied in the study area. So if efficacy of anthelmintics is not preserved, anthelmintic treatment failures will result leading to the probable emergence of anthelmintic resistance.

In view of the current Ethiopian context, anthelmintic resistance merits urgent attention for several reasons of which some of them that can be mentioned are absence of clear information on anthelmintic efficacy or susceptibility or resistance on regional and country basis, lack of livestock owner and public awareness about the effect of anthelmintic resistance on the economy of the country, absence of functional drug use policy, lack of effective drug quality control, drug smuggling involving anthelmintics at the top, unknown source of anthelmintics, relatively cheap cost of drugs from open market, absence of regular drug efficacy evaluation, use of expired drugs. Hence the base of the emergence of anthelmintic resistance can be anywhere from the manufacturer to the final person administering drugs to animals.

In Ethiopia very scant information is available and no research has been conducted in the area of anthelmintic efficacy, susceptibility or resistance. This piece of information may therefore help the coming researchers to understand the status and the situation of the study area. More over the egg hatch assay technique being for the first time to be used in the country may also initiate other workers to apply it for anthelmintic efficacy evaluation.

In view of the findings of abomasal parasites of the present study and to maintain and conserve the efficacy of the evaluated drugs so as to prevent the probable inevitable development and spread of anthelmintic resistance in the study area and all over the country the following recommendations are forwarded:

- The problems of Helminthosis and anthelmintic resistance should receive special attention at all levels.
- Integrated parasite control programmes that combine all possible control methods involving strategic anthelmintic control, grazing management and breeding genetically resistant animals have to be implemented.
- Creation of society awareness about the problem of Helminthosis and risk of anthelmintic resistance
- The status of efficacy of anthelmintics in use should be made certain by encouraging researchers.
- Functional drug use policy should be developed urgently.
- Regular and effective drug efficacy control centers need to be come into effect.
- Accurate dose of drugs should be administered to animals or should be based on the dose of the heaviest animal in the flock.
- Anthelmintics should be used on basis of annual rotation.
- Anthelmintics treatments should be integrated with better management practices.

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8 LIST OF ANNEXES

Annex 1: Summary of previous studies conducted on GIT Nematodes of Small Ruminants in different parts of Ethiopia.

Year	Author	Study Area	*Haem.*	*Tricho*	*Oster.*	*Buno.*	*Oeso.*	*Trichu.*
1973	Graber	Shewa	72.7	-	-	-	-	11.3
1975	"	Wollo	58.3	-	-	-	-	-
1982	Bekele	A.A.	67.0	89.0	-	34.0	53.0	83.0
1983	Brook	D.Zeit	-	-	7.8	-	22.9	-
1983	""	D.Berhan	-	84.3	-	-	-	-
1983	"	Assela	32.8	-	1.8	-	-	-
1983	"	Awassa	3.0	1.4	-	-	82.1	-
1986	Gebreyesus	Gonder	36.36	6.81	-	34.09	77.27	54.55
1987	Solomon	Ogaden	93.61	33.0	-	32.0	52.0	92.0
1988	Ahmed	Wellega	88.23	29.4	-	-	-	76.47
1989	Tesfalem	Bale	59.21	38.1	-	55.2	76.32	61.84
1990	Gebrekiros	Awassa	75.7	18.4	-	-	31.1	9.7
1990	Njau	D.Bilca	2.9	19.4	1.5	-	25.4	-
1991	Bayou	Illubabor	58.0	20.0	-	44.0	86.0	24.0
1991	Melkamu	E.Showa	65.48	39.29	-	50.0	72.62	60.71
1992	Dereje	W.Soddo	80.0	10.0	-	44.0	90.5	50.0-
1992	Etagegnehu	Wollo	42.9	34.0	44.0	-	-	59.52
1993	Yosef	Assela	63.0	54.76	-	40.48	85.71	63.44
1994	Genene	Kombolcha	83.87	63.44	-	40.7	79.56	65.51
1997	Achenef	D.Berhan	62.87	51.72	-	-	13.79	6.67
1998	Getachewu	Mekele	95.55	28.89	-	-	82.22	36.04
1999	Donald	Eastern Ethiopia	98.8	49.6	-	42.4	50.0	67.6
2001	Abebe & Esayas	Eastern Ethiopia	90.82	55.8	-	38.97	74.8	51.75
2002	Haileleul	W.Soddo	61.63	22.09	-	41.86	74.42	

Annex 2: Differential parasite counting of the Abomasum

- The abomasums was ligated at both ends and removed from omasum and duodenum then it was opened along the greater curvature and its contents was thoroughly washed in to a graduated bucket under a slow jet of water.

- The mucous membrane being carefully rubbed with fingers to remove any worms adhering to it. The contents and washings were made to a total of 4 litters.

- Then vigorously stirred until all food material, mucous and water were thoroughly mixed. Then a total of 200ml of the contents was transferred to measuring cylinder in five steps of 40 ml per step while stirring the mixture.

- Then a sub sample of 20 ml was transferred in to a small graduated beaker to which 2-3ml iodine was added to stain the worms and 2-3ml sodium thiosulphate solution was also added to decolorize debris.

- Then about 3-4ml of the sub sample placed in a petridish having parallel lines marked on it 5mm apart, diluted with water and the worms counted under a stereomicroscope. Samples were examined for the presence of parasites, identified and counted as male, female and juvenile using a stereomicroscope (10x objective).

- The total number of worms counted in the 20 ml sub sample is then multiplied by 100 to get the total number of worms present in the abomsum (MAFF, 1979).

Annex 3: Recovery and Count of Mucosal Larvae of *Haemonchus species*

- The opened and washed abomasums with the mucous membrane facing down was placed in a tray containing warm saline solution of 0.8% and left soaked for 8 hours.
- Then the abomasum was removed; rinsed well with warm saline solution and discarded. The saline solution in the two containers was sieved on 25 μm sieve aperture to retain the larvae.
- Then the total volume of the saline solution was made up to 200ml out of which an aliquot of 10 ml was examined in a petridish under a stereomicroscope and the larvae counted and the species identified. The total number of larvae was determined as the number of larvae in the sub sample multiplied by 20, which gives the total abomasal larval count.

