Anthelmintic utilization practices in Ada'a district and in vitro anthelmintics efficacy study against *Haemonchus.contortus* and *Trichostrongylus.columbriformis in Oromia, Ethiopia*

A THESIS SUBMITTED TO THE COLLEGE OF VETERINARY MEDICINE, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF DEGREE OF MASTER OF SCIENCE IN TROPICAL VETERINARY PARASITOLOGY

BY

GEBEYEHU ALKADIR BACHA

Bishoftu, Ethiopia

June, 2020

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DEPARTMENT OF VETERINARY PARASITOLOGY AND PATHOLOGY

As members of the examining board of the final **MVSc** open defense, we certify that we have read and evaluated the thesis prepared by Gebeyehu Alkadir entitled: **Anthelmintic utilization practices and In vitro efficacy study against local isolate of *Haemonchus.contortus* and *Trichostrongtylus.columbriformis* in Ada'a woreda, Oromia Region, Ethiopia,** and recommend that it be accepted as fulfilling the thesis requirement for the degree of Master of veterinary science in Veterinary Parasitology

Dr. Jirata Shiferew (Chair person	Signature	Date
Dr. Solomon Gebru (External Examiner	Signature	Date
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Dr. Bersissa Kumsa (Co-adviser)	Signature	Date

Statement of the Author

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Gebeyehu Alkadir Bacha	Signature	Date

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Dedication

This Paper is dedicated to the memory of my father Ato Alkadir Bacha Erena, who was absolute, reasonable and genuine father.Whom I like than everything but missed due to unexpected sudden death on January 22, 2016. But I am left with his wise remarkable ideas, sayings, suggestions and quotes that have special area and with me throughout my life. All of his encouragements during him alive was very remarkable and strengthen me in challenging of my life situations and due to this I have great memory throughout my life.

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List of abbreviations

AChR-Acetyl-Choline Receptors

AAD = Amino-acetonitrile derivatives

ADM =Adult Motility Test

ALB – Albendazole

AR – Anthelmintic -Resistance

AS-PCR- An allele-specific polymerase chain reaction

BZ – Benzimidazoles

BZ-R-Benzimidazoles Resistance

EHT – Egg Hach assay

FEC – Faecal egg Counts

FECRT – Faecal Egg Count Reduction Test

GIN Gastro-Intestinal nematodes

GIT-Gastro-intestinal Tract

IVM- Ivermectin

LDA-Larval Development Assay

LEV - levamisole

MBD - Mebendazole

MLs – Macrocyclic Lactones

SNPs-Single Neuclotide Polymorphism

TCBZ = Triclabendazole,

TM =Tetramisole

WAAVP=World animals advanced veterinary parasitology

LMT=Larval Motility Test

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Summary

The present study was conducted to assess anthelmintic (AH) utilization practices in Ada'a District of East Showa zone and evaluate the efficacy of commonly used anthelmintic classes of different brands collected from local markets A structured questionnaire survey was undertaken with 100 respondents farmers owning sheep and/or goats. For the Ah efficacy study, four drugs of different brands (Albendazole (ABZ) for egg hatch test, levamisole (LEV) and ivermectin (IVM) for larval motility test, and tetramisole (TMZ) for adult motility test) were investigated against local isolate of *Haemonchus.contortus and Trichostrongylus columbriformis.* ABZ and LEV brands originated from China (CN), East Africa (EA) and India (IN), Ivermectin from CN, IN and Uruguay (UG), whereas Tetramisole was from CN and IN. Serial dilutions of the drugs were made to achieve concentrations of 1.0, 0.5, 0.25, 0.125, and 0.0625µg/ml. To undertake egg hatch test 100 eggs/well of *H. contortus or T. colubriformis* were incubated with the drugs at 27^0C for 48 hours. Larval motility test was done with 50L3 of *H. contortus* or *T. colubriformis* mixed with the drug of choice and incubated at 25 ^{0}C for 24hrs after which motile and non-motile worms were counted. Similarly, the adult motility test was done with 15 adult *H. contortus*/well mixed with the test AH and incubated at 37^0c for 10 hours. All tests were done in triplicates and the median values were taken. Control wells contained eggs, L3 or adult worms with distilled water. The findings show that: a) the questionnaire survey indicated ivermectin was the most commonly used drug (39 %) followed by albendazole (36 %), tetramisole (17 %) and levamisole (8%), b) in the *in vitro* assays, all tested anthelmintics brands showed concentration-dependent responses on eggs, larvae and adult parasites; c) in egg hatch test on *T.colubriformis* eggs, CN and IN brands of albendazole performed >50% egg hatch inhibition at all drug concentrations; d) the EC50 for inhibition of *T.colubriformis* egg hatching was 2.375µg, 0.087µg and 1.199µg respectively for EA, CN and IN brands. The EC50 against *H.contortus* eggs was 0.504µg, 0.017µg and 1.886µg respectively for EA, CN and IN brands; e) East Africa brand of levamisole was more effective at reducing *H.contortus* larval motility than Indian brand (92% and 76% respectively) at 0.5µg while it is better at 0.5µg, 0.25µg and 0.125µg against *T. colubriformis* ($P<0.05$). The EC50 for inhibition of motility of *T.colubriformis* L3 by levamisole was 0.057µg and 4.028µg respectively for EA and IN brands suggesting a much better performance of EA brand over the Indian brand. The EC50 of levamisole against *H.contortus* L3

was 0.058µg and 0.048µg respectively for EA and IN brands; f) the three brands of ivermectin at all concentrations tested had similar efficacy against motility of *H. contortus* L3. The linear regression for concentration-response curve indicates that the EC50 for inhibition of motility of *T.colubriformis* L3 by ivermectin was 0.073µg, 0.178µg and 0.040µg respectively for CN, UG and IN brands suggesting a lower performance of the Uruguay brand compared to the others. Similarly, the EC50 of ivermectin against *H.contortus* L3 was 0.025µg, 5.040µg and 3.061µg respectively for China, Uruguay India brands; g) in the adult motility test, both China and India brands of tetramisole have performed similarly against *H. contortus*. However, the EC50 was 0.134µg and 5.576µg respectively for CN and IN brands suggesting a marginal performance of the former and a much lower efficacy of the latter brand. In conclusion, excepting for few cases, ABZ, IVM and TMZ brands from china origin are more effective against eggs, L3 and adults of the parasites concerned. On the other hand, LEV from East Africa Company is proven effective against L3 of both parasites whereas LEV and IVM from Indian origin are effective only against L3 of one species at acceptable concentration. Therefore, while recommending anthelmintics of choice such variations must be taken in to account.

Key words: anthelmintics, *H. contortus, T. colubriformis*, egg hatch test, larval motility test, adult motility test, questionnaire survey

1. INTRODUCTION

Livestock is an important and integral component of agriculture, which is the pillar of the Ethiopia economy and is believed to have the largest livestock population in Africa (Afras *et al*.,2018). Among all the livestock that constitute Ethiopian farm animals, ruminants comprising of cattle, sheep and goats are among the main source of draft power (cattle), wealth accumulation purposes and income generation. Sheep and goat play an important economic role in the overall production system of large and small scale farmers, where most shoat production is for wool, leather and meat production (Welay *et al*., 2018). Ethiopia lies within the tropical latitudes of Africa and has an extremely diverse topography, wide range of climatic features and multitude of agro-ecological zones, which make the country suitable for different agricultural production systems. This in turn has contributed to the existence of a large diversity of farm-animal genetic resources in the country. The current livestock production of Ethiopia is estimated as 53.99 million heads of cattle, 25.49 million sheep, 24.06 million goats, 1.91 million horses, 6.75 million donkeys, 0.35 million mules, 50.38 million of poultry, 0.92 Million of camels and 5.21 million of bee hives. Sheep and goats are among the major economically important livestock in Ethiopia (Mengist *et al*., 2014).

Despite the large number of small ruminants and their contributions to the livelihood of the farmers and the national economy small ruminants productivity in Ethiopia is low due to different factors including, Weak attention from scientists, administrators and legislators, low genetic potential and policy issues, Market and institutional problem and problem of credit facilities; shortage, seasonal unavailability and low nutritive (poor nutrition) value of feed and/or ; prevalence of different diseases and parasites labor shortage, lack adequate veterinary service, water shortage, capital shortage, market problem and capital shortage (Afras *et al* ., 2018).

Helminthosis in small ruminants is of considerable significance in a wide range of agro-climatic zones of Africa. This situation is not an exception in Ethiopia. About 80% of the national sheep population was reported to harbor varying degrees of infection with different species of nematode parasites; the most prevalent and economically important one being Heamonchus spp (Chaka *et al*. 2009 ;Sissay *et al*. 2007). Nematode parasites commonly known as strongylids belonging to the order Strongylida and superfamily Trichostrongyloidea significantly affect the health of livestock.

Among these strongylid species, *Haemonchus contortus* and *Trichostrongylus* spp. are reported to be the most prevalent and highly pathogenic in livestock, particularly in small ruminants. *Haemonchus contortus* is the most notorious parasite in livestock due to its biotic potential and blood sucking ability. Among the *Trichostrongylus* species, *T. colubriformis* is considered as the most common species in goats (Tan *et al*., 2014).

Haemonchus contortus is one of the most fecund strongylid nematodes; individual females are capable of producing thousands of eggs per day, which can lead to rapid larval pasture contamination and associated outbreaks of haemonchosis (Roeber., *et al* 2013). As compared to *H. contortus*, *Trichostrongylus* infection may show milder clinical signs, which may result in appetence, weight loss, poor body condition, emaciation, diarrhea, hypoproteinaemia and death in the case of heavy infection, particularly in malnourished animals(Tan *et al*., 2014). The control of these parasites relies heavily on the administration of anthelmintic drugs. Between 1960 and 1990, the pharmaceutical industry made major progress in developing deworming compounds with excellent broad-spectrum activity and safety. This led to the discovery of three major drug classes available for ruminants, each with distinct modes of action: benzimidazoles (BZs), imidothiazoles and tetrahydropyrimidines (I/Ts) and macrocyclic lactones (MLs). The modern broad-spectrum anthelmintics are currently widely used in prophylaxis and treatment of helminth infections in farm animals (Papadopoulos *et al*., 2011; Magzoub *et al*., 2009).

Anthelmintic resistance of gastrointestinal nematode (GIN) parasites constitutes a major problem for livestock health and productivity around the world. The common practice of intensive farming methods coupled with heavy reliance on anthelmintics has resulted in a serious escalation in the prevalence, distribution, and scope of AR in many of the most important GIN parasite species. In sheep and goats the situation is the most severe, with increasing number of farms around the world experiencing resistance to all classes of available anthelmintics. Multiple anthelmintic resistances in GIN parasites of cattle and horses are also being increasingly reported raising the level of concern in these hosts(Storey *et al*., 2014).

In Central Ethiopia, including Ada'a district, the existing method to control gastro-intestinal nematodes has increased its dependence on the treatment with anthelmintics. The use of anthelmintics has been practiced for a long time, and constitutes a considerable share of the costs

spent by the country in the control of helminthosis. Meanwhile, the risk of under dosing and a continued use of very limited classes of anthelmintics, irrespective of efficacy status are frequently encountered on many farms. Also, smuggling and misuse of veterinary drugs involving anthelmintics is a wide spread practice in the country. Some of these drugs, particularly albendazole and tetramisole, have been continuously imported and distributed to every corner of the country under different trade names and by different manufacturers. The extensive and indiscriminate use of the drugs has resulted in the development of resistances (Kumsa *et al*.,2010,Belina *et al*. 2017 and Aga *et al* ., 2015). Effective methods that can preserve and maintain the efficacy of anthelmintics, and delay or prevent the emergence of anthelmintic resistance are not practiced in any part of the country (Kumsa *et al*., 2010).

On the other hand, no systematic surveys have been carried out to evaluate anthelmintic resistance situation in small ruminants particularly in sheep and goats. The incidences of anthelmintic resistance in small ruminant nematodes have been reported from studies conducted in different parts of the country. This is perhaps an alarming signal for the incoming challenges with worm management in small ruminants in Ethiopia (Aga *et al*., 2013, Kumsa *et al* ., 2007, Desalegn *et al* ., 2009,Terefe *et al*., 2013).

Therefore, the objectives of the present study were:-

- To assess the efficacy of four commercially available anthelmintics (Albendazole, Ivermectin, Levamisole and Tetramsiole) in small ruminants.
- To compare in vitro susceptibility of H.contortus and T.columbriformis to commonly used Anthelmintic.

2. LITERATURE REVIEW

2.1. Gastrointestinal nematode parasitism

2.1.1. Classification and description

Nematodes are the most numerous animals on earth. Nematodes make up a large assemblage of worms of relatively simple structure with a widespread distribution, their cylindrical, non-segmented bodies distinguishing them easily from other helminthes. Gastrointestinal helminths are ubiquitous parasitic agents of livestock especially ruminants and are known to limit ruminant production in many areas and countries (Adedipe *et al*.,2014). Helminth parasites of ruminants are broadly grouped into two phyla, namely nemathelminthes which are nematodes or roundworms such as Haemonchus, Trichostrongylus, Bonostomum, Oesophagostomum and Chabertia and platyhelminthes which include cestodes (e.g. Avitellina, Moniezia, Stilesia and Taenia) and trematodes such as, Fasciola and Paramphistomum. (Karshima *et al*., 2018). The parasites in general have a digestive tube consisting of mouth, esophagus and the intestine and rectum. In most species, adult female nematodes produce eggs that are passed out of the host with the faeces(Belina *et al*., 2017) These parasites cause a range of diseases in their hosts, from diarrhoea to anaemia, and cause significant economic losses to farmers and their keepers in terms of reduced production and treatment costs, as well as being a major welfare issue for the infected animals. They also reduce production efficiency, thereby potentially raising food prices and damaging the environment (Sargison *et al* .,2016). Nematode parasites commonly known as stronglids belonging to the order Strongylida and superfamily Trichostrongyloidea significantly affect the health of livestock. Among these strongylid species, *Haemonchus.contortus* and Trichostrongylus spp. are reported to be the most prevalent and highly pathogenic in livestock, particularly in small ruminants.

Haemonchus.contortus

Haemonchus is a cylindrical gastrointestinal nematode commonly known as the red stomach worm, large stomach worm, the wire worm or the Barber pole worm(Selemon, 2018). It is a pathogenic haematophagous nematode that infecting the abomasum of ruminant hosts (Vadlejch *et al*., 2014). The genus Haemonchus is in the subfamily of Haemonchinae and consists of four main species in domestic ruminants, namely, *H. contortus* (in ovine and caprine), H. placei and H. similis (in bovine) and H. longistipes (in dromedary). 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 color and considerable size. In both sexes there is pair of wedge shaped cervical papillae in the esophageal region and a tiny lancet inside the buccal capsule used for piercing small blood vessels. 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 (Gharbi*et al*., 2013). 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 (Kumsa *et al*., 2004; Gharbi*et al*., 2013).

Haemonchus species have been differentiated by morphological analyses and by the use of molecular techniques. Measurements of male spicules and their barbers are the most common method employed to differentiate both species; H. placei usually present longer spicules and barbs than *H. contortus* (Lichtenfels *et al*., 1994; Jacquiet *et al*., 1996). Jacquiet *et al*. (1996) developed a discriminate function combining these measurements to differentiate such species. Differences in the morphology of the infective larvae (L3) have also been reported, with H. placei L3s longer, more robust, and with longer sheath tail than those of *H. contortus*(Francisco *et al*., 2014). Although some species were defined based on relatively few specimens, consistent structural differences were apparent especially in attributes of the spicule tips and dorsal ray among male nematodes. Subsequently, evaluation of the synlophe (a system of longitudinal cuticular ridges present in male and female nematodes served to provide separation of species based on female specimens, provided the possibility of linking male and female conspecifics in mixed infections and recognition of hybrids between Haemonchus contortus and Haemonchus placei (Hoberg *et al* , 2016).

Two or sometimes three Haemonchus spp. are sympatric in many regions of the world especially in areas where sheep, goats, cattle and one humped dromedaries are reared together and share the same grazing pasture (Kumsa *et al* ., 2008). Previous investigators reported that the vulvar process of Haemonchus species worms varies both in shape and size (Vadlejch *et al*., 2014 ; Lichtenfels *et al*. 1994). Study of vulvar morphology of female Haemonchus worms helps to understand the biology, considered as the marker of ecological adaptation possess great taxonomic importance. (Kumsa *et al* ., 2008) indicated that vulvar morphology is the manifestation of some genetic factors necessary to establish and develop inside hosts. 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 (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).

Trichostrongylus *species*

Trichostrongylus colubriformis is the predominant small intestine worm of sheep and goats. These small, thread-like worms measure approximately 4.3-8.6 mm long. The males have a large bursa with unequal, dark brown spicules and the females have a slit shaped vulva without distinctive exterior lips. Both sexes have an excretory pore on the neck. These nematodes thrive under cool and wet conditions. In small ruminants, this worm is generally the next most common and important after *H.contortus. T.colubriformis* follows the general trichostrongyle life cycle. Once in the small intestine, T.colubriformis feeds on nutrients in the mucosa, thereby causing irritation to the mucosa and interference with digestion. Diarrhea, swelling of the intestinal wall and edema, are common with large infections. The worm is called the bankrupt worm because death of an animal is uncommon but the animal develops poor condition, leading to production and income loss (Qasim,

2015); (Weingartz, 2017). 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. 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 shape gubernaculum is present (Whitlock, 1985; Jambre,1988.).

T.axei is the smallest member of the genus and parasitizes the true stomachs of ruminants, horses, and leporids (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).

2.1.2. Life cycle of Haemonchus and Trichostrongylus

Most GIT nematodes have the same life cycle. Majorities are oviparous, and the eggs are similar and very characteristic type, and immediate transfer of infection from one host to another does not occur. The life cycle of the nematode may be direct or include an intermediate host. In this simple cycle, adult female parasites in the abomasum or intestines produce eggs that are passed in the manure (Tibebu, *et al*., 2018). Under optimal condition in external environment first-stage larvae (L1) can develop and hatch out of the egg within 24 hours. L1 grows and develop to the second stage larvae (L2) which in turn grow and develop in to third-stage larvae (L3), which is the infective stage (Figure 2). After ingestion L3 develop into fourth stage larvae (L4), which then develop into immature adults (L5). Sexually mature adult nematodes develop within 2 to 4 weeks after ingestion of the L3 unless arrested larvae development occurs (Belina *et al.*, 2017). Figure 1 shows life cycle representing gastrointestinal nematodes (order Strongylida) of small ruminants; adapted from(Roeber *et al*., 2013).

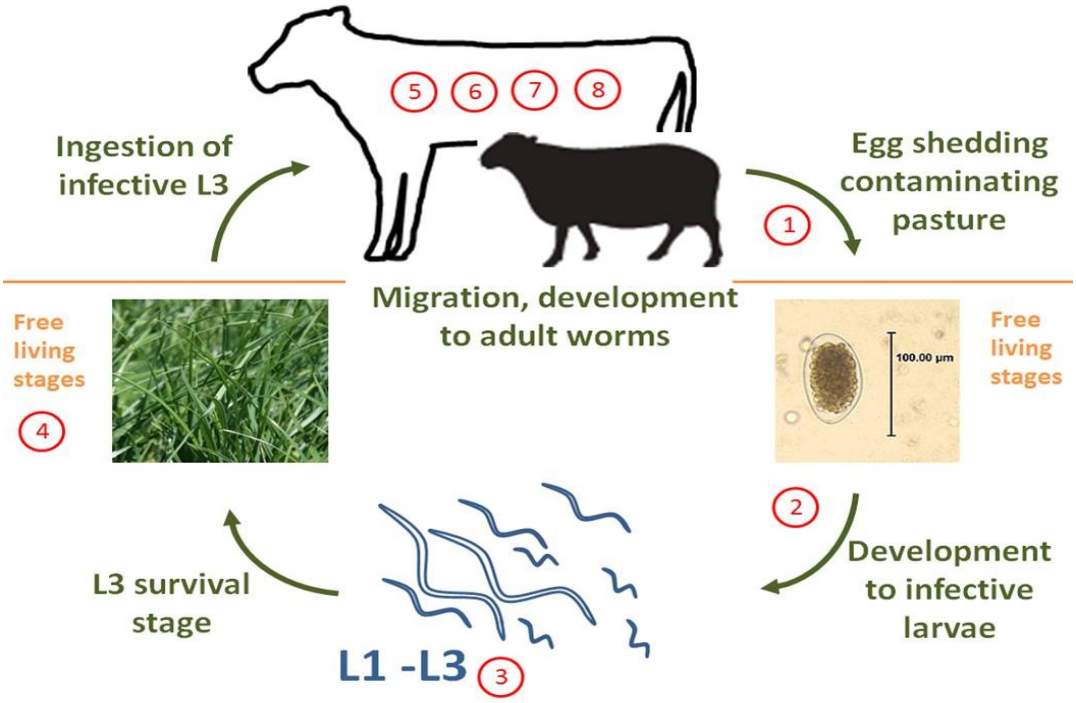

Figure 1. Life cycle representing gastrointestinal nematodes (order Strongylida) of small ruminants (adapted from Roeber *et al*., 2013)

2.1.3. Distribution and risk factors

It is indisputable that *H. contortus* is the most notorious parasite in livestock (i.e., ruminants) due to its biotic potential and blood sucking ability (Tan *et al*., 2014). Trichostrongylus colubriformis is intermediate in temperature preference and does well in both cool and warm climates. Both parasites cause a more classical parasitic gastroenteritis, characterized by reduced appetite, reduced weight gain and/or weight loss, and diarrhea. In contrast, *H. contortus* rarely causes diarrhea. Because any one or all of these parasite species may be infecting an animal, it is important to determine which species are present before optimal control measures can be implemented (Kaplan, (2014).

The most important predisposing factors of helminth infections are grazing habits, climate, nutritional deficiency, pasture management, immunological status, vector, presence of intermediate host, and the number of infective larvae and eggs in the environment. The effect of helminth infections is determined by a combination of factors, of which the varying susceptibility of the host

species, the pathogenicity of the parasite species, the host/parasite interaction, and the infective dose are the most important(Adedipe *et al*., 2014). The problem of nematode parasitism is of particular importance throughout the developing world since nutritional resources available to small ruminant livestock are often inadequate and, as a consequence, natural immunity is compromised resulting in low productivity and high mortality. Due to suitable geographic and climatic conditions of the country, parasitic GI nematodes are perhaps the leading cause of productivity losses in small ruminant production in Ethiopia(Mengist *et al.*, 2014).

2.2. Impact of gastrointestinal parasitic nematodes of sheep, and goats

Diseases caused by gastrointestinal nematodes (GINs) in livestock are a major production constraint, causing economic losses, especially in small ruminants in the tropics and subtropics. Nematode parasites are amongst the most important production-limiting diseases of ruminant livestock worldwide. *Teladorsagia circumcincta*, *H. contortus*, *Trichostrongylus vitrinus/colubriformis* and *Nematodirus battus* are of particular relevance. In tropical countries, Haemonchus contortus is the most prevalent nematode in herds, causing severe losses due to the high pathogenic pressure it exerts through hematophagy. Other parasites also contribute to losses through diarrhea, spoliation of intestinal mucosa and anemia, mainly because of mixed infections, which cause a variety of clinical signs and low productivity (Salgado *et al*., 2016). The infection of sheep and goats by nematodes is rampant in most African countries, where the environmental conditions are conducive to nematode growth and transmission(Wondimu *et al*.,2017). Among the parasitic diseases that constrain the survival and productivity of sheep and goats, Haemonchus contortus is recognized as a major production limiting parasite. These disease have major impact on morbidity and mortality rates with annual losses as high as 30 to 50% of the total value of livestock production of Ethiopia (Wondimu *et al*.,2017). In general, GINs reduce productivity of small ruminants due to lowering fertility, reduction in milk production and loss of weight when feed intake is reduced. Losses due to Gastroitestinal tract (GIT) parasitism can be categorized as direct or indirect. Direct losses are due to acute illness and death, forced premature slaughter and rejection of parts of the carcass at meat inspection in abattoirs. Indirect losses can arise from impaired growth, reduced milk, meat and wool production, reduced reproduction performance, condemned organs and high cost of control measures (Granroth-Wilding., *et al*. 2015; Jegede *et al*.,2015).

Apart from losses in production and productivity, their harmful effects on these animals range from gastroenteritis, anorexia, abdominal distention, diarrhoea, emaciation, and so forth; all of which result in serious economic losses to the farmer and the nation in general (Oluwole *et al*., 2015). The pathogenic effect of *Haemonhus.contortus* results from the inability of the host to compensate for blood loss (Radostits *et al.,* 1994; (Lichtenfels *et al*. 1994). *H. contortus* is an important, voracious blood sucking parasite of small ruminants found in abomasum, cause major production losses world-wide and heavy burden of this blood feeding parasite causes anemia, diarrhoea, loss of weight, oedema, recumbency, severe debility and ultimately death (Kasim *et al*. 2016 ; Brik *et al*., 2019). *H. contortus* sucks about 0.05 mL of blood per day by ingestion or liberation from lesions. Haemonchosis is widespread wherever sheep and goats are raised, but the greatest economic losses occur in temperate and tropical regions(Brik *et al*., 2019 ; Tehrani *et al*. 2012;Saminathan *et al*.,2015). It has been ranked as the most important parasite of small ruminants in all regions across the tropics and subtropics and causes an insidious drain on production, retarded growth, loss of appetite, anemia, edema, decrease in protein and even mortality in young animals as reported by Paddock as well as the emerging anthelmintic resistance (Mengist *et al*.,2014). The anemia of Haemonchosis is generally considered to be moderately macrocytic normochromic in nature. 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 (Karshima *et al*., 2018)**.**

Haemonchus.contortus infection (i.e., haemonchosis) may exhibit clinical signs such as anemia, followed by lack of appetite, lethargy, loss of weight, dehydration, oedema and death as a consequence of the disease(Qamar *et al.*, 2009). As compared to Haemonchosis. contortus, Trichostrongylus infection may show milder clinical signs, which may result in inappetence, weight loss, poor body condition, emaciation, diarrhea, hypoproteinaemia and death in the case of heavy infection, particularly in malnourished animals(Tan *et al*., 2014).Haemonchus contortus is one of the most fecund strongylid nematodes; individual females are capable of producing thousands of eggs per day, which can lead to rapid larval pasture contamination and associated outbreaks of haemonchosis. In sheep, the pre-patent period of Haemonchus is 18–21 days; adult worms are short-

lived, surviving in their hosts for only a few months. The main pathogenic effects are caused by the L4s and adults, which both feed on blood, causing severe anaemia which usually becomes apparent after two weeks of infection(Roeber *et al*.,2013).

Trichostrongylus genus parasites of domestic animals and specially ruminants are a most important cause of economic loss throughout the world. The economic impact of these parasites including weight loss, impaired wool and milk production and poor reproductive performance. Decrease in appetence, is the main aspect of *Trichostrongylosis* and is generally recognized as a major feature in the pathogenesis of this parasite.Sheep is an important animal protein source in third world countries where veterinary care is much less than cows. So the prevalence of trichostrongylus in sheep improves control measures and reduces the mortality of the population (Shahbazi *et al*. 2012).

2.3. Treatment and control of gastrointestinal nematodes

2.3.1. Treatment options and principles

Treatment of gastrointestinal helminthosis mainly involves commercially available anthelmintic such as the benzimidazole, imidazothiazole and macrocyclic lactone groups. However, the extensive use of anthelmintics for the control of helminth infections in grazing livestock has resulted in the development of resistance that has become a major practical problem in many countries of Africa (Waruiru 1997; van Wyk *et al*. 1997) and elsewhere in the world (Coles 1998; Chandrawathani *et al*. 1999; Eddi *et al*. 1996; Le Jambre *et al*. 1995). A similar situation has been reported in eastern Ethiopia (Sissay *et al*. 2006) where nematodes have shown resistance to albendazole, tetramisole and ivermectin at prescribed dosages in small ruminants. Their long-term utilization, inappropriate handling and under dosage may be some of the risk factors for their reduced efficacy and for the increasing development of drug resistance (Taylor *et al*. 2007). The maintenance of good efficacy and prevention of the emergence of anthelmintic resistance requires periodic assessment of risk factors that could eventually contribute to reduced efficacy and the development of resistance (Terefe *et al*., 2012).

The best forms of control of helminthes will require a basic understanding of certain principles bordering on the parasite's developmental cycle, mode of transmission and predisposing factors to

infection. Apart from the conventional chemical agents or anthelmintics, a number of alternatives are being practiced or on trial with three main principles of action. The first one is to limit the contact between the hosts and the infective larvae in the field through grazing management methods. The latter were described since the 1970s and, at present, they benefit from innovations based on computer models (Hoste and Torres-Acosta, 2011). Several biological control agents have also been studied in the last three decades as potential tools to reduce the infective larvae in the field. The entire philosophy of using biological control agent against GIN nematodes in animals is to reduce the number of infective stages that are available to be picked up by grazing susceptible individuals of the different species of livestock (Larsen, 1999).

The second principle aims at improving the host response against GIN infections relying on the genetic selection between or within breeds of sheep or goats, crossbreeding of resistant and susceptible breeds and/or the manipulation of nutrition. These approaches may benefit from a better understanding of the potential underlying mechanisms, in particular in regard of the host immune response against the worms. The third principle is the control of GIN based on non-conventional AH materials (plant extracts or mineral compounds). Worldwide studies show that non conventional AH materials can eliminate worms and/or negatively affect the parasite's biology (Hoste *et al*.,2011). Copper wire particles have also been tried with varying success (Worku *et al., 2017)*.