Annex 4: Culture and Recovery of infective larvae of *Haemonchus contortus* from Ogaden sheep

- The contents of the abomasum were poured a little at a time on to a sieve mesh screen with an aperture of 0.15mm.
- Then washed with a stream of until no more food matter passes through. Then the sieve is inverted over a tray and by means of stream water the worms and food material collected on the sieve are washed in to it. Then sufficient number of Ogaden strain female worms were picked by forceps and collected in to a small clean beaker as outlined by (MAFF, 1979).
- Then the collected female worms were thinly sliced by scalpel blade in 0.8% NaCl solution to release their eggs. The egg suspension was spreaded over finely broken up horse faeces that was sterilized for two hours at 140^{oc}. Moist and crumbly consistency was obtained by adding dry faeces or water to ensure sufficient moisture for development of eggs in to infective larvae (MAFF, 1979 ; Hansen and Perry, 1994; Wood *et al.*, 1995).
- Then the mixture was transferred to petridish and placed in an incubator running at 27^{oc} and left there for 7-10 days to get the infective L_3 stage.
- The cultured petridish was stirred each day to avoid the growth of fungi and aerate the lower layers of the culture and water was added to the cultures every 1-2 days. Sufficient number of infective larvae was obtained after several culturing of the eggs obtained from freshly collected female worms (Bowman, 19955).
- To harvest infective larvae the culture was removed from the incubator and the faeces were tipped out of the culture pertridish in to a 300ml wide mouthed jars. Then lid of the culture petridish and its cover were washed with small quantity of water so as to remove any migrated larvae. Then water was added to the culture until the jar is full to the brim.
- A standard petridish was inverted over the mouth of the jar and the whole was turned upside down so that the inverted jar stands in the petridish. About 15ml of water was poured into the petridish and allowed to stand over night. The next day the fluid in the petridish containing many L_3 was pippetted into a conical centrifuge tube and concentrated by sedimentation (MAFF, 1979; Hansen and Perry, 1994; Wood *et al.*, 1995).
- The larvae were passaged once in sheep before transit in that this Ogaden isolate of *Haemonchus contortus* served as initial infective larval source and was maintained in

apparently nematode free sheep for production of subsequent source of infective larvae for experimental infection.The L_3 used for the experimental infections was obtained through faecal culture at 27^{oc} for 7- 10 days, baermaniztion of the faeces as stated above, purification by sedimentation and recovery of the clean larvae. The larvae harvested were stored in tape water at +4 ^{o}C until use (MAFF, 1979; Wood *et al.,* 1995).

Annex 5: Preparation and Counting of Larval incoula

- Freshly harvested larvae of 10 days old were used for experimental infection as recommended by Wood *et al*., 1995. The larval suspension was removed from refrigerator and its volume was measured, recorded and then transferred to a tightly stoppered flask and was vigorously shaken for several minutes to get a homogenous suspension.
- Then 100µl of the larval suspension was immediately withdrawn with a micropipette and transferred to a microscope slide. The drop was then spread out and covered with a 38 x 22mm cover slip. The slide was methodically searched under a microscope and the larvae counted. This shaking, sampling and counting was repeated five times and the final count was based on the mean count of these five samples.
- Then the number of viable L_3 per milliliter was established. Then the desired number of L_3, which was 4000 in the present study, in an inoculum volume of 15ml per animal was dispended into syringes that was labeled with the ear tag number of the recipient animal.
- Then the inoculum was given orally and the syringe was rinsed with 15ml water and the washings administered (Wood *et al*., 1995; MAFF, 1979).

Annex 6: Modified McMaster Egg Counting Technique

- 3g of faeces is weighed and broken up thoroughly in 42ml of tape water. The mixture is then poured through a fine mesh sieve of 250µm aperture.
- The filtrate is then collected, agitated and filled into 15ml centrifuge tubes and centrifuged at 2000rpm for 2 minutes.
- The supernatant is poured off; the sediment is agitated very well and replaced by saturated salt solution flotation fluid to the previous level.

- The suspension is then inverted six times so as to have very good homogenous distribution of the eggs in the mixture.
- Then using a Pasteur pipette the fluid is removed to fill both chambers of the McMaster slide with out interruption to avoid the formation of bubbles in compartment. After 5 minuets the eggs float to the cover slides and transferred under the microscope and all the eggs in the ruled areas of the McMaster are counted.
- The number of eggs in both chambers of the McMaster are counted and multiplied by 50 for determination of the total number of eggs per gram of faeces (epg).

Annex 7: The Egg Hatch Assay techinque for Albendazole

- Pretreatment samples were pooled for all lambs and Ogaden isolate Haemonchus eggs recovered using Magnesium sulphate as a flotation fluid.
- Faecal samples were soaked in clean tape water and sieved through 25, 150, 60 and 25µm, the eggs being retained on the 25µm sieve.
- The egg suspension was then centrifuged at 3500 rpm for 10 minutes. Then supernatant was decanted and replaced by Magnesium sulphate at 1:9 ratios and then centrifuged at 2500 rpm for 10 minutes.
- Then Magnesium sulphate was removed from eggs with excess tape water. 50-100 eggs in 100 µl of water were incubated for 48 hours at 23^{oc} in serial concentrations of albendazole dissolved in 1% DMSO.
- The concentrations of Albendazole ranges from 0-8.96µg/ml. Lugol's iodine was used to stop further hatching and all eggs and larvae at each Albendazole concentration counted as dead, embryonated or hatched to L_1.

Annex 8: Egg recovery from faeces

- Freshly collected faes in less than 2hrs was that faeces mixed very well with tape water to have good mixed suspension of eggs in 1-2 litters.
- Then a small amount of the faecal suspension was put on the first top largest mesh sieve material that was arranged in order of mesh size, the smallest mesh sieve being at the bottom.
- Then washed with jet of pressurized water until the liquid passing through the smallest mesh sieve is clear and transparent.