The current methods of gastrointestinal nematode (GIN) control are based on repeated use of synthetic anthelmintic drugs (Table 1). Anthelmintic Drugs are chemotherapeutic agents commonly used either for prophylactic purposes, in which the timing of treatment is based on a knowledge of the epidemiology, or for therapeutic purposes to treat existing infections or clinical outbreaks. However, most anthelmintics leave residues in meat, milk and their products. On the other hand, ivermectin is excreted in faeces in sufficient quantity to have a detrimental effect on invertebrates that usually degrade dung heaps, and hence on organisms higher up the food chain (Worku *et al.*, 2017). Anthelmintics are drugs that are used to treat infections caused by parasitic worms (helminths). Anthelmintics either kill worms or cause their expulsion from the body, without causing any significant damage to the host. Although there is a high prevalence of parasitic worms, the progress of anthelmintic drug discovery and development by pharmaceutical companies has been slow over the years. One contributing factor is that the majority of those suffering from

helminth infections live in developing nations who lack the resources to support a profitable drug market (Abongwa *et al*., 2017).

Table 1. Some drugs used in the treatment of helminths in livestock

Nematodes	Trematodes	Cestodes
Benzimidazoles	Praziquante	Benzimidazoles
Ivermectin	Closantel	Niclosamide
Levamisole	Triclabendazole	
Pyrantel	Nitroxynil	
Piperazine	Oxyclozanide plus	

Source: (Abongwa *et al*., 2017).

2.3.2. Anthelmintics: Benzimidazole group and modes of action

The antihelmintic drugs derived from benzimidazole are the largest chemical family used to treat endoparasitic diseases in domestic animals and humans (Khokra *et al*., 2014). The first of this class, thiabendazole, was discovered in 1961 and subsequently a number of further benzamidazoles were introduced as broad spectrum anthelmintics. There is an extensive literature on these compounds reporting a number of different biochemical effects. Nonetheless, it is clear that their anthelmintic efficacy is due to their ability to compromise the cytoskeleton through a selective interaction with β-tubulin. BZD anthelmintics are extensively metabolized in all mammalian species studied. Thiabendazole, mebendazole, and albendazole belong to this class of drugs(Ahire *et al*., 2020). They are the first chemical class of modern anthelmintics developed. They include drugs such as albendazole, cabendazole, fenbendazole, flubendazole, mebendazole, oxfendazole, oxibendazole, parbendazole and triclabendazole some of which are used against both nematodes and flukes as broad- spectrum anthelmintics (Khokra *et al*., 2014). The benzimidazole compound can be grouped as follows: a. Benzimidazole thiazolyls: Eg thiabendazole, cambendazole. b. Benzimidazole methylcarbamates: which include: Parbendazole, mebendazole, Flubendazole, Oxibendazole, luxabendazole, albendazole, albendazole sulphoxide (ricobendazole), fenbendazole, oxfendazole. c. Halogenated benzimidazole thiols: Example is Triclabendazole. d. Pro-benzimidazoles: thiophanate, febantel and nitobimin(Enejoh *et al*., 2017). There are two major modes of action of anthelmintics. There are the drugs that act on parasite membrane ion-channels and which usually have a more rapid therapeutic effect; the other group acts more slowly on a range of 'biochemical' target sites found in parasites. The target ion-channels include: the excitatory nicotinic acetylcholine receptor

on muscle of nematodes; the inhibitory (γ-) gamma aminobutyric acid) (GABA) receptor channel also present on nematode muscle; and the glutamate-gated Cl− channel(Martin and Robertson ,1997). The target site (enzyme or ion-channel) of the anthelmintic may be present in the host animal as well as the parasite; it is usually pharmacologically distinct in the parasite in order to permit selective drug action. For example, the benzimidazole anthelmintics bind selectively to nematode β-tubulin to effect their action. β-tubulin is present in the host animal as well but is sufficiently different in its three dimensional protein structure in the mammalian host so that benzimidazole anthelmintics only bind to the nematode β-tubulin molecule(Martin and Robertson ,1997).

Furthermore, the major genetic determinant of BZ resistance (BZ-R) in most, if not all, trichostrongylid nematode species is the possession of single nucleotide polymorphisms (or SNPs), in the parasite's isotype-1 b-tubulin gene. Pivotal amongst these is a tyrosine for phenylalanine substitution at codon 200 (the so-called F200Y SNP), encoded by a change from TTC to TAC(Kotze *et al*., 2014). The initial mode of action of benzimidazoles was thought to be inhibition of various parasite metabolic enzymes including fumarate reductase and malate dehydrogenase. However, it is now established that benzimidazoles selectively bind with high affinity to parasite ß **tubulin** and inhibit microtubule polymerization. This results in the destruction of cell structure and consequent death of the parasite(Abongwa *et al*., 2017).

Table 2. Summary of Ion-channel target sites of anthelmintic drugs

Target site (and parasite group)	**Generic drug name**
Nicotinic acetylcholine receptor (in nematodes)	Levamisole, butamisole, pyrantel, morantel,
GABA receptors (in large intestinal nematodes)	Piperazine
GluCl receptor (in nematodes and insect parasites)	Ivermectin, abamectin, doramectin,
Membrane calcium permeability (cest and tremat)	Praziquantel
Nicotinic acetylcholine receptor (in nematodes)	Levamisole, butamisole, pyrantel,
GABA receptors (in large intestinal nematodes)	Piperazine

(Source: Martin, 1997)

It inhibits the microtubule formation. So the parasite loses its cytoskeleton and motility and dies. It also impairs glucose uptake and decrease (↓) ATP formation. However, beside the tubulin, other mechanisms of action have been described for the BZs including disruption of the energy metabolism of the host. In fact initial studies of the mode of action of BZs focused on their role in carbohydrate metabolism as these compounds have been shown to inhibit glucose uptake both in vitro and in vivo in many helminth species. Albendazole has been shown to block glucose uptake by larval and adult stages of susceptible Parasites, depleting their glycogen stores and decreasing formation of ATP leading to the death of the parasite (Lacey, 1988).

Mebendazole and flubendazole induce the loss of cytoplasmic microtubules of the tegumental and intestinal cells of cestodes and nematodes, and this is followed by loss of transport of secretary vesicles, a decreased glucose uptake and an increased utilization of stored glycogen. The literature survey recently, shown that mebendazole (MBZ), a marketed benzimidazole (BZ) Anthelmintic, is an effective anti-melanoma agent given its ability to disrupt microtubule stability at clinically achievable concentrations, thereby inducing apoptosis(Ahire *et al*., 2020). Benzimidazole anthelmintics bind selectively to nematode β-tubulin to effect their action. β-tubulin is present in the host animal as well but is sufficiently different in its three dimensional protein structure in the mammalian host so that benzimidazole anthelmintics only bind to the nematode β-tubulin molecule. Other anthelmintics act on targets only present in parasites. Anthelmintics acting on a target protein only present in parasites are often favoured because of advantages of greater safety and selectivity(Martin and Robertson,1997).

Table 3. Summarizes the modes of action of anthelmintics that act at more 'biochemical' target sites other than ion-channels

Target site (and parasite group)	Generic drug name
β -tubulin(in nematodes)	Thiabendazole,cambendazole,oxibendazole, albendazole, albendazole sulphoxide
β-tubulin (in nem, cest and trem)	Fenbendazole, oxfendazole, mebendazole
Proton ionophores(H.contortus, O. ovis)	Closantel, rafoxanide, oxyclozanide,nitroxynil,
Malate metabolism (in immature Fasciola)	Diamphenethide
Phosphoglycerate kinase and mutase (inFasciola)	Clorsulon
Arachidonic acid metabolism (filaria)	Diethylcarbamazine

Source ; (Martin and Robertson,1997)

Albendazole (ABZ): Albendazole acts by blocking the glucose uptake of larvae. The adult worm stored Depletes of glycogen hence, decreases the formation of ATP, as a result the parasite is immobilized and dies. It is another marketed antihelminthic that is structurally related to MBZ. ABZ, however, has the unique advantage of crossing the BBB, a characteristic that is used to treat parasitic infections of the central nervous system and may be harnessed to potentially target brain metastasis (Hong *et al*.,2018).

Albendazole a benzimidazole carbamate (methyl 5 propylthio-1H-benzimidazole-2-yl carbamate) is a broad spectrum ant parasitic which is used worldwide against a variety of parasites (Waller *et al*., 1997; Delatour *et al*., 1983). Studies conducted on the mechanism of action of BZs have demonstrated that, by binding to tubulin, these drugs inhibit micro- tubule polymerization (Hanjeet *et al*., 1991). Inhibitors of microtubule polymerization have been shown to exhibit experimentally and clinically, useful antitumor activity (Ahire *et al*., 2020).

Albendazole imported by private companies takes the largest share in Ethiopian market, in a limited extent the drug is also produced domestically. The quality of veterinary drugs imported, manufactured and distributed in the country is controlled by Ethiopian Veterinary Drug and Feed Control and Administration Authority. The authority has checkpoints at potential entry sites. However, there may be importation and distribution of substandard drugs). There are also complaints from animal health professionals and animal owners regarding the effectiveness of available drugs in the market(Seifu *et al*., 2019).

Thiabendazole : The benzimidazole drugs bind selectively to beta-tubulin of nematodes, cestodes and fluke, and inhibit microtubule formation. Among the anthelminthics available for the treatment of human strongyloidosis, the benzimidazole compound, thiabendazole is considered effective in 75–96% of cases, although with considerable adverse effects; while its therapeutic alternative, albendazole has a cure rate of 42–100%, depending on the dose schedule and length of follow-up (Rossignol et .,1984).

2.3.3. Anthelmintics: Macrocyclic lactones group and its mode of action

The MLs act as gamma-aminobutyric acid (GABA) and antagonists and glutamate-gated chloride (GluCl) channel potentiators, acts on the same receptor as the GABA neurotransmitter in nematodes. that is a ligand-gated Cl- channel found on the synaptic and extrasynaptic membrane of nematode muscle membrane. Ivermectin induces release of GABA which leads to the complete paralysis and immobilization of the worms. The effect is irreversible and the consequence is paralysis and death of the nematode (Demessie *et al*.,2016). The biological targets for MLs are glutamate-gated chloride ion channel receptors (GluClRs) expressed in the neurons and muscle cells of nematodes. ML drugs irreversibly activate these channels, thereby inhibiting neuronal activity and muscle contractility, and thus inducing flaccid paralysis and death. MLs also activate other ligand-gated ion channel receptors, namely the c-aminobutyric acid (GABA) and glycine (Gly) receptors, however, this activation requires much higher drug concentrations than required for the GluClRs, and hence the GluClRs are considered to be the principal target for this class of anthelmintics (Kotze *et al*., 2014).

The ability of the drug to enter the worm and interact with its target receptor in order to trigger a harmful physiological effect (shown at top for a drug- susceptible worm) is diminished through four principal mechanisms. These mechanisms apply to varying degrees to the major anthelmintic drug classes, as indicated by the relative font of the drug class names at the base of the figure; ML = macrocyclic lactones, TCBZ = triclabendazole, Lev = levamisole (as a representative of the nicotinic agonist drug class), BZ =benzimidazoles, AAD = amino-acetonitrile derivatives; ⁄denotes that resistance to the AADs is only characterised in laboratory-selected isolates(Kotze *et al*., 2014)

Ivermectin : Ivermectin is endectoparasiticide of Macrocyclic lactone class which is commonly used as broad spectrum an the lmintics. It has wide spectrum of activity against gastrointestinal and lung nematodes, mites, ticks, biting flies and larvae of parasitic dipteran flies. It is also active against larvae of canine heartworm Dirofileria immitis(Udhavrao *et al*.,2017). Ivermectin is a member of the family of compounds produced by the soil microorganism Streptomyces avermitilis and known generically as avermectins. It is believed to act on susceptible nematodes and arthropods by potentiating the release and binding of gamma-aminobutyric acid (GABA) in certain nerve synapses, and thus blocking GABA-mediated transmission of nerve signals. While paralysis is the

most evident effect, suppression of reproductive processes has been observed, and the biochemical basis of the compound's several biological properties requires further elucidation. The avermectins are also highly active against many free-living and plant- parasitic nematode and arthropod pests (Putter *et al*., 1981), but these properties will not be considered herein (Campbell *et al*., 2000).

Ivermectin is highly active against a wide spectrum of nematode species, including most larvae and adult forms; it is also highly effective against many arthropod parasites of domestic animals. All important gastrointestinal and lung nematodes are susceptible to the drug, including sensitive mites, ticks, biting flies, and parasitic dipteran larvae (Campbell *et al*.,2000;Jose *et al*.,2009). in dogs, ivermectin is also active against developing larvae of Dirofilaria immitis and is used in heartworm prophylaxis. Ivermectin's extremely low water solubility and its precipitation in SC tissues favour slow absorption from the injection site, resulting in a prolonged presence in the bloodstream. On the other hand, the erratic SC absorption of ivermectin could relate to variability in pharmacokinetic parameters (Barragry *et al*.,1987).

Gamma-amino-butyric acid (GABA) is the neurotransmitter substance mediating transmission of inhibitory signals from the intemeurons to the motor neurons in the ventral nerve cord of parasites. It is now established that ivermectin acts as a GABA agonist. The function of this GABA transmitter is to open the chloride channels on the postsynaptic junction, allowing inflow of Cl - ions and the induction of the resting potential (Sakthikarthikeyan *et al*.,2016).

Ivermectin potentiates this effect by stimulating the presynaptic release of GABA and by increasing its binding to the postsynaptic receptors. In the presence of ivermectin, the chloride channels are open when they should be closed, the net effect being that signals and impulses are not received by the recipient cell. Although the motor neuron and muscle cells are both capable of individual excitation, passage of the electrical impulse across the synapse is blocked(Barragry *et al*., 1987). Early reports on the mechanism of ivermectin resistance in parasitic nematodes highlighted the presence of mutations in GluClRs (Kotze *et al*., 2014) reported an increased frequency for an allele of a GluCl a-subunit gene in ivermectin and moxidectin resistant *Haemonchus.contortus* isolates, suggesting that a mutation in this gene was associated with ML resistance(Sakthikarthikeyan *et al*., 2016).

2.3.4. Anthelmintics: Imidazothiazole and its modes of action

Imidazothiazoles act as nicotinic acetylcholine receptor (nAChR) agonists. They bind to nAChRs on body wall muscles, causing spastic paralysis of the worm, and hence, its expulsion from the host. Somatic muscle cells of nematodes possess both synaptic and extrasynaptic nicotinic acetylcholine receptors(Abongwa *et al*., 2017).

Tetramisole is the first imidazothiazole anthelmintics which was introduce into the veterinary market in 1967. However, the current and the most available imidazothiazole anthelmintic worldwide is the levamisole. The other compound available is the butamisole which is a deravative of levamisole (Martin *et al*.,1997).

Levamisole: is an anthelmintic agent that exerts its therapeutic effect by acting as a full agonist of the nicotinic receptor (AChR) of nematode muscle. Its action at the mammalian muscle AChR has not been elucidated to date despite its wide use as an anthelmintic in humans and cattle. By single channel and macroscopic current recordings, we investigated the interaction of levamisole with the mammalian muscle AChR. Levamisole activates mammalian AChRs. However, single channel openings are briefer than those activated by acetylcholine (ACh) and do not appear in clusters at high concentrations(Rayes *et al*.,2004) .

2.3.5. Anthelmintics: Amino-acetonitrile derivatives and their modes of action

The AADs are a new class of synthetic anthelmintics with broad spectrum activity against nematodes that are resistant to the benzimidazoles, imidazothiazoles and macrocyclic lactones. Monepantel, also known as AAD 1556, is the first member of this class to be developed for the control of a broad range of parasitic nemtaodes in sheep. Monepantel acts as a positive allosteric modulator of *H. contortus* MPTL-1 and *C. elegans* ACR-20 receptors, and at high concentrations (>0.1 μM), it acts as a direct agonist of these receptors(Abongwa *et al*., 2017).

2.3.6. Anthelmintics: Tetrahydropyramidines and its modes of action

These anthelmintics are nicotinic receptor agonists and elicit spastic muscle paralysis due to prolonged activation of the excitatory nicotinic acetylcholine (nACh) receptors on body wall muscle. Examples of this anthelmintic drug class include pyrantel, oxantel and morantel. Pyrantel is an imidazothiazole-derived tetrahydropyrimidine that was discovered in 1966 as an anthelmintic agent with broad spectrum activity against roundworms and hookworms in domestic animals(Holden-dye *et al*.,2013). Pyrantel however lacks activity against whipworms. Studies on the mode of action of pyrantel at the single-channel level identified the L-subtype nAChR in *A. suum* as also preferentially activated by pyrantel. Pyrantel, like levamisole, also causes open channel-block. Contrary to pyrantel, oxantel preferentially activates the N-subtype nAChRs in *A. suum*. Oxantel, like levamisole and pyrantel, also causes open channel-block in *A. Suum* (Abongwa *et al*., 2017)..

Morantel is a methyl ester analog of pyrantel which also targets the L-subtype nAChR in *A. suum*. At the single-channel level, morantel causes the activation and block of this receptor subtype. Recently, morantel was shown to act as an agonist of the nAChR subtype comprising ACR-26/ACR-27 subunits from *H. contortus* or *Parascaris equorum* expressed in *X. Laevis*oocytes (Abongwa *et al*., 2017).

2.3.7. Anthelmintics: Salicylanilides and their modes of action

Salicylanilides are a very large group of compounds, originally developed as fungicides for topical use and as antimicrobial agents in soaps. Halogenated salicylanilides, in particular closantel and rafoxanide, are important anthelmintics that are used extensively in the control of Haemonchus spp. and Fasciola spp. infestation in sheep and cattle, and Oestrus ovis in sheep in many parts of the world. Niclosamide is widely used for the treatment and control of cestode infections in several animal species. The primary action of salicylanilides has generally been associated with the uncoupling of oxidative phosphorylation. Early in vitro studies, using houseflies as well as rat liver mitochondria, showed several salicylanilides as potent inhibitors of this electron transport associated phosphorylation(Swan, 1999).

Piperazine:-Piperazine was discovered in 1900, and its anthelmintic moiety was discovered in 1954. The drug has good efficacy profiles against ascarid and nodular worm infections of all species of domestic animals, moderate for pinworm infections, and zero to variable to other veterinary helminths. Its use is limited in ruminants because ascarids are not a significant problem in this species. Piperazine is available as hexahydrate and a variety of salts such as citrate, phosphate, tartrate or hydrochloride(Enejoy *et al*.,2017).

2.4. Anthelmintic drug resistance

The development of resistance to all the older anthelmintic groups in sheep is a serious increasing problem and resistant GIN are a great hazard for sheep flocks and farmers, at the point that, sometimes, the inability to control the parasites infestation results in the farm closures or culling of entire flocks. Anthelmintic resistance and multiple drug resistance is very problematic in Australia, New Zealand, South Africa and many Latin American countries (Castagna *et al*., 2019). Anthelmintic drug resistance is the heritable reduction in the sensitivity of a parasite population to the action of a drug. The reduction is expressed as the decrease of the frequency of individual parasites affected by exposure to the drug, compared to the frequency observed in the same population upon initial or prior to exposure. Although not unequivocal but generally considered the most adequate, this dentition encompasses two biologically distinct but not always distinguishable processes: (i) existing drug-tolerant parasite lines may become more frequent particularly under drug pressure, and (ii) previously susceptible parasites may undergo genetic mutations, possibly induced by drug exposure, and be selected under drug pressure (Conder *et al*., 1995).

There are the different types of resistance that are side-resistance, cross-resistance and multiple resistances. The side and cross resistances are condition in which a drug-selected population has a gene coding for a mechanism that defeats the toxicity of the drugs within a mode of action families and from different mode of action families, respectively whereas multiple drug resistance (MDR) is a state in which a population has been selected independently by drug from different mode to produce different but concurrent mechanism of evasion (Nega *et al.*,2018).

Resistance to anthelmintics has particularly become a major problem in small ruminants infected with gastro intestinal nematodes of the family Trichostrongylidae. The nematode Haemonchus contortus, which parasitizes the abomasum of small ruminants, was the first parasite ever to develop resistance. Resistance to phenothiazine was reported in the USA in 1957 within two decades of the drug's introduction onto the market. Resistance has developed mainly in *H. contortus*, Teladorsagia circumcincta, Trichostrongylus colubriformis, Ostertagia spp. and Cooperia spp., affecting Australia, New Zealand, South Africa, many European countries, several Asian countries and both American continents(Holden dye,(2011).

2.4.1. Definition and distribution of Anthelmintic drug resistance

The control of GI nematode infections in livestock, over the past decades and still today, is primarily based on the preventive or curative use of chemotherapeutics. However, by way of their inherent genetic diversity, GI nematodes have consistently found ways to circumvent existing control measures. As a consequence, we are currently faced with an escalating spread of anthelmintic resistance (AR) and infection patterns that may be altered by a changing climate, altered land□use and associated farm husbandry changes (Charlier *et al*.,2018). Anthelmintic resistance has grown from a curiosity to an important economic problem in several animal industries and is now set to threaten the control of human parasites (Sangster *et al*., 1999).

Resistance is probably an inevitable consequence of the use of anthelmintic and the history of resistance to anthelmintic starts with the first report on phenothiazine resistance approved in 1957. In most regions of Africa, the development of anthelmintic resistance could be expected to be slow, because of high refugia and low frequency of treatment. The exception is south Africa, where in large-scale commercial sheep farms the intensive use of anthelmintics for several decades has led to very high levels of multiple anthelmintic resistances. However, the overall prevalence of anthelmintic resistance has not been extensively investigated throughout the African continent, anthelmintic resistance in sheep and goat parasites has been reported from at are least 14 countries(Nega *et al*.,2018).

2.4.2. Anthelmintic drug resistance situation in Ethiopia

Gastrointestinal helminth infections are very common in many parts of Ethiopia and their control is almost exclusively based on anthelmintic treatment (Dereje , (2009); Fikru Regassa *et al*., 2006), Tembely *et al*.,1997). Aberra , (1992) reported the prevalence of helminth parasites in Bedelle wereda (District) and its environs to be around 90% based on fecal examination(Getachew *et al*.,2013). Unsound use of anthelmintics in veterinary practice, for both food producing and companion animals, favors the development of either intrinsic or acquired anthelmintic resistance. Anthelmintic drug resistance is a growing problem, and indeed developing new drugs may not be the solution for this problem. Some of the common causes that contribute to the development of anthelmintic resistance are unnecessary use of anthelmintic drugs, inappropriate dose, inadequate duration of therapy, use of irrational drug combinations (Kassahun *et al*., 2016).

The extensive use of anthelmintics for the control of helminth infections on grazing livestock has resulted in the development of resistance that has become a major practical problem in many countries of Africa (Waruiru, (1997); Van Wyk *et al.,* 1997), Europe (Várady and Corba, 1999; Chartier *et al*., 1998); Coles, (1998), Asia (Chandrawathani *et al*., 1999), South America (Eddi *et al*., 1996; Nari *et al*.,(1996) and Australia (Green *et al*., 1981); Le Jambre *et al*., 1995). A similar situation has been reported in eastern Ethiopia by Sissay *et al*., (2006) where nematodes have shown resistance to albendazole, tertramisole and ivermectin at prescribed dosages in small ruminants. On the other hand, an experimental study on Heamonchus contortus infection in sheep has shown 100% efficacy of ivermectin (Yacob *et al*., 2008). Highly prolific species such as *H. contortus* with relatively short life expectancy of adult worms have a higher risk of developing diverse resistance-alleles due to spontaneous mutations than the less prolific *T. colubriformis* (Silvestre *et al*.,2002).

Their long-term utilization, inappropriate handling and under dosage may be some of the reasons for their reduced efficacy and for the increasing development of drug resistance. A study done on the blood feeding parasite, *H. contortus* has demonstrated the existence of multiple-resistance to repeated applications of benzimidazoles, levamisole and ivermectin (Waruiru, (1997)). In this study, all the three drugs were almost 100% effective against ivermectin susceptible isolates while only closantel proved efficacious on the ivermectin resistant strain. Since anthelmintics within each drug class act in a similar manner, resistance to one anthelmintic in a given drug class is likely to be accompanied by resistance to other anthelmintics of that same class (side resistance). There is also the likelihood for the development of cross resistance from anthelmintics of one drug class to those

of another, if the two drug classes share similar targets. Hence, the widespread occurrence of resistance across the majority of anthelmintic drug classes(Sisay *et al*.,2006).

Anthelmintic resistance has increased to become an important economic problem in several animal industries. The modern broad-spectrum anthelmintics are currently widely used in prophylaxis and treatment of helminth infections in farm animals. The problem of resistance to chemotherapeutic drugs has gradually grown from its rather sporadic occurrence in the early 1960s to the current status where anthelmintic resistance threatens the sustainability of many intensive systems of production(Várady *et al*.,2011). The history of parasite resistance to anthelmintics starts with the first report on phenothiazine resistance in 1957. *H. contortus* was the first nematode to develop resistance against the different anthelmintics. Ivermectin was tested as a single oral dose in sheep experimentally infected with avariety of nematode parasites (Egerton *et al*., 1980). A dosage of 0.05 mg/kg was effective against some species (especially Haemonchus contortus and Oesophagostomum columbianum) but not all. On the other hand, a dosage of 0.2 mg/kg was 95-100% effective against all tested stages and species, viz. adult *H. contortus*, third stage (L3) and fourth stage (L4) larvae and adult Ostertagia circumcincta, adult Trichostrongylus axei and *T. colubriformis*, L4 and adult Cooperia sp. and adult Oesophagostomum columbianum. Two of the species (*H. contortus* and *T. colubriformis*) were represented by strains known to be resistant to benzimidazole anthelmintics(Campbell *et al*., 2000).

Benzimidazoles are the oldest class of authorized anthelmintics; thiabendazole was introduced in the 1960s. The first report of decreased efficacy of thiabendazole against *H. contortus* strains dates from 1964, just 3 years after its introduction to the market. The problem of anthelmintic resistance in GI nematode of Ruminant is worldwide and well documented reports of anthelmintic resistance have been made from South Africa, Australia, New Zealand, Malaysia,Spain, France, Denmark, UK, Brazil, and the United States (Verma *et al*.,2018).

Table 4. Major reported resistances to commonly used anthelmintics in Ethiopia

		Broad spectrum						Specific group/Narrow spectrum					
		Izs		Ms				Snl					
Host	Helminth parasite	Bzs	M/P	Lev	Ivm	Mxd	Dmt	Mbc	Cst	Rxn	Opp	Oxa	Ppz
Sheep		+		+	+	+		+	+		+		
Trichostrong.spp.		+	+	+	+	+			+	+			
	H. contortus	+		+	+	+		+	+				
	Trichuris spp.												
	O.ostertagi												
	Cooperia spp.	+							+				
	F.hepatica												
Goat	**Trichostrong**	+		+									
spp.		+	+	+	+				+		+		
	H. contortus	+											
	O.ostertagi												
Cattle	**Trichostrong.**	+											
spp.		+		+	+	+	+						
	H. contortus							+					
	Oesophag spp.	+			+			+					
	Trichuris spp.	+						+					
	O.ostertagi	+			+	+	+	+					
	Cooperia spp.												
	F.hepatica												

Source ; (Nega and Seyoum, (2017).

Bzs = benzimidazoles; Izs = imidazothiazoles [M = morantel, P = pyrantel]; Mls = macrocyclic lactones [Ivm = ivermectin, Mxd = moxidectin, Drm = doramectin]; Sns = salicylanilide [Mbc = milbemycin; Cst = closantel]; Rxn = rafoxanide; Opp = organophosphate; Oxa = oxamniquine; Ppz = piperazin.

2.4.3. Mechanisms of anthelmintic resistance

Anthelmintic Resistance mechanisms includes mutation or deletion of one or more amino acids in the target genes, reduction in the number of receptors, decreased affinity of receptors for drugs, and absence of bioactivating enzymes. Due to modern molecular technology, mechanisms of resistance

in worms are becoming further understood. Resistance in worms can be the result of a variety of mechanisms and can be categorized as genetic changes in the drug target, in the drug transport or in the drug metabolism. The cause of resistance in worms is often complex. Whereas nematode resistance to benzimidazoles can be due to a mutation in the gene coding for the target site, the same mutation(Furgasa *et al*.,2018).

There are several phases in the process of resistance development. Firstly, there is an initial phase of susceptibility where the number of resistant individuals within the parasite population is low with continued exposure to the same drug group. An intermediate phase then follows in which the frequency of heterozygous resistant individuals within the population increases. Finally, sustained selection results in a resistant phase where homozygous resistant individuals predominate within the population. The speed of this process will depend on how severe selection pressure is on the parasite population. It is known that this is linked to the frequency of treatment and the fact that widespread and excessive use (8 to 12 times per year) of the drugs without considering the ecology of the parasites, has led to the development of resistance of the parasites to drugs(Verma *et al*., 2018) .

Analysis of resistance mechanisms in several organisms is warranted as their general biochemical framework of resistance is often similar. Cells may evade drug action by hiding in sanctuaries; drug uptake may be thwarted by loss of uptake systems or alteration of membrane composition; once inside, drugs may be inactivated, excreted, modified and excreted, or routed into vacuoles; drug activation mechanisms may be suppressed or lost; the interaction of drug with target may be made less effective by increasing the level of competing substrates or by altering the target to make it less sensitive to the drug; the cell may learn to live with a blocked target by passing the block(Ouellette, (2001) .

The general consensus is that anthelmintic resistance appears to be a pre-adaptive herita-ble phenomenon with the gene or genes con-ferring resistance being present within the parasite population even prior to the drug being used for the first time. Under these circumstances resistance arises as a result of selection through exposure of the worm population to an anthelmintic. When an animal is optimally exposed to an anthelmintic the only worms that should survive are those that carry the genes that confer resistance. For a short period (until the animal becomes re-infected with drug susceptible worms from pasture) the resistant survivors are the only worms laying eggs and in

this way the gene pool for resistance is increased. The rate of development of resistance is influenced by many factors, of them, significant ones are described here (Hatam *et al*.,2013) .