- Then the material remaining on the smallest sieve size of 25 µm was collected in a small beaker. All the prepared faecal suspension was treated in the same fashion to recover the remaining eggs.
- Then all the recovered egg material was kept at $+4^{oc}$ until processing to avoid embryonation of the eggs.
- Then after homogenizing the feacal material was centrifuged at 3500 rpm for 10 minutes and the supernatant was discarded since eggs sediment.
- Then a solution of magnesium sulphate at 1.27 density was added in proportion of 9:1 faeceal sediment, mixed very well by glass rod and then centrifuged at 2500 rpm for 10 minutes.
- Then to remove magnesium sulphate from eggs the supernatant was filtered by jet water in 60µm sieve mesh size at the top and 25µm sieve at the bottom and the egg suspension remaining on the 25µm sieve was collected.
- Then the concentration of eggs in the suspension was determined by counting under a microscope and adjusted at 50-100eggs per 100µl of water and left for 1hr at $+4^{oc}$ until processing.
- Known susceptible and resistant reference strains of parasites are maintained to compare with the response of the test isolate.
- Eggs from susceptible nematodes rarely hatch at concentrations of more than 0.1µg/ml of thiabendazole (Whitelock, 1980). This concentration is often used as the discriminating dose (cutoff value) for determining whether a parasite is susceptible or resistant to the benzimidazole group of anthelmintics.

Annex 9: Preparation of different dilution series of the test anthelmintic solution

- In the first instance solution No1 (mother solution) was prepared at concentration of 1000 µg /ml by dissolving 50 mg of albendazole Greece in 10ml DMSO (dimethylsulphoxied) solution and 40ml distilled water.
- Then solution No 2 at concentration of 480 µg/ml was prepared by mixing 24ml of mother solution (solution No 1) with 26ml-distilled water. Also solution No3 was prepared at concentration of 60µg/ml by mixing 3ml of mother solution (solution No1) with 47ml of distilled water. All the solutions were shaked very well while mixing at least for 15 minutes.

- Then 56 small tubes of 1.8ml size were prepared and labeled. The control was prepared in 5 replicates where as each of the 17 different dilution series were prepared in 3 replicates.
- Then 100µl of the egg suspension was added to all the 56 tubes including the control where as 50µl of albendazole Greece solution was added to the tubes No 1-17 but not in the control.
- Then all the tubes were incubated at 23^{oc} for 48hrs after which 10µl of iodine was added and kept at -20^{oc} in deep refrigerator to stop further development of the eggs. Then all the contents of each tube were examined under a microscope to count the number of eggs that were died, embryonated or hatched in to first larval stage.
- Then the lethal death (LD_{50}) of the anthelmintic under evaluation was determined after correting the natural mortality in the control test tubes containing only distlled water and eggs.

Annex 10: The No of solution, volume of the different test solution, volume of distilled water and the concentration of each test tube are in the egg hatch assay indicated in the following table:

Code	No of solution used	No of replicates	Volume of solution taken inµl	Volume of distilled H_2O taken in ml	Final Concentration of the anthelmintic
Control	Distilled H_2O	5	0	50.0000	0
1	3	3	25µl	49.9975	0.01
2	3	3	50	49.9500	0.02
3	3	3	100	49.9000	0.04
4	3	3	150	49.8560	0.06
5	3	3	200	49.8000	0.08
6	3	3	300	49.7000	0.12
7	3	3	400	49.6000	0.16
8	3	3	600	49.4000	0.24
9	2	3	800	49.2000	0.32
10	2	3	150	49.8560	0.48
11	2	3	200	49.8000	0.64
12	2	3	300	49.7000	0.96
13	2	3	400	49.6000	1.28
14	2	3	600	49.4000	1.92
15	2	3	800	49.2000	2.56
16	2	3	1200	48.8000	3.84
17	2	3	1600	48.4000	5.12

Annex 11: Monthly Sex ratio of *Haemonchus species* in sheep and goats.

Host spp	Worm sex	Month								
		Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Average M: F
Sheep (n=114)	Male	5100	3600	3400	3000	1000	2300	2200	2500	2887.5
	Female	10200	7300	6400	5200	1800	4100	3400	3600	5262.5
	M: F	0.5	0.5	0.5	0.6	0.5	0.6	0.6	0.7	0.5
Goat (n=82)	Male	ND	ND	1400	1700	3100	2500	2800	2600	2350.0
	Female	ND	ND	3000	3600	5500	4100	3800	4300	4050.0
	M: F	ND	ND	0.5	0.5	0.6	0.6	0.7	0.6	0.6

Annex 12: Monthly Sex ratio of *T.axei* in sheep and goats.

Species	Worm sex	Month								
		Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Average M: F
Sheep (n=114)	Male	2500	1400	600	1400	100	900	1000	700	134.4
	Female	5800	3200	1100	3500	300	1600	1700	1600	293.7
	M: F	0.4	0.4	0.5	0.4	0.3	0.6	0.6	0.4	0.5
Goat (n=82)	Male	ND	ND	800	1300	2100	1900	2400	1700	283.3
	Female	ND	ND	2100	2500	4200	2900	2700	2500	469.4

M: F	ND	ND	0.4	0.5	0.5	0.6	0.9	0.7	0.6

Annex 13: Average value of Morphometrics of spicules in μm in sheep and goats on monthly basis

Animal species	Parameters measured	Month							
		Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Sheep	TL	484.6	482.4	472.8	454.1	469.4	470.5	462.7	455.0
	THr	43.9	43.7	42.6	41.6	42.7	42.4	41.4	42.1
	THl	22.1	23.9	22.7	21.1	23.4	22.0	22.8	22.0
	DF	-0.2	-0.7	-0.3	-0.7	-0.2	-0.4	-0.5	-0.5
Goat	TL	ND	ND	484.1	468.4	450.1	447.3	468.9	472.7
	THr	ND	ND	42.9	41.6	41.0	41.0	42.2	41.5
	THl	ND	ND	21.0	21.6	21.1	21.5	21.1	21.7
	DF	ND	ND	-0.5	-0.6	-0.8	-0.7	-0.6	-0.6