Table 5. Anthelmintic family and mechanism of resistance

Anthelminthic family	**Mechanisms of resistance**	**Comment**
Benzimidazoles	B-tubulin isotype 1 mutations: f200y, f167y,	The best studied mutations and probably the most important.
	B-tubulin isotype 2 mutations: f200y, f167y, deletion.	F200y seems to be the most important mutation in *haemonchus contortus*, but this might not be true for all species.
Avermectins and milbemycins	Mutations in glucl and/or gaba-r genes	Molecular evidence from *cooperia oncophora*:genetic evidence from *H. contortus*.
	Overexpression of p-glycoproteins population	Population genetic and some pharmacological evidence.
		The relative importance of these two mechanisms is yet to be determined.
Levamisole	Changes in nicotinic	Physiological and pharmacological evidence: no molecular data
		to date.

(*Source* ; Nega and Seyuom,2017)

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2.4.4. Detection of anthelmintic drug resistance

Different methods have been described to detect a presence of resistance to anthelmintic. These methods can be divided into in vivo (Fecal egg count reduction test, worm burden reduction test)and In vitro (egg hatch assay, larval paralysis assay, larval migration inhibition assay etc) techniques. The in vivo methods are suitable for all types of anthelmintic, including those that undergo metabolism in the host to chemically active compounds. In vitro techniques offer rapid, sensitive and considerably more economic methods of screening but suffer from certain limitations(Furgasa *et al.*,2018).

Faecal Egg Count Reduction Test (FECRT) : This is the most common test to study anthelmintic resistance. The ability of the anthelmintic in question to reduce the concentration of eggs per gram of faeces (EPG) by more than 95 percent, measured 10-14 days after treatment, in comparison with the EPG measured at the time of treatment. Failure to do so is indicative of resistance. This test was originally designed for sheep, but can be used also for cattle, swine and horses. Cut-off value for drug efficacy in FECRT 95% and 90%, macrolides and benzimidazoles / pyrantel, respectively(Verma *et al.*, 2018). For The worm reduction test, animals are necropsied at the end of the trial, after which the remaining worms in the intestinal tract of the treated animals are compared with those from animals that did not receive any treatment (Rinaldi *et al*., 2014) . Controlled test is considered the gold standard in measuring efficacy of anthelmintics ,which is the most reliable method of assessing anthelmintic efficacy against mixed nematode infections. This tests the efficacy of an anthelmintic by comparing parasite populations in groups of treated and recommended untreated animals. Basically, the procedure compares worm burdens of animals artificially infected with suspected resistant isolates of nematodes. The parasitized animals are randomly separated into medicated and non medicated groups and the animals are necropsied after treatment interval (10 to 15 days) and the parasites are recovered to be identified and counted. This test must be compulsorily done before the registration of a new drug and is not extensively used except in cases of special interest or when confirmation of resistance is required at species level and for evaluation of the effect on larval stages. In an attempt to reduce the cost and labor required for this test, laboratory animal models have been used and guidelines for evaluating anthelmintic efficacy using the controlled test have been published(Demessie *et al*., 2016) .

Several different in vitro tests are available but the majority is almost exclusively used for research purposes. These tests can be used to quantify the level of resistance but they require considerable

technical expertise and in some cases, expensive laboratory equipment. Ideally, these tests require mono-specific infections. The maintenance of standard laboratory strains, both drug susceptible and resistant is necessary for comparative purposes. The main in vitro bioassays are listed in Table 2 (Nega *et al*.,(2018) .

Table 6. Bioassays for the diagnosis of anthelmintic resistance

List of Assays	**Application**
Egg hatch Assay	Benzimidazoles/levamisole/morantel
Larval paralysis	Levamisole/morantel
Tubulin binding	Benzimidazoles
Larval development	All drugs
Adult development	Benzimidazoles

(*Source* ; Nega *et al*.,(2018)

The egg hatch assay has been developed to differentiate between resistant and susceptible strains of gastrointestinal nematodes for the BZs and for the levamisoles that used to calculate the 50% of lethal dose of the drug on freshly collected nematodes eggs. It provides an accurate method for assessing the susceptibility of mixed nematode populations and comparatively more rapid and economic to conduct than the FECRT(Demessie *et al*., 2016) .The principle is based on determination of the proportion of the eggs that fail to hatch in solution of increasing drug concentration in relation to the control wells enabling the user of the test to develop a dose response line plotted against the drug concentration(Nega *et al*.,2018). The long term stability of thiabendazole in solutions of DMSO is not known but reduction in anticipated concentrations may occur when stock solutions are diluted in water(Coles *et al*.,2006).

To obtain meaningful data, eggs for the egg hatch test must be fresh and should be used within three hours of being shed from the host as sensitivity to some BZs decreases parasites, as embryonation proceeds. The test has only been shown to work on nematode species in which eggs hatch rapidly. There are several variations of the egg hatch assay, but the essential aim is to incubate undeveloped eggs in serial concentrations of the anthelmintic(Demessie *et al*., 2016).

The larval paralysis and motility assay depends on the principle that estimates the proportion of the third stage larvae in tonic paralysis after incubation with a range of levamisole drug concentrations to differentiate between resistance and susceptible strain of parasites. It is relatively easy to carry out, fairly good reproducibility of test (Nega *et al*., 2018).

The larval development assay (LDA) is based on culturing a known number of GIN eggs in the presence of different anthelmintics. It is reported be relatively easy to perform, more sensitive than the FECRT and allows for the identification of parasite larvae to the genus level. LDA is the only one that allows to the detection of resistance against all the drugs irrespective of their mode of action. In this test, nematode eggs isolated from fecal samples are applied to the wells of a micro-titer plate and larvae hatch and develop to the L3 stage in the presence of anthelmintic. The concentration of anthelmintic required to block development is related to an anticipated in vivo efficacy(Demessie *et al*., 2016) .

Tubulin Binding Assay is based on the differential binding of benzimidazoles to tubulin, an intracellular structural protein from susceptible and resistant nematodes. Tubulin binding assay involves the incubation of a crude tubulin extract from adult parasites, infective larvae or eggs, with a titrated benzimidazole until equilibrium is reached(Verma *et al.*,(2018). The mechanism of benzimidazole resistance appears to be associated with a reduced affinity of tubulin for the anthelmintics. The free, unbound drug in test suspension after incubation is removed using charcoal and the tubulin-bound label is sampled and counted by liquid scintillation spectrophotometry. Tubulin extracts from resistant parasites bind substantially less strongly than do those from susceptible parasites. The test is considered to be rapid, highly reproducible and sensitive to minor changes in the resistance status of parasite populations, but it is unsuitable for routine field assays(Nega *et al*., 2018) .

The adult development assay is used for detecting benzimidazole resistance in trichostrongylid nematodes has advanced significantly and (Nega *et al*., 2018), (Verma *et al.*,2018) and *H. contortus* has been cultured through to the adult egg-laying stages, although this test is mainly for research purposes(Demessie *et al.*, 2016).

The most common molecular mechanism that confers benzimidazole resistance in trichostrongyles in small ruminants involves a phenylalanine to tyrosine mutation at residue 200 of the isotype 1 β-tubulin gene. However, in addition a similar mutation at codon 167 may be involved in benzimidazole resistance in nematodes. An allele-specific polymerase chain reaction (AS-PCR) has been used to detect this mutation in *H. contortus* and Teladorsagia circumcincta adult and larval stage(Verma *et al.*,2018). The key issue is that only when a diagnosis based on using pooled larval DNA samples can be obtained will it be possible to bring molecular resistant testing to routine use. Testing of representative numbers of single stages is prohibitively expensive. Also the available molecular tests mainly address resistance in species where the problem is widespread and in some cases may be too common to justify testing. The most common molecular mechanism that confers BZ resistance in trichostrongyles in small ruminants involves a phenylalanine to tyrosine mutation at residue 200 of the isotype 1 b-tubulin gene(Coles *et al*.,2006).

2.5. Management of anthelmintic drug resistance

The key areas of concern in the management of anthelmintic resistant throughout the world are: A) Drug related factors (pharmacokinetics, formulation and mode of application of anthelmintics). B) Management related factors (incorrect dosing of anthelmintics, frequency of anthelmintic treatment, and use of the same anthelmintic class for several years, pasture management of livestock). C) Parasite related factors (number of nematodes in refugia, frequency of genes for resistance in an unselected parasite population, genetic factors as mode of inheritance, fitness and fecundity of resistant nematodes, generation time(Verma *et al*., 2018). Considering the increasing concern regarding the development of drug resistance, the use of pharmacology-based information is critical to design successful strategies for future helminth parasite control in livestock. Integrated pharmacokinetic/pharmacodynamic and clinical pharmacology knowledge is required to preserve both well-established and modern anthelmintics. Assessment of drug disposition in the host and comprehension of the mechanisms of drug influx/efflux/detoxification in different target helminths, have signified relevant progress in anthelmintic therapy in ruminants. Moreover, different pharmacokinetic-based approaches to enhance parasite exposure (pharmacokinetic optimization) and the use of a mixture of molecules from different chemical families (drug combinations) have

been assessed as valid strategies to control resistant parasites and to slow the selection for further resistance (Lanusse *et al*., 2018).

Alternatives to the use of chemical compounds such as grazing management, improving resistance of the parasites through selective breeding, by vaccination and provision of good nutrition are also of paramount importance. Control of pasture can reduce the impact of worm infection in livestock. Another approach is through the use of the pasture for different animals at different times such as bringing equine or cattle to the pasture for one season and using the pasture for sheep grazing in the next season. The reason is that sheep and cattle or equines do not share much of the important helminth parasites such as *Haemonchus contortus*. However, implementation of this method needs a good knowledge about the epidemiology of the helminth parasites that are endemic to that area, such as the knowledge about the time at which the helminth eggs are hatched and the larval populations reach the infective stage(Verma *et al.,*2018)

A safe pasture is one that has not had sheep or goats grazed on it for 6 months during cool/cold weather or 3 months during hot, dry weather. Weaning sheep and goats at 2 months of age and rotating them through pastures ahead of the adults will minimize the exposure of susceptible animals to large numbers of infective larvae (L3). There is considerable evidence that part of the variation in resistance to nematode infection is under genetic control. Resistance is most likely based on inheritance of genes that play a principal role in expression of host immunity. On the basis of survival of the fittest management conditions, several breeds of sheep around the globe are known to be relatively resistant to infection. Such breeds include Scottish Blackface, Red Maasai, Romanov, St. Croix, Barbados Blackbelly, and the Gulf Coast Native *(*Waller *et al*.,2006) .

The most promising vaccine for small ruminant worms is based on a ‘‘hidden gut’’ antigen and specifically targets H contortus. This antigen is derived from the gut of the worm and, when administered to the animal, antibodies are produced. When the worm ingests blood during feeding, it also ingests these antibodies. The antibodies then attack the target gut cells of the worm and disrupt the worm’s ability to process the nutrients necessary to maintain proper growth and maintenance, thus killing the worms. This vaccine has been tested successfully only in sheep under experimental conditions and has had limited success under field conditions(Dyary, (2016)). On the other hand, reducing hosts exposure to infection through biological control on pasture such as by

using nematophagus or nematode trapping fungi has also shown great promise. Research with nematode-trapping fungi has documented the potential as a biological control agent against the free-living stages under experimental and natural conditions. These fungi occur in the soil/ rhizosphere throughout the world where they feed on a variety of free-living soil nematodes. These fungi capture nematodes by producing sticky, sophisticated traps on their growing hyphae. Of the various fungi tested, Duddingtonia flagrans, has the greatest potential for survival in the gastrointestinal tract of ruminants(Mahlatse, (2011)) .

The need to provide refugia through modification of worm control programs depends largely upon the environment and importance of local factors in drench resistance. Where environmental conditions promote the continual survival of worm larvae on pasture, a substantial pool of larvae in refugia (not exposed to drenches) is usually available on the livestock property involved. This presumably explains the relatively lower levels of anthelmintic resistance in non *Haemonchus* species in temperate countries. However, where the treatment interval is close to the pre-patent period of the nematode species involved, non-resistant worms do not have an opportunity to contribute to the population, and resistance can develop rapidly. This almost certainly explains the high levels of resistance in the *H. contortus*, where the need to combat a highly pathogenic nematode has created a conflict with the sustainability of anthelmintic use (Besier, (2003)).

Where strategic control programs based on the seasonal absence of worm larvae on pasture explain high levels of anthelmintic resistance (Besier, (2003)), it may be necessary to deliberately allow the survival of some worms not recently exposed to anthelmintics. In Western Australia, where the commonly-used "summer drenching" program provides excellent worm control but is associated with high levels of resistance in *T.circumcincta* and *Trichostrongylus* spp., the tactic of leaving a proportion of the flock undrenched when strategic treatments are given has reduced the development of resistance (Besier, (2001)). However, the failure to suppress worms in summer has been shown to increase the risk of winter parasitism, especially in immature, worm-prone, animals (Besier ,(2003).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Bishoftu town and its surroundings(Figure 2). The town is located 45 km East of Addis Ababa at 9°N latitude and 4°E longitudes, at altitude of 1850 m above sea level in the central Oromia Region. The area has an annual rainfall of 86.6-mile meter (mm) of which 84% falls during the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures of the town are 26 and 14°C, respectively, with mean relative humidity level of 61.3%. Mixed crop-livestock production system is the common managementsystem inBishoftu town National Metrological Service Agency (NMSA, 2011). Bishoftu is the capital town of Ada'a district, which is one of the districts in East Shoa Zone of Oromia Region and located at 47 km South East of Addis Ababa with a human population of about 95,000.. The district covers an area of 92, 751.33 ha. The average altitude is about 1880 meter above sea level. The average annual rainfall is about 839 mm and the average temperature is 24°C.