Annex 14: Number and percentage of animals infected by different Haemonchus species on monthly basis

Animal Species	Haem. Spp	Month							
		Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Sheep		10	9	9	9	9	11	10	9
	H.c	5 (50)	3 (33.3)	6 (66.7)	7 (77.8)	4 (44.4)	7 (63.6)	(60.0)	5(55.5)
	H.c & H.p	1(10)	3(33. 3)	0 (0)	1 (11.1)	2 (22.2)	4(36.4)	2(20.0)	4(22.2)
	H.c& H.l	0 (0)	3(33.3)	0 (0)	0 (0)	2(22.2)	0(0)	1(10)	0(0)
	H.c,H.p& H.l	4(40)	0 (0)	3(33.3)	1(11.1)	1(11.1)	0(0)	1(10)	0(0)
Goat		ND	ND	9	9	9	9	9	10
	H.c	ND	ND	1(11.1)	7(77. 8)	9 (100)	7(77. 8)	4(44.4)	4 (40)
	H.c & H.p	ND	ND	6(66.7)	2(22.2)	0(0)	2(22.2)	5(55.5)	6 (60)
	H.c& H.l	ND	ND	2(22.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	H.c H.p& H.l	ND	ND	0(0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

ND= not done, ( ) H.c H.p H.l

Annex 15: Average Body Weight of experimental animals infected with *H. contortus* on Weekly basis

Group	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Exiptol Greece	11.3(8.2-14.4)	12.1(9.0-15.2)	11.9(8.8-15.0)	12.4(9.3-15.5)	12.0(8.9-15.1)	11.5(8.4-14.6)	12.7(9.6-15.9
ABZ Pakistan	13.5(12.7-14.3)	15.1(14.3-15.9)	15.0(14.2-15.9)	15.7(14.9-16.5)	15.5 (14.7-16.3)	15.0(14.2-15.8)	16.45(15.6-17.3)
TTM Greece	12.8(11.0-14.6)	13.8(12.0-15.6)	13.9(12.1-15.7)	14.00(12.2-15.8)	14.3(12.5-16.1)	13.5(11.7-15.3)	14.7(12.9-16.5)
Duxamintic Pakistan	13.1(10.8-15.4)	14.0(11.7-16.34)	14.0 (11.7-16.4)	14.40(12.1-16.7)	14.4 (12.1-16.7)	14.0(11.7-16.3)	14.7(12.4-17.0)
Positive control	12.2(9.8-14.6)	12.80(10.4-15.2)	13.1(10.8-15.5)	13.5(11.1-15.9)	12.9(10.6-15.3)	12.5(10.1-14.9)	12.5(10.1-14.9)
Negative control	14.2(10.9-17.5)	15.1(11.8-18.4)	15.2(11.9-18.5)	15.5(12.2-18.8)	16.0(12.7-19.3)	16.3(13.0-19.6	16.9(13.6-20.2)

*=Group average Body weight.

( )= Lower and upper bound of 95% Confidence interval

Day 0: day of infection

Day 35: day of treatment

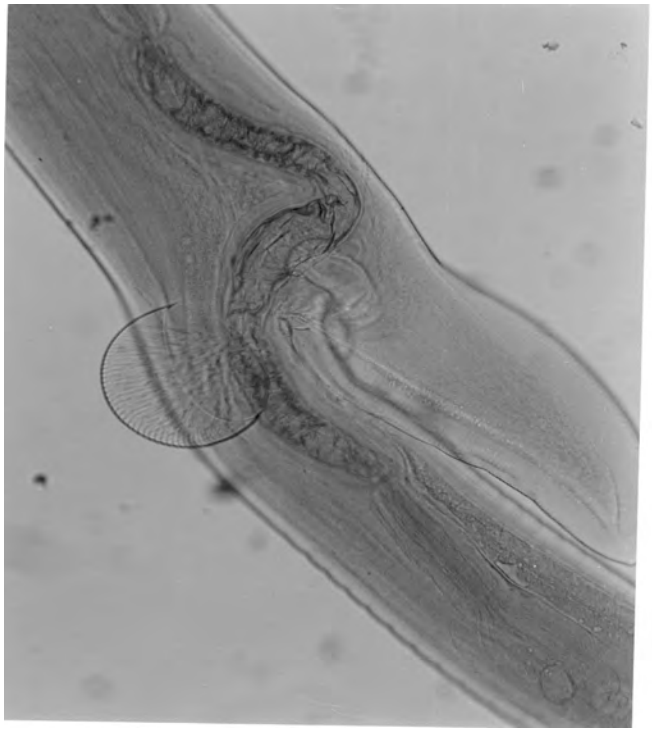
Day42: day of slaughtering

Annex 16: Estimated LD_{50} values of Ogaden isolate compared to known susceptible and resistant reference strains

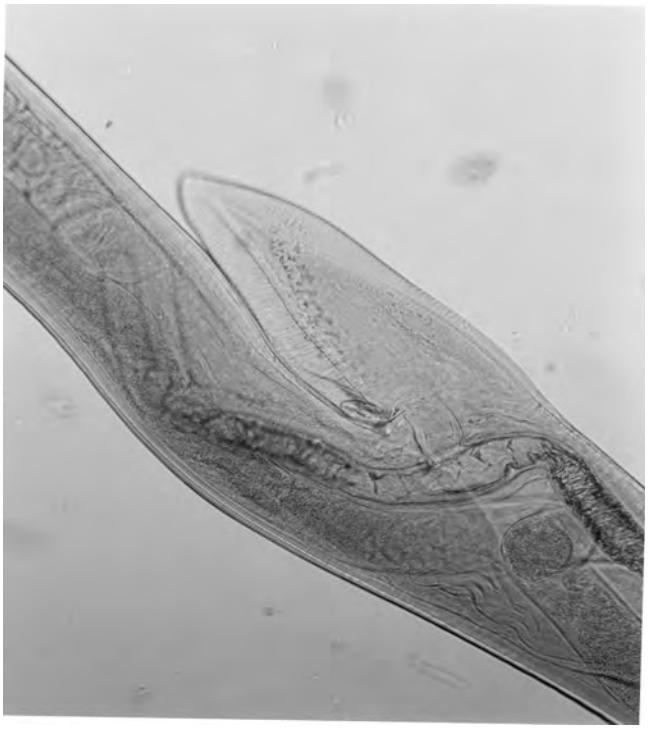
No	ABZ conc.	Ogaden Isolate			Susceptible strain			Resistant Strain		
		Corrected death	Total Sum	Corrected death %	Death	Total Sum	Death %	Corrected Total death	Total Sum	Death %
1	0.01	9.00	58.33	15.43	4.47	46.80	9.55	3.93	53.93	7.29
2	0.02	29.33	81.00	36.21	8.13	47.13	17.25	6.27	55.60	11.28
3	0.04	27.67	79.34	34.87	14.47	47.47	30.48	7.60	61.60	12.34
4	0.06	31.00	62.00	50.00	11.80	44.47	26.53	7.93	58.93	13.46
5	0.08	32.35	62.35	51.88	20.80	40.80	50.98	10.60	61.60	17.21
6	0.12	46.00	89.00	51.68	16.80	42.47	39.56	10.93	54.26	20.14
7	0.16	40.67	79.67	51.05	29.47	57.47	51.28	11.93	57.93	20.59
8	0.24	42.33	81.66	51.84	26.13	58.13	44.95	14.27	57.27	24.92
9	0.32	33.67	73.67	45.70	26.80	50.80	52.76	10.93	53.26	20.52
10	0.48	61.67	88.34	69.81	33.47	60.47	55.35	24.26	55.27	44.51
11	0.64	67.66	90.99	74.36	33.14	48.81	67.89	19.27	49.94	38.59
12	0.96	77.00	90.67	84.92	25.47	44.80	56.85	20.60	47.60	43.28
13	1.28	89.67	93.34	96.07	25.80	45.80	56.33	23.26	45.93	50.64
14	1.92	80.00	84.00	95.24	31.46	47.13	66.75	20.60	44.27	46.53
15	2.56	80.67	81.00	99.59	26.80	41.47	64.62	21.60	42.93	50.31
16	3.84	77.33	78.33	98.72	30.13	44.46	67.77	20.93	40.60	51.55
17	5.12	80.00	80.00	100.00	30.13	42.46	70.96	19.27	38.27	50.35
18	6.4	-	-	-	32.80	36.13	70.96	20.60	37.60	54.79
19	7.68	-	-	-	38.80	41.80	92.80	19.93	39.60	50.33
20	8.96	-	-	-	39.13	39.13	100	31.26	38.86	80.44

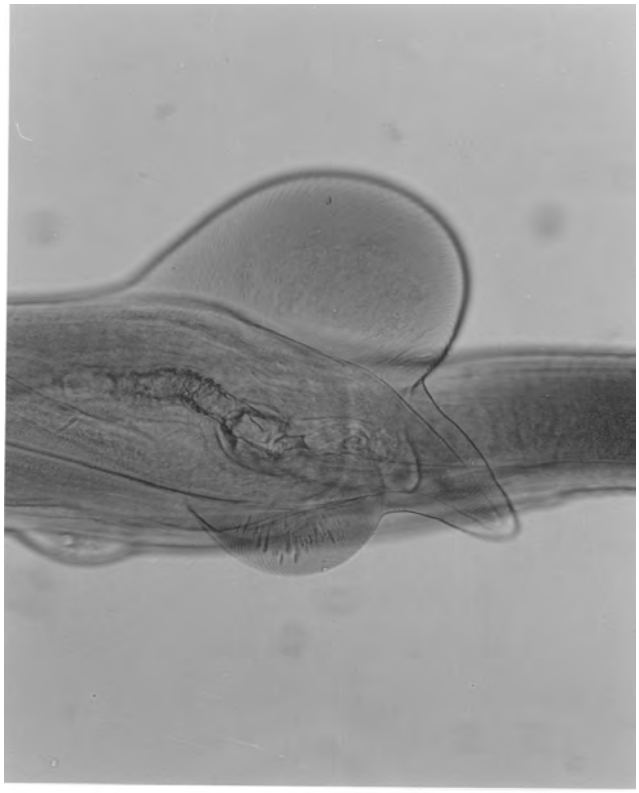
Annex 17: Plates to show the different vulvar flaps of female Haemonchus species, spicules of male Haemonchus and T.axei