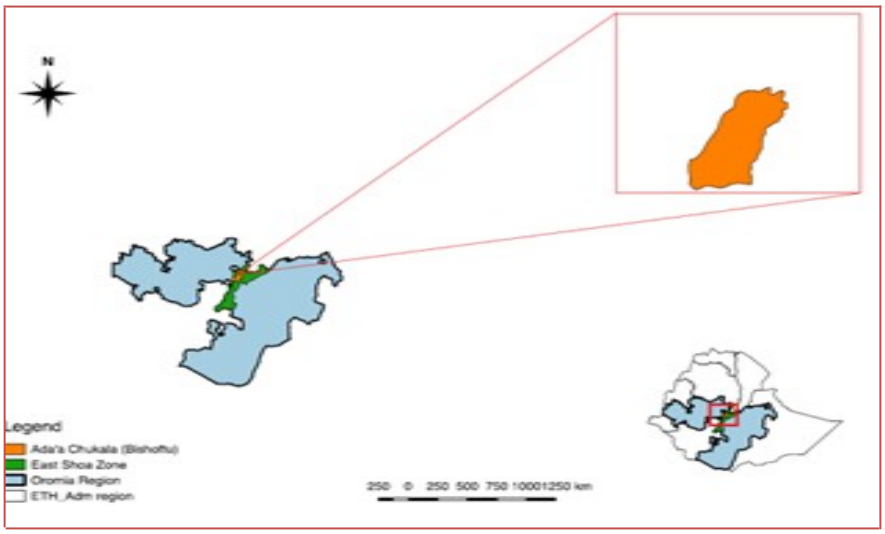

Figure 2. Map of Bishoftu town in East Shoa Zone of Oromia Regional State (Source; Worku and Bedanie, 2019)

3.2. Questionnaire survey

Questionnaire survey was performed on the 100 sheep and goat owners in the different PAsof Ada'a district. The survey was conducted by way of personal interview of farmers in language they can communicate to complete the questionnaires data. After introducing the objectives of the study and

obtaining their full consent, one hundred (100) small ruminant owners and fifteen (15) veterinary drug stores and pharmacies were included and interviewed for the purpose of data collection in the study area according to the previous methods (Terefe *et al* .,2012 ; Kumsa *et al* .,2010).The sample size of the respondents was determined using the formula ($n = 0.25/SE^2$) proposed previously (Arsham, 2007) at the standard error (SE) of 0.05 with 95% confidence interval. Based on this, hundred (100) small ruminant owners (farmers) having 5 to 15 animals were randomly selected and interviewed according (Arsham, 2007). The questionnaire focused mainly on information on the the frequency of use, criteria for selection or choice, main source, and rotation of anthelmintics, who administer the animals,observations on the responses of treatment (efficacy), and educational background of each participant. Only those owners who have reared their sheep and goats were included in questionnaire survey. Animal traders were not included as they keep the animals for short duration and hence were not expected to have enough information about their animals according previously done (Terefe *et al*., 2012; Kumsa *et al*., 2010). To know the exact number of sheep owners to be interviewed, we don't know the total number of households owning livestock in Ada'a districts. Therefore using the worst case scenario; the fifty of (50%) of the respondents giving a "Yes" response to a yes or "No" questions have been expected .Then sample size was calculated according to the earlier formula (Arsham, (2007).Therefore, taking a standard error of 5%, and use the formula which says "Sample Size (N) = $0.25/SE^2$", one hundred (100) respondents were interviewed to compile the data designed for questionnaire survey.

3.3. Study Animals and parasites

A survey of gastrointestinal parasites was done in different places of Ada'a district to identify donor sheep with high nematode egg count. Two animals were purchased by negotiation with the owners. These animals were humanely slaughtered to recover adult parasites to get pure *Haemonchus* from the abomasum and pure *Trichstrongylus* from the small intestine. Then, the worms were washed and crushed to liberate eggs. The eggs were then cultured in a plastic jar filled with sterilized catttle faeces for 10 days at room temperature according to previous method (Mcintyre *et al.*, 2018). At the end of the ten days, the infective **(**L3**)** larvae were harvested (Wyk *et al*.,2013). Two new sheep free of helminth parasites and aged 12 and 24 months were purchased from the local market for propagation of the parasites to be used in in vitro studies. They were acclimatized for 2 weeks in a

fly-proof animal facility found in the College of Veterinary Medicine and Agriculture. Then, the recovered L3 were drenched to the animals; one species for each animal. The animals were provided with grass hay supplemented with concentrate (wheat bran). What was provided adlibitum. All animals were handled according to the guidelines for experimental animal use and management. Following, establishment of the infections, daily fecal collection was done for harvesting L3 to be used in larval motility test (LMT) and for recovering eggs to be used in egg hatch assay (EHA). Adult parasites required for the adult motility test were obtained by humanely slaughtering sheep infected by mono specific parasites after the EHA and LMT were completed.

3.4. In vitro anthelmintic efficacy studies

3.4.1. Anthelmintics used in this study

The anthelmintics used for the experiment were bought from local retail markets and composed of three drug classes (Table 7). Before, the anthelmintic were purchased; information was gathered on anthelmintic utilization practices in the area and the common drugs available on the local market. Then the efficacy of different brands of albendazole, Ivermectin and Levamisole and Tetramisole were tested using the LMT and EHA methods (Table 8) and interpreted according to the guidelines provided by WAAVP recommendations for efficacy evaluations of anthelmintic (Coles *et al* 1992, 2006).

Table 7. Details of the anthelmintic drugs used in vitro experiment

Drug groups	Generic name	Trade name	Manufacturer
Benzimidazole	Albendazole	Alzole	East Africa Pharmaceuticals, Ethiopia
	Albendazole	Albenda	Chengdu Qiankun veterinary Pharma
Imidazothiazol	Tetramisole	Tetsole®	East Africa Pharmaceuticals, Ethiopia
e	Tetramisole	Ashitetra 600 mg	Ashish Life Science Pvt Ltd, India
	Levamisole	Nizal-QK bolus	Ashish Life Science Pvt Ltd, India
	Levamisole	Zanisole	East Africa Pharmaceuticals, Ethiopia
Macrocyclic	Ivermectin1%	Tecmectin (1%)	Chengdu Qiankun veterinary Pharma
Lactones	Ivermectin1%	Ivermic (1%)	Uruguay, Veterinary drug store , Belgium
	Ivermectin1%	Ashiver (5 mg)	Ashish Life Science Pvt Ltd, India brands

Table 8. List of in vitro evaluation techniques of efficacy of anthelmintic performed against isolate of *H.contortus* and *T.columbriformis*

Anthelmintics Tested	**Type of brands of tested AH**	**In vitro test conducted**
Albendazole	East African Pharmaceuticals ,Ethiopia ,Sudan brands	Egg Hatch Assay (EHA)
	Chengdu Qiankun veterinary Pharmacy, Chinese brands	
Levamisole	East African Pharmaceuticals ,Ethiopia ,Sudan brands	LMT
	Ashish Life Science Pvt Ltd, Indian brands	
Tetramisole	Chengdu Qiankun veterinary Pharmaceuticals	Adult motility Assay (AMT)
	Ashish Life Science Pvt Ltd, India	
Ivermectin	Chengdu Qiankun veterinary Pharmaceuticals	LMT
	Uruguay ,Belgium veterinary drug store	
	Ashish Life Science Pvt Ltd, India	

3.4.2. Preparation of serial dilutions of Anthelmintics for in vitro efficacy study

For egg hatch assay: The stock solutions of albendazole of Chinese, East Africa and Indian brands was prepared at concentrations of 1000µg/ml by dissolving 100mg of albendazole in 20 ml of Dimethylsulfoxide solution and 80 ml of distilled water. The second mother solution of dissolved Albendazole was prepared at concentrations of 500µg/ml by mixing 50 mg of mother solutions with 50 ml of distilled water. The third concentration of Albendazole solution was prepared at concentrations of 250µg/ml by mixing 25 mg of first mother solution with 75 ml of distilled water. The fourth concentration of Albendazole solution was prepared by dissolving 12.5 mg of mother solutions in 87.5 ml of distilled water. The fifth final solution of Albendazole was prepared by dissolving 6.25 mg of Albendazole solutions in 93.75 ml of distilled water. The prepared anthelmintic serial concentrations were then added to the micro dilution plates at final dilutions of (1 µg, 0.5 µg, 0.25 µg 0.125 µg and 0.0625 µg/mL) according to (Sileshi *et al.*,2012) to be used directly for the in vitro cultivation of isolated parasite eggs.

For Larval motility test: Macrocyclic lactones group of anthelmintic were tested using larval motility test, to evaluate the percentage mortality of infective stage (L3) recovered from *Haemonchus.contortus* and *Trichostrongylus.columbriformis* in sheep. Therefore, the stock

solutions of different ivermectin and levamisole brands were prepared at final concentrations of (1 µg, 0.5 µg, 0.25 µg 0.125 µg and 0.0625 µg/mL) as described in Albendazole serial preparations.

For adult motility test: tetramisole of Indian and Chinese brands were tested against adult parasites of *Haemonchus.contortus* and each tested concentrations were the same used in albendazole, ivermectin, and levamisole solutions. The stock solutions of tetramisole of Chinese and Indian brands was prepared at concentrations of 1000µg/ml by dissolving 100mg of tetramisole in 20 ml of Dimethylsulfoxide solution and 80 ml of distilled water. The second mother solution of dissolved tetramisole was prepared at concentrations of 500µg/ml by mixing 50 mg of mother solutions with 50 ml of distilled water. The third concentration of albendazole solution was prepared at concentrations of 250µg/ml by mixing 25 mg of first mother solution with 75 ml of distilled water. The fourth concentration of albendazole solution was prepared by dissolving 12.5 mg of mother solutions in 87.5 ml of distilled water. The fifth final solution of albendazole was prepared by dissolving 6.25 mg of tetramisole solutions in 93.75 ml of distilled water. The prepared anthelmintic serial concentrations were then added to the micro dilution plates at final dilutions of (1 µg, 0.5 µg, 0.25 µg 0.125 µg and 0.0625 µg/mL) according to (Sileshi *et al.*,2012)to use directly for the in vitro cultivation of isolated parasites eggs.

3.4.3. Egg Hatch Assay or Test (EHA/EHT)

In egg hatch assay, benzimidazole group of anthelmelmintic was tested to evaluate the percentage inhibition of eggs on eggs recovered from *Haemonchus.Contortus* and *Trichostrongylus .columbriformis* in sheep. This test was conducted for evaluation and comparison of efficacy of albendazole of Chinese, East African Pharmaceuticals and Indian brands such as sold on Ethiopian markets. For efficacy evaluation of these anthelmintic, albendazole of Chinese, East African pharmaceuticals and Indian brands were purchased from local private veterinary drug stores and prepared in serial concentration in solutions of Dimethylsulfoxide (DMSO) and distilled water. The eggs of *H. contortus* were adjusted as 100 eggs/0.1ml and 100 eggs were added into 1.5ml test tubes containing various serial concentrations (1.0, 0.5, 0.25, 0.125, and 0.0625µg/ml) of test substances (albendazole) and incubated at 25°C for 48 hr according to guidelines of (Sileshi *et al*.,2012). Untreated eggs in distilled water were used as a negative control. After 48 hours, the test substances were mixed with one drop of diluted iodine solutions. Then 100µLof agitated sample

was transferred into a petridish marked with grid and hatched or embryonated, unhatched eggs and dead eggs were counted as described in detailas protocols described by C.elegans model (Viviane *et al*.,2017) and World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) Results were expressed as %inhibition of eggs hatch as a representation of three independent experiments performed in triplicate using stereo microscope. The mean number of eggs and larvae at each concentration was calculated and percentage hatch was derived using the following formula.

$$\text{Percentage hatch} \quad \frac{\text{Number of hatched eggs}}{\text{Number of hatched eggs + Number of unraced eggs}} \times 100$$

The concentrations were expressed in percentage (weight/volume) for each serial dilutions considering that the effectiveness of selected anthelmintic compound is generally higher in vitro than in vivo, and it was decided to continue the screening of each tested compound at lower concentrations, only if at the highest concentration tested some inhibiting effects were observed in each triplicates of the assays performed in this study according to Ferreira *et al*. (2013).

3.4.4. Larval motility test (LMT)

In this method, infective larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis* were exposed to an anthelmintic for 24 hours and subjected to test under larval motility test. This test was designed to evaluate the efficacy of ivermectin and levamisole in the small ruminants nematode. Therefore, ivermectin of Chinese, Indian and Uruguay and levamisole of East Africa and Indian brands were purchased and tested to compare their efficacy within their brands. For these anthelmintic class, the tested concentration was the same as used in albendazole.For evaluation of the motility of third-stage (L3) larvae in the presence or absence of increasing dilutions of levamisole and ivermectin of each tested brands, eggs were initially cultured according to the method of(Van Wyk & Mayhew, (2013). Two sheep prepared as donor animals were infected orally with 2500 infective larvae (L3). The infected sheep were checked for the establishment of the infection as of day 21 post-infection using faecal examinations according to (Chaka *et al*., 2009). Then 15 gram of feces with nematode eggs were crashed and homogenized with sterile pestle and mortar. The culture material was moistened daily by spraying distilled water to provide the appropriate humidity and air to grow the larvae. After incubation for 10 days at room temperature,

L3 were recovered by spontaneous migration after downward inverting the culture material and adding warm water. The concentrated larvae were washed three times and total counted were stored in separate tubes. The storage of counted concentrated larvae was finally diluted and counted three times to distribute the required amount of larvae in ech well. Finally, dilutions of counted larvae were adjusted at 50 µL of suspensions containing 50 L3 per 10µL of serial concentrations of ivermectin and levamisole solutions according to guidelines of (Dolinská *et al*., 2016).

The plates were then incubated for 24 hour at 27 0_C and the number of motile and non-motile larvae was counted, particularly focusing on the presence or absence of smooth sinusoidal movement, respectively. The larvae were also incubated in distilled water as negative controls. After taking from incubator the result was recorded based on movement of larvae. Therefore,if smooth sinusoidal movement is present, it is additionally stimulated through exposition of larvae to microscopic light or shaking of well plates. Then results were expressed as % inhibition of larval motility as a representation of three independent experiments performed in triplicate as described by (Ferreira *et al.*, 2013).

3.4.5. Adult Motility Test (AMT)

Adult motility assay was conducted on mature *H. contortus* worms, collected from abomasa of freshly slaughtered sheep, following the technique of(Avinash *et al*.,2017). It was conducted in petri dishes at room temperature (27–30°C). Fifteen worms were exposed in triplicate to each of the following treatments in separate Petri dishes at (1.0, 0.5, 0.25, 0.125, and 0.0625µg/ml) five different concentrationsand distilled water alone for negative control). Accordingly, immediately after animal´s death, the abomasum was removed, opened and washed for the collection of adult worms. Then fifteen actively moving adult stages of *H. contortus* were manually picked up from the mucosal surface and the contents of the abomasum, collected and washed in distilled water were placed in petridishes containing tetramisole dissolved in DMSO and diluted in distilled water at final concentrations of (1.0, 0.5, 0.25, 0.125, and 0.0625µg/ml) for efficacy evaluation of tetramisole of Chinese and Indian brands. Again, fifteen (15) adult parasites were placed alone in distilled water for the negative controllfollowing the technique of (Avinash *et al*.,2017). The adult parasites placed in petridishes were incubated at 37 ^{0}C and checked their motility at 2 hour intervals until 10 hours according to the previous method (Ferreira *et al.*, 2013) Three replications per each

treatment concentration were employed. After 10 hours, the tetramisole were washed away and the parasites suspended in distilled water for thirty minutes for possible recovery of parasite motility. The numbers of motile and immotile worms were counted. Motility and viability of the worms was assessed by gently prodding the worms using a pointed syringe. The response was recorded as either live or dead. Worms were considered dead when a minimum reaction to touch was observed. Observations were made on the motility or survival of parasites at zero, two, four, six and eight hour post-exposure (PE) after the beginning of the test under our experimental conditions. Results were expressed as % of motility as a representation of three independent experiments preformed in triplicate according to the method of (Ferreira *et al.*, 2013).

3.5. Ethical approval

All experimental procedures involving the use of animals were approved by the Ethics Committee of Addis Ababa University, College of Veterinary Medicine and Agriculture (Ref No. VM/ERC/18/01/12/2020). Questionnaire surveys were performed after informed consent from participants.