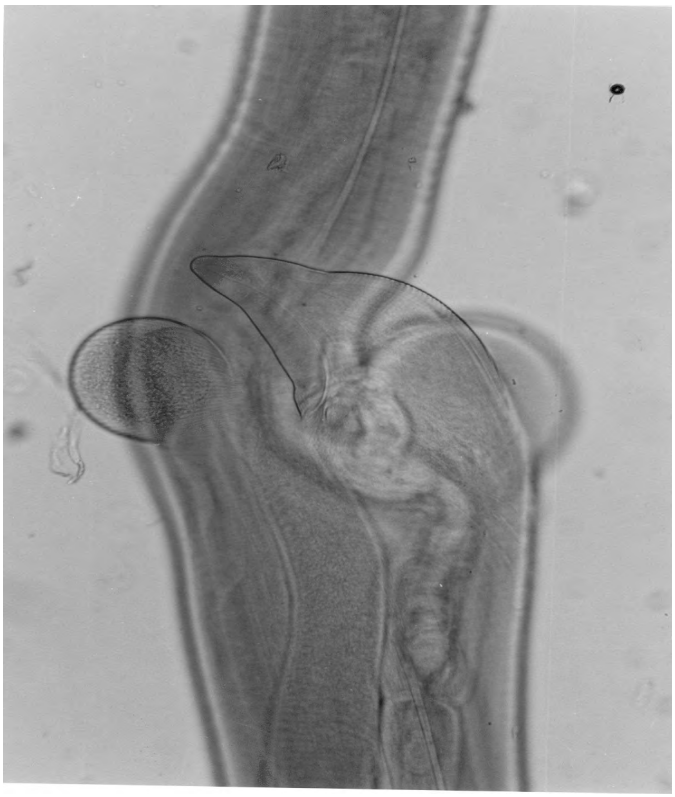
Linguiform A



Linguiform B

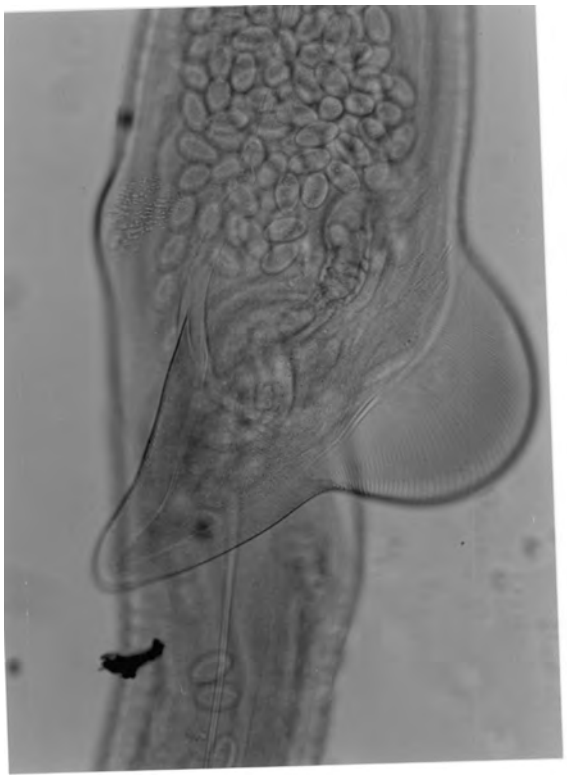




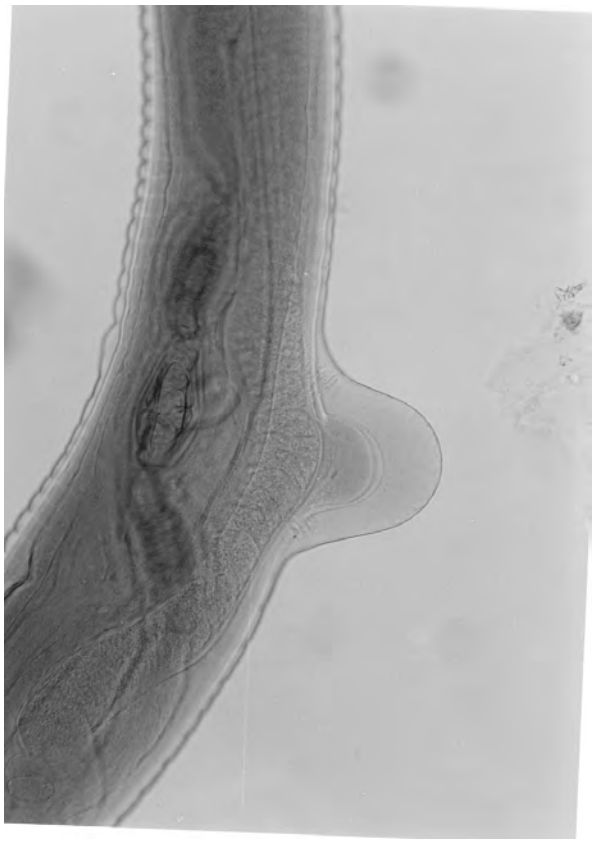


inguiform C

Linguiform I



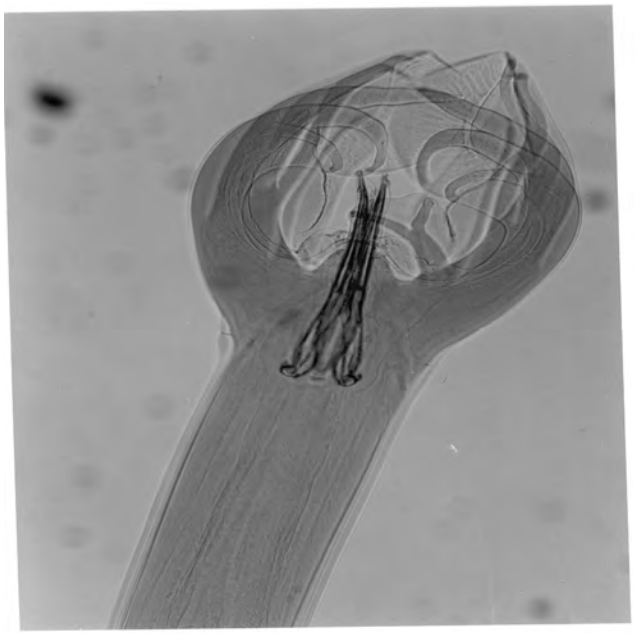
Linguiform I



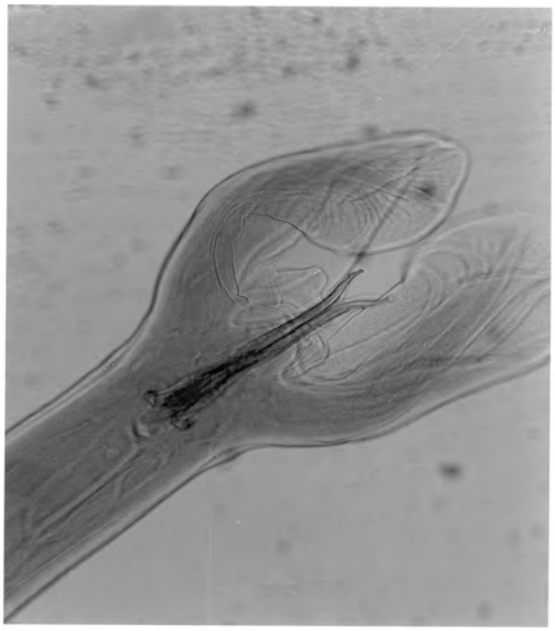
Knobbed Vulvar flap

Smooth Vulvar Flap

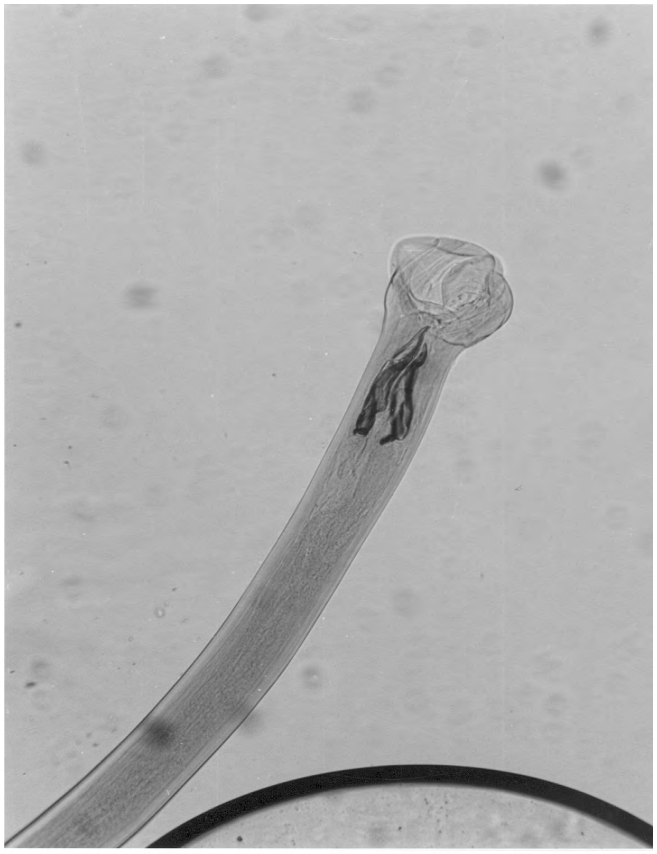
Male Haemonchus Spicules



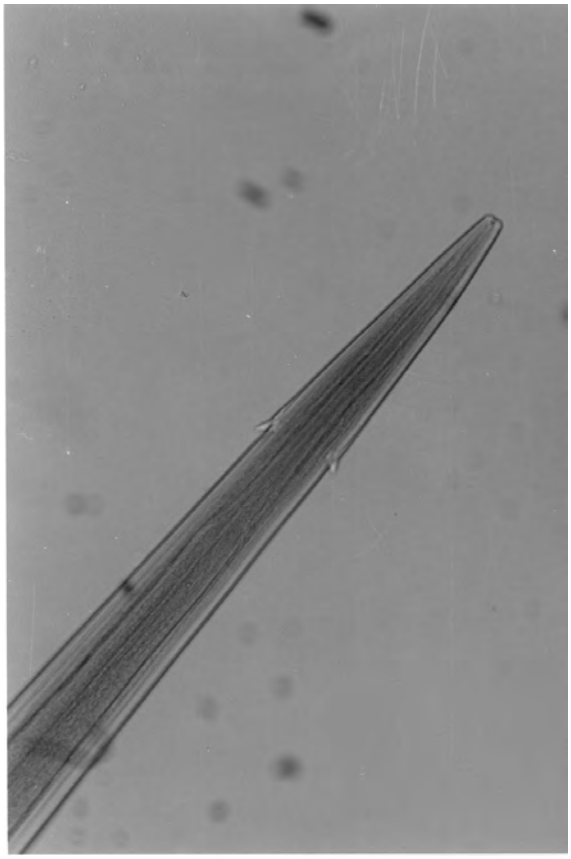
T. axei Spicules



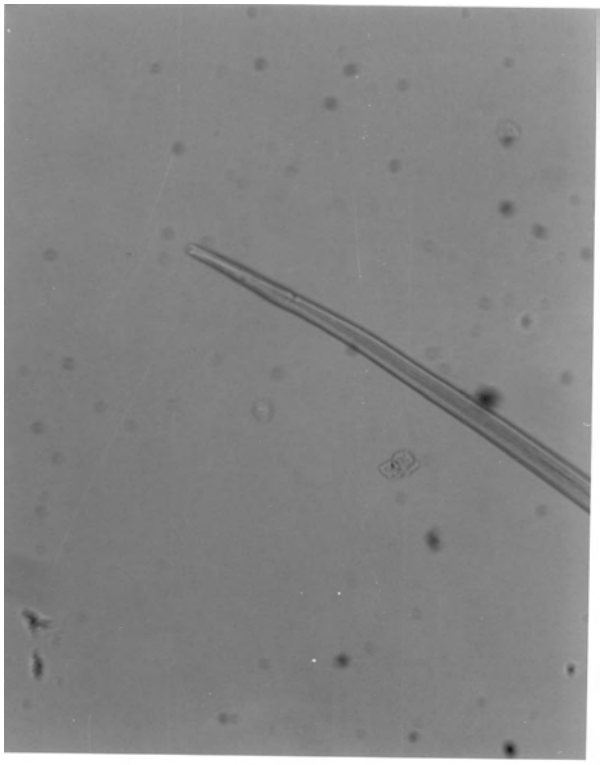
Haemonchus cervical papillae

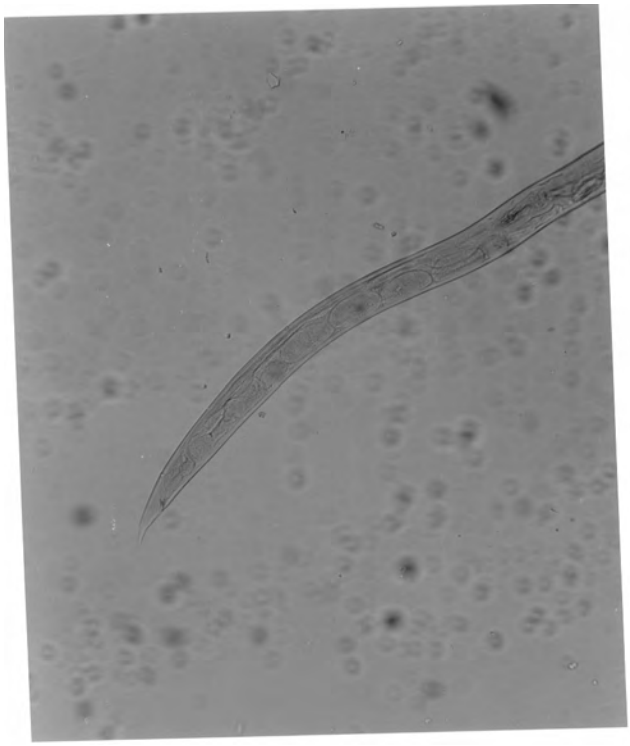


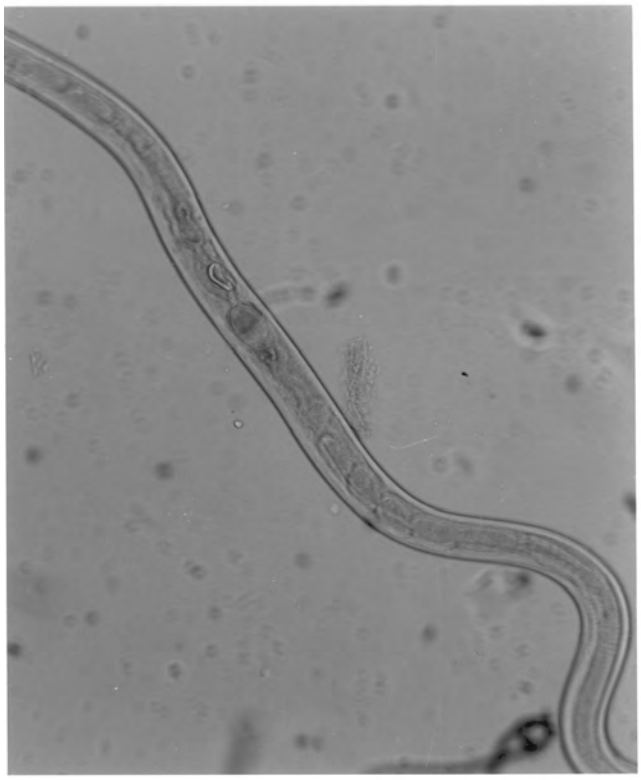
Esophageal Notch of *Taxei*



Pole to pole arranged eggs of female *T. axei*







Pole to pole arranged eggs of female *T. axei*

9. CURRICULUM VITAE

1). Name: Bersissa Kumsa Eseta

Address: Bedelle Regional Veterinary Laboratory.P.O.Box 15, Bedellee, Ethiopia.

Phone (07) 45-01-69/70

Nationality: Ethiopian

Date of birth: 26th August 1972.

2). Educational Background

Year	Institution	Award
1976-1984	Shambu Elementary School	
1985-1988	Shambu Singer secondary School	Ethiopian School leaving certificate Examination