3.6. Data management and Analysis

The data for questionnaire survey were analysed using Statistical Package for Social Sciences (SPSS) version-20 statistical software. Descriptive statistics (percentages) were used to measure the results describing the respondents' responses to the questionnaire format. Results are presented as percentages and the absolute numbers on which these percentages are in parentheses from a questionnaire survey. The data were analysed by using R software and SPSS statistical package to test for the significant differences within variables to correlate associated factors in evaluation of egg hatch inhibition, larval and adult inhibition potential among the tested anthelmintics. Two way Analysis of variance (ANOVA) was used to detect the significant effect of different anthelmintics on the parasites eggs, larvae and adult parasites in performance of each in vitro trials. $P < 0.05$ was considered to be statistically significant. The median effective concentration (EC50) which is the concentration at which 50% of the eggs fail to hatch or the larvae become non-motile as a result of

anthelmintic treatment was calculated by using the 'Quest Graph™ EC50 Calculator', an online EC50 calculator tool (https://www.aatbio.com/tools/ec50-calculator).

4. RESULT

4.1. Questionnaire survey

The questionnaire survey indicated ivermectin (39 %) was the most commonly used drug followed by albendazole (36 %), tetramisole (17 %) and levamisole (8%). All sheep owners responded that they use anthelmintic only when animals show symptoms like poor body condition, diarrhea or coughing. Anthelmintic are administered either by prescription from animal health professionals or owners' decisions. Most of the respondents indicated that they selected their drug of choice by color (drug type) and route of administration. The survey indicated that farmers prefer boluses of green color (Albendazole) followed by white (Tetramisole), white but longer in size (levamisole) and subcutaneously injectable (ivermectin) in order of preference. Significantly higher number of respondents administers anthelmintics by animal health personnel (including all ivermectin injections) compared to those who do it by their own ($P< 0.05$). The frequency of treatment with anthelmintics was, on average, twice per year (Table 9).

Table 9. Responses of farmers to questionnaire survey on anthelmintic utilization practice

Content of questionnaire format	Responses of the farmers	Percent
Type of commonly used AH	Albendazole	36.0
	Ivermectin	39.0
	Levamisole	8.0
	Tetramisole	17.0
Who administer AH	Professional	79.0
	Farmers by their own	21.0
Frequency per year	One times	12.0
	Two times	51.0
	Three times	32.0
	Five times	2.0
	More than five times	3.0
Source of AH	Private clinic	42.0
	Government clinic	44.0
	Open market	14.0
Alternative if treatment not respond	Change another AH	37.0
	Give other drugs	40.0
	Send to vet clinic	1.0
	Use medicinal plant	10.0
	Sell it to the market	5.0
	Lost after died	7.0
	Total	100.0

4.2. In vitro anthelmintic efficacy

4.2.1. Egg hatch test (EHT) for Albendazole brands

In this study, different brands of albendazole utilized by the local farmers of the district were analyzed for their anthelmintic activity against local isolate of *H.contortus* and *T.columbriformis* The percentage of eggs that hatch (or conversely die) at each concentration is determined, corrected for natural mortality from control plates, and a dose-response line plotted against drug concentration. For the three tested anthelmintic brands, the egg hatch inhibition potential between treatment and control wells showed significant difference **($p<0.05$)** against both local isolate of *H.contortus* and *T.columbriformis*. Egg hatch test on *T.colubriformis* eggs showed no significant difference between China and India brands of albendazole at 1µg, 0.125µg and 0.0625µg ($P> 0.05$). Both brands performed >50% inhibition in egg hatching even at the lowest concentration of 0.065µg. On the other hand, the egg hatch inhibition potential of the East African (EA) brand was

much lower than that of the above two brands (P<0.01). It has given egg hatch inhibition of 50% only with 1µg concentration while it remained below this cut off point for the rest of the drug concentrations (Figure 3). A similar test on *H. contortus* eggs revealed a different performance. No significant difference between China and EA brands of albendazole at 1µg and 0.5µg (P> 0.05) with values of 72 and 78% for China brand and 78% at both concentrations for EA brand. However, from this concentration downward, the EA brand remained below 50% egg hatch inhibition performance whereas the China brand was good (68%) even at 0.065µg (Figure 4).

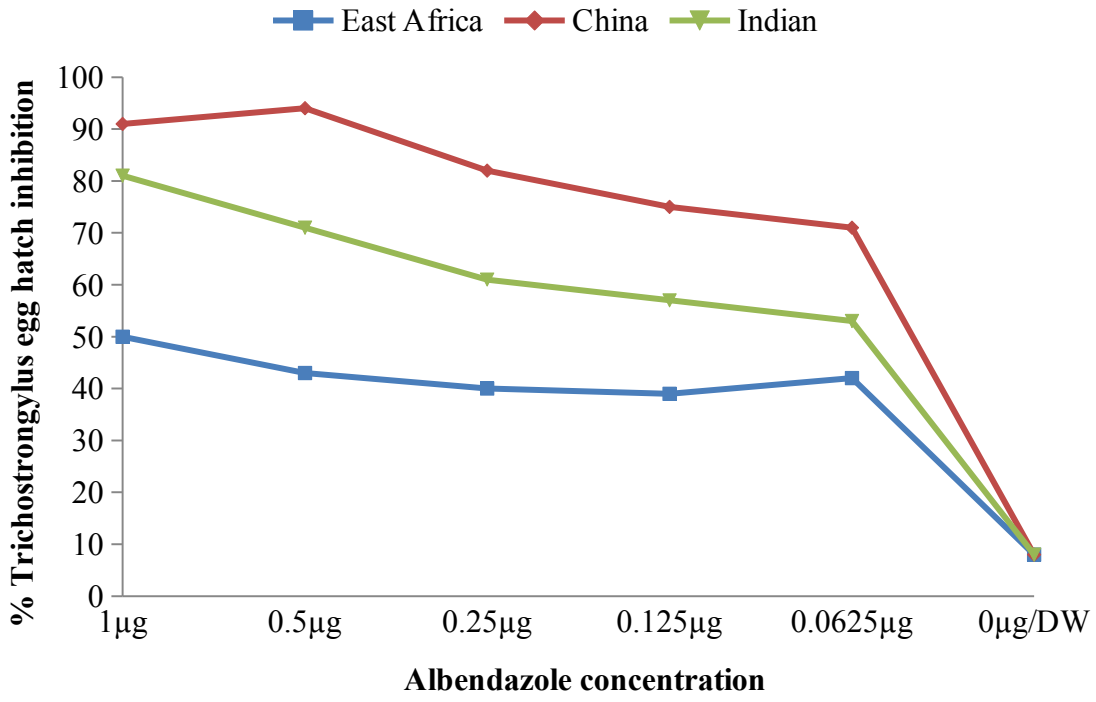

Figure 3. Egg hatch assay performance of different brands of Albendazole against eggs of *T. colubriformis*

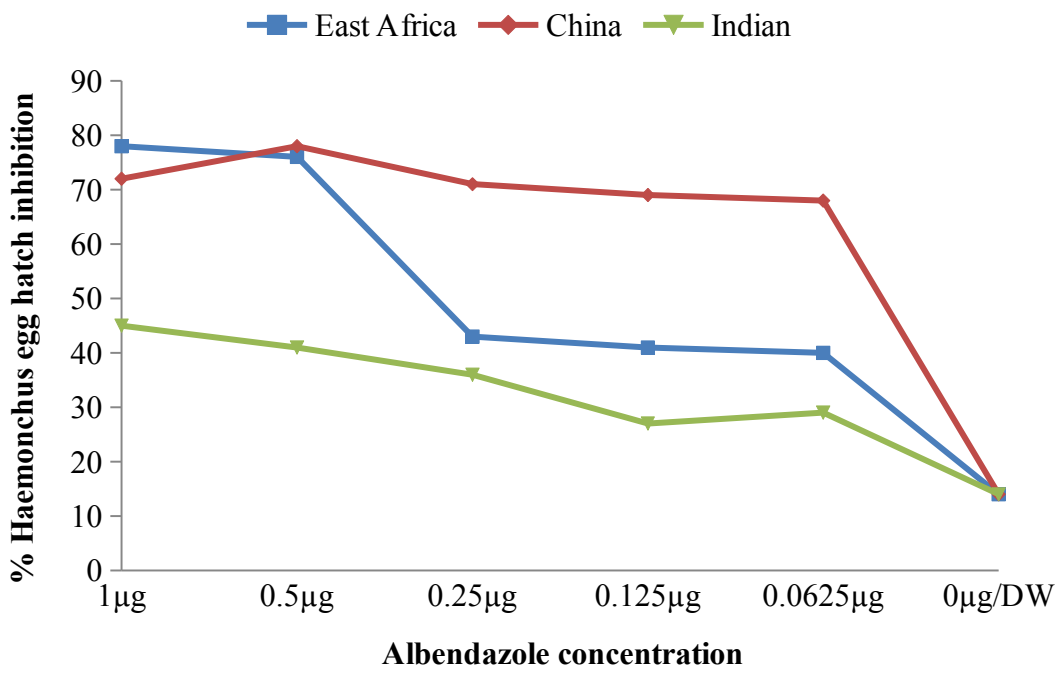

Figure 4. Egg hatch assay on eggs of *H. contortus* with different concentrations of Albendazole brands.

The linear regression for concentration-response indicates that the EC50 for inhibition of *T.colubriformis* egg hatching by albendazole was 2.375µg, 0.087µg and 1.199µg respectively for EA, CN and India brands. The EC50 of Albendazole against *H.contortus* eggs (Figure 5) was 0.504µg, 0.017µg and 1.886µg respectively for EA, CN and India brands. Taking EC50 cutoff value in excess of 0.1µg/ml as an indicator of benzimidazole resistance (Coles *et al*., 2006), except the brand from China, the other two do not qualify for dependable efficacy against eggs of both parasites. The transformed drug concentration-response curve for Haemonchus is shown in Figure 6.

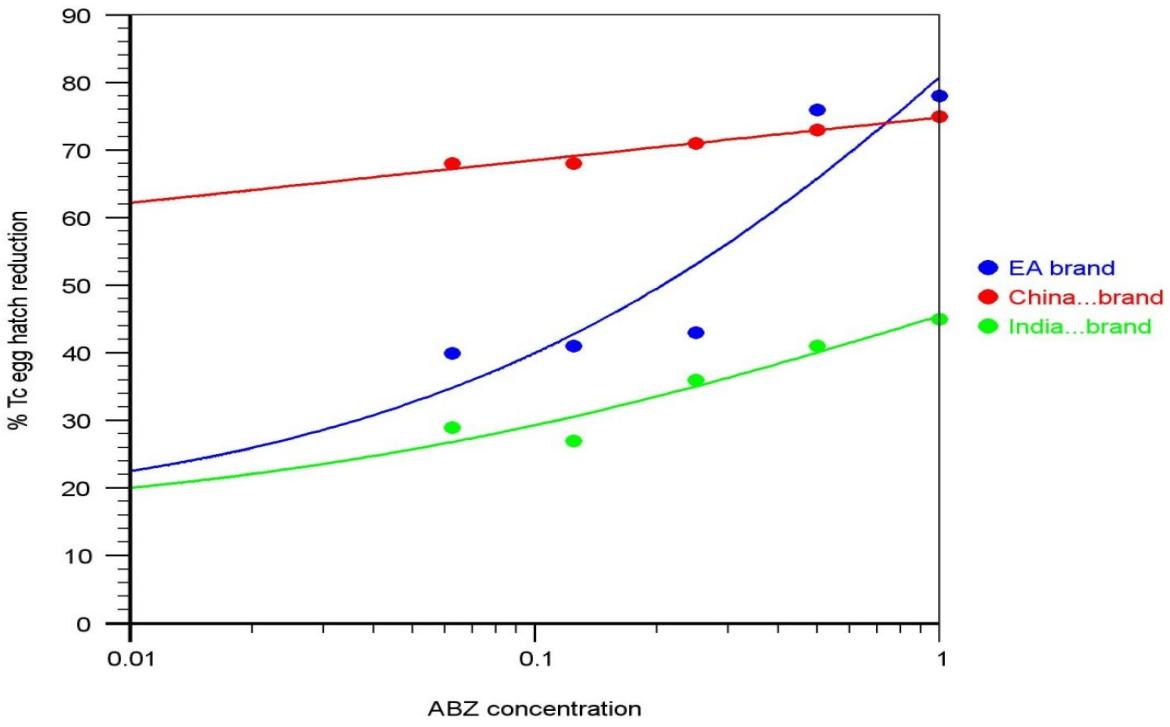

Figure 5. Egg hatch test log transformed concentration-response curve for Albendazole against eggs of *T. colubriformis*

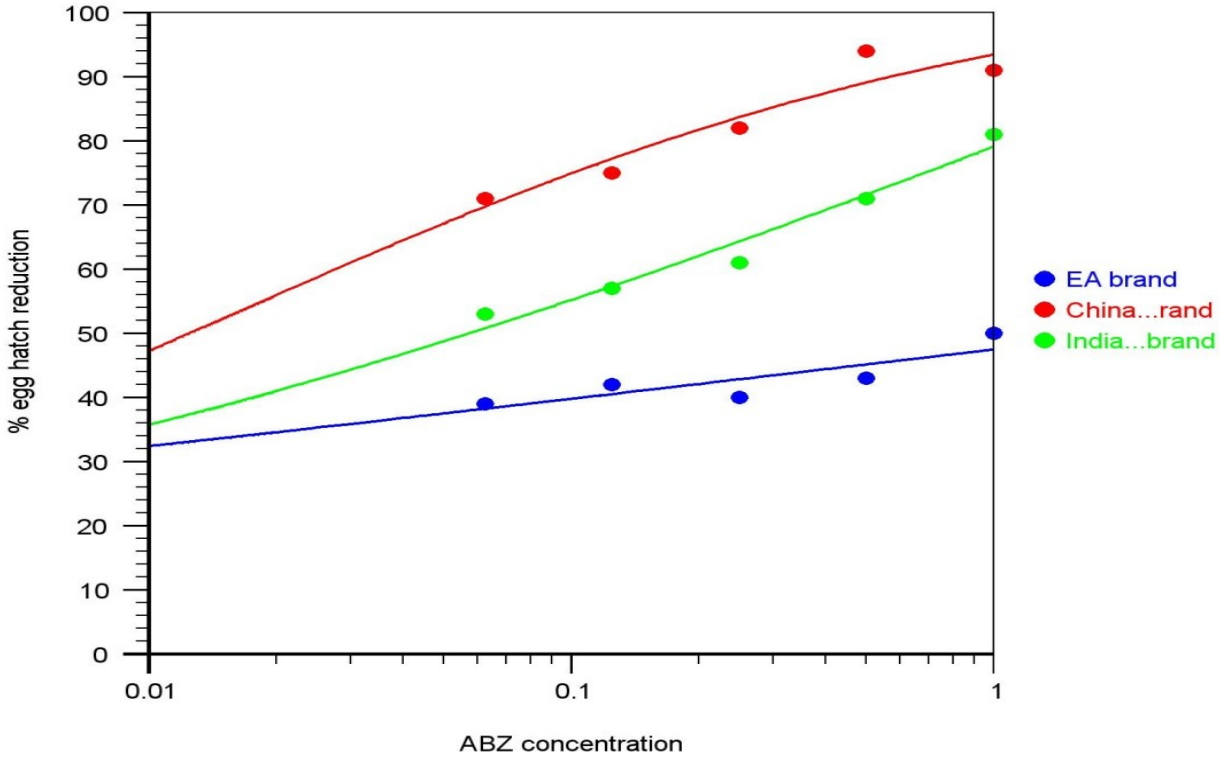

Figure 6. Egg hatch test log transformed concentration response curve for Albendazole against eggs of *H.contortus*

4.2.2. Larval motility test (LMT)

In this study, larval motility test with different levamisole and ivermectin brands were conducted. The test can be used to determine the EC50 which is the median effective concentration required to kill 50% of the worm population. It is measured by detecting presence or absence of sinusoidal movement of larvae in serial concentrations of anthelmintic dissolved in Dimethylsulfoxide (DMSO). The final concentration of levamisole and ivermectin for each brands were (1, 0.5, 0.25, 0.125, 0.0625 µg/ml). Then data were log-transformed, EC50 values calculated and concentration-response curves created as shown below.