1989-1994	Addis Ababa University Faculty of veterinary Medicine	Doctor of Veterinary Medicine
2003-2004	Addis Ababa University Faculty of veterinary Medicine	MSc in Tropical Veterinary Medicine (Parasitology)

3. Work Experience

Year	Institution	Responsibility
1993-1994	East Wollega at Nekempte Oromia Agricultural development Bureau	Research as undergraduate on Hydatidosis at Nekempte
1995-1996	Oromia Agricultural development Bureau Illubabor Zone Diddessa district	District field veterinarian
1996-1998	Oromia Agricultural development Bureau Illubabor Zone Diddessa district	District veterinary section team leader and field veterinarian
1998-2000	Oromia Agricultural development Bureau Illubabor Zone	Illubabor Zonal veterinary Officer and Tsetse & Trypanosomosis control project coordinator (funded by MfM (NGO)
2000-2001	Oromia Agricultural development Bureau Illubabor Zone	Illubabor Zone Veterinary Section NLDP coordinator
2001-2002	Oromia Agricultural development Bureau, Bedelle Regional Veterinary Laboratory	Immunology Research Officer

4). Language Skill

Oromiffa----------Mother language

Amaharic----------Writing and Speaking

English---------- Writing and Speaking

5). Membership

Member of the Ethiopian veterinary Association

6) Research Output

a). Present Status of Rabies in Ethiopia (Seminar Paper, 1993).

b). Hydatidiosis at Nekempte, Prevalence and economic significance (DVM thesis paper, 1994)

c). Community based Tsetse and Trypanosomosis control by NZI trap technology in 5 districts of Illubabor Zone of Oromia Administrative region with MfM (NGO).

7) Short term Trainings

a) Certificate in Training on community based Tsetse and Trypanosomosis control by Nzi trap technology by Ethiopian Science and technology commission.
b) Certificate in Training on Tsetse & Trypanosomosis by Target and Spot on technology by NTTCC.

10. SIGNED DECLARATION SHEET

I the under signed, declare that the thesis is my original work and has not been presented for a degree in any University.

Name: Bersissa Kumsa Eseta

Signature_____

Date of Submission: June, 11,2004.

This thesis has been submitted for the examination with my approval as University advisor.

Dr. Abebe Wossene(DVM, MSc, Associate pro.)_____