Levamisole efficacy

As depicted on the graph of Levamisole efficacy test (Figure 7), at 1µg and 0.0625µg concentrations, there is no difference in efficacy between the two brands tested against both *H. controtus* and *T. colubriformis*. East Africa brand of levamisole was significantly more effective at reducing *H.contortus* larval motility than Indian brand (92% and 76% respectively) at 0.5µg while it is significantly better at 0.5µg, 0.25µg and 0.125µg against *T. colubriformis* (P<0.05).

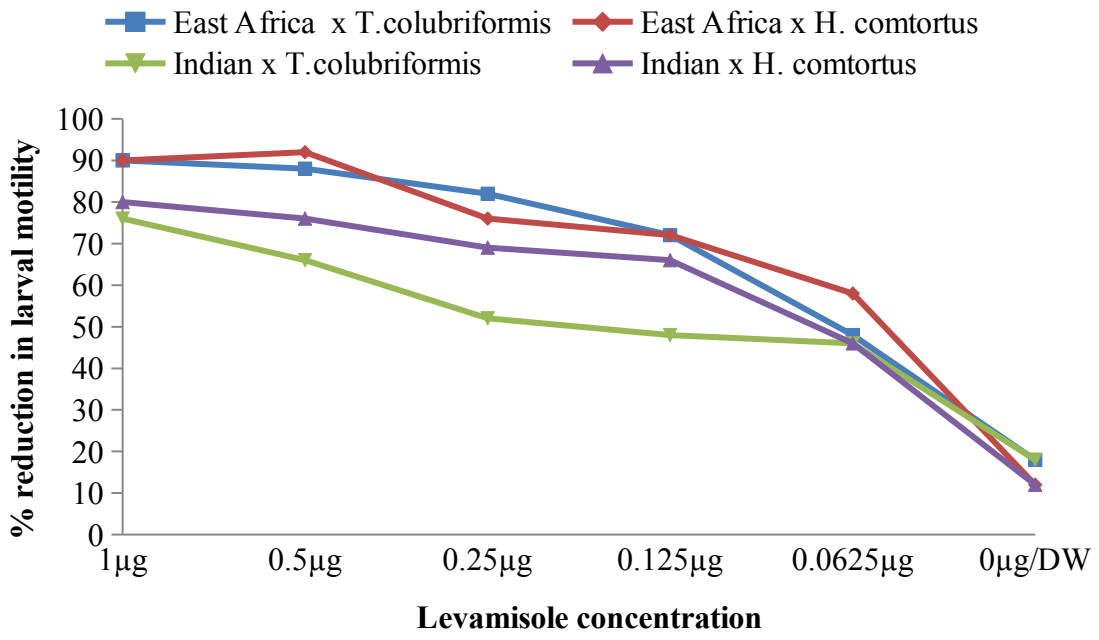

Figure 7. Effect of different brands of levamisole on motility of *H. contortus* and *T. colubriformis* L3

The log transformed concentration-response curve indicates that the EC50 for inhibition of motility of *T.colubriformis* L3 by levamisole was 0.057µg and 4.028µg respectively for EA and India brands (Figure 8) suggesting a much better performance of EA brand over the Indian brand. The EC50 of levamisole against *H.contortus* L3 (Figure 9) was 0.058µg and 0.048µg respectively for EA and India brands; both being in the range of good efficacy at 0.1µg cutoff point.

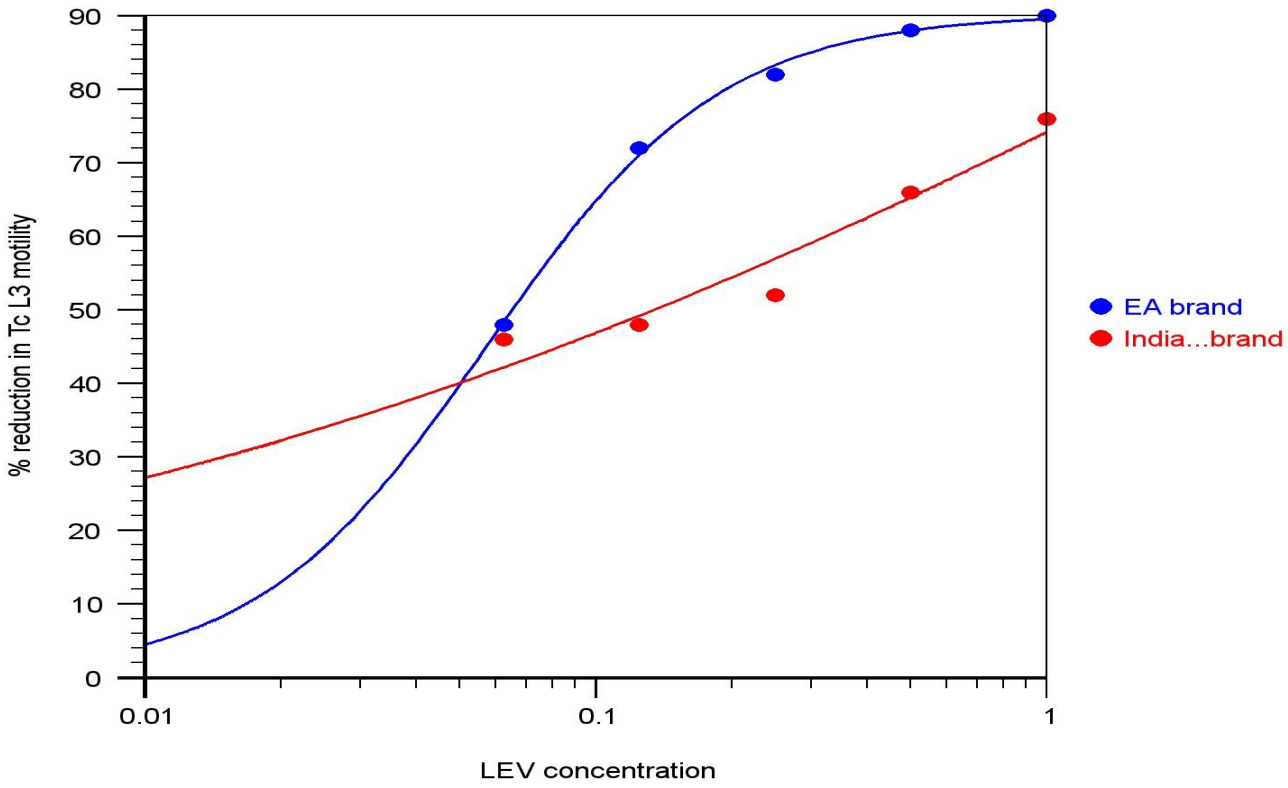

Figure 8. Larval motility test log transformed concentration-response curve for levamisole against eggs of *T. colubriformis*

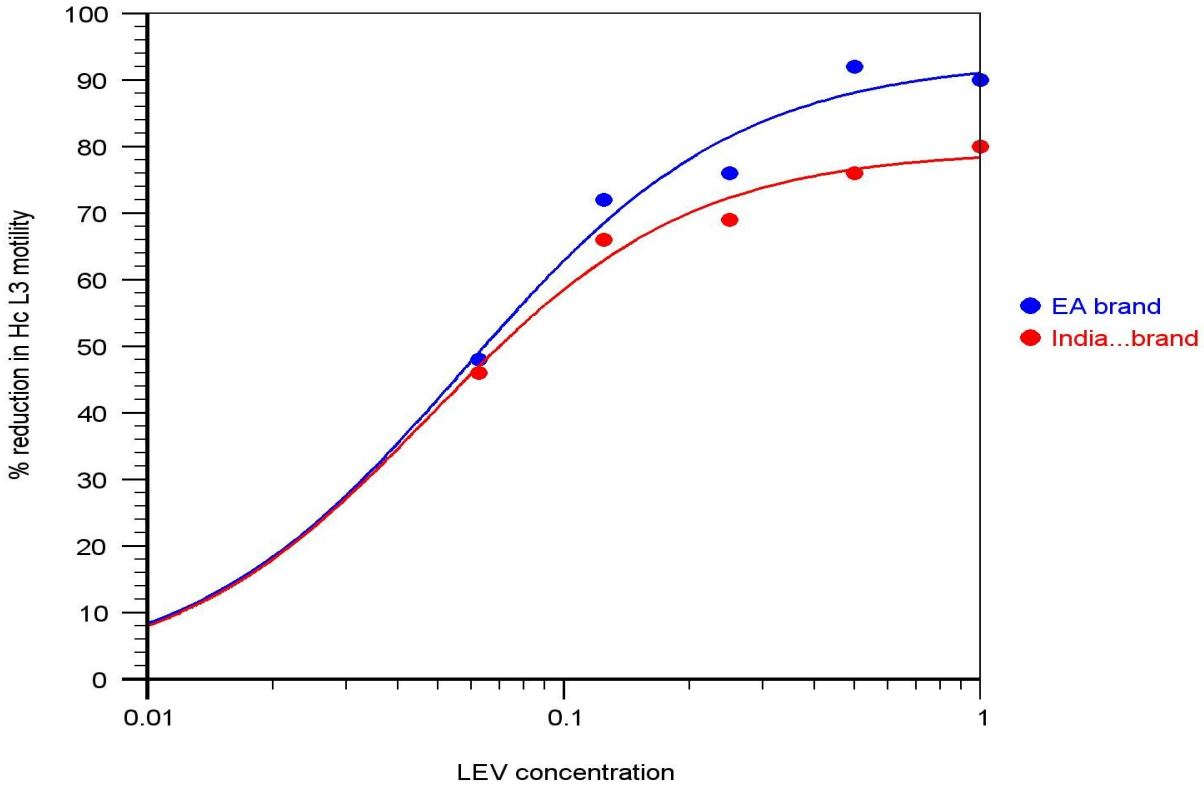

Figure 9. Larval motility test log transformed concentration-response curve for levamisole against eggs of *H.contortus*

Ivermectin efficacy

Except for higher reduction in L3 motility by China brand of ivermectin at 1µg, there was no significant difference between the three brands (China, Uruguay, and India) of the drug against *T. colubriformis* L3 with all other concentrations. On the other hand, the three brands of ivermectin at all concentrations tested had similar efficacy against motility of *H. contortus* L3 (Figure 10). At 0.5µg China and India brands were better than Uruguay brand ($P<0.05$) on the same parasite. China brand also had better performance than Uruguay brand at 0.25µg and 0.125µg concentrations ($P< 0.05$).

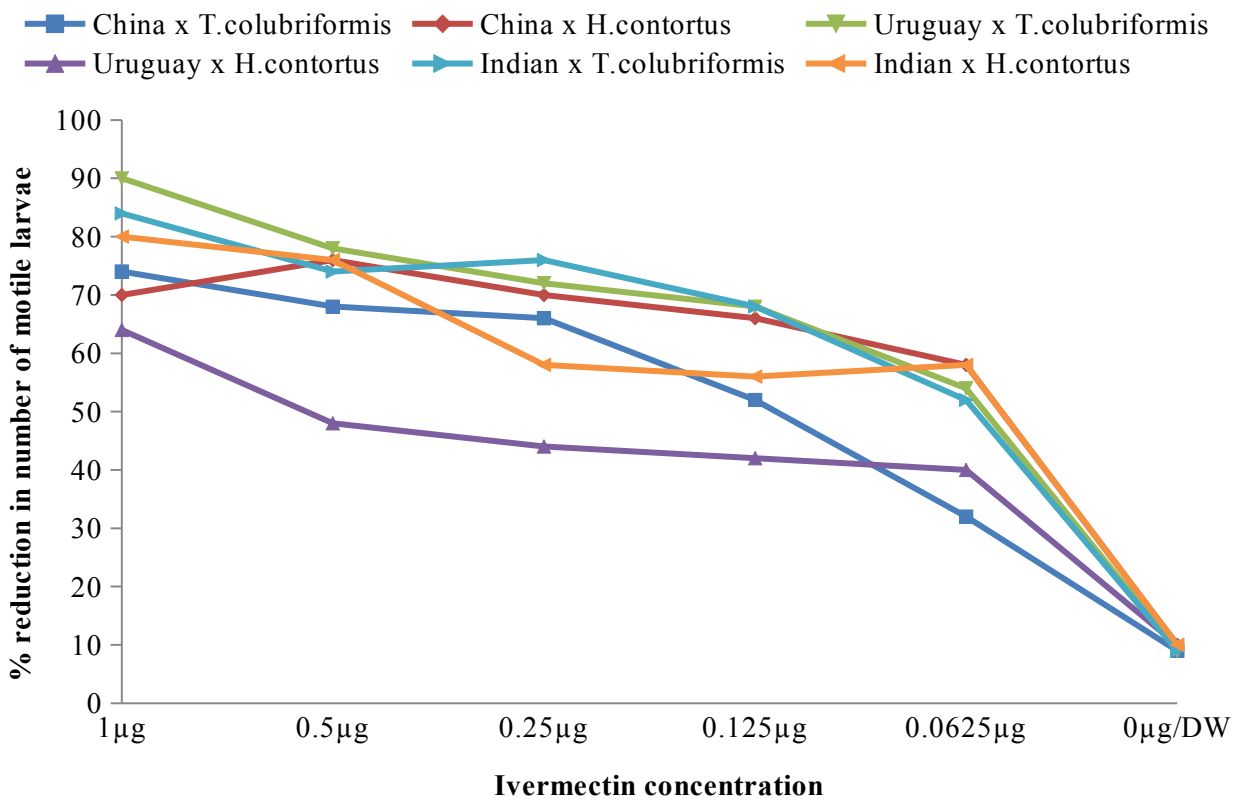

Figure 10. Effect of different brands of ivermectin on motility of *H. contortus* and *T. colubriformis* L3

The linear regression for concentration-response curve indicates that the EC50 for inhibition of motility of *T.colubriformis* L3 by ivermectin was 0.073µg, 0.178µg and 0.040µg respectively for China, Uruguay and India brands (Figure 11) suggesting a lower performance of the Uruguay brand compared to the others. Similarly, the EC50 of ivermectin against *H.contortus* L3 (Figure 12) was 0.025µg, 5.040µg and 3.061µg respectively for China, Uruguay India brands; only the first one being in the range of good efficacy at 0.1µg cutoff point.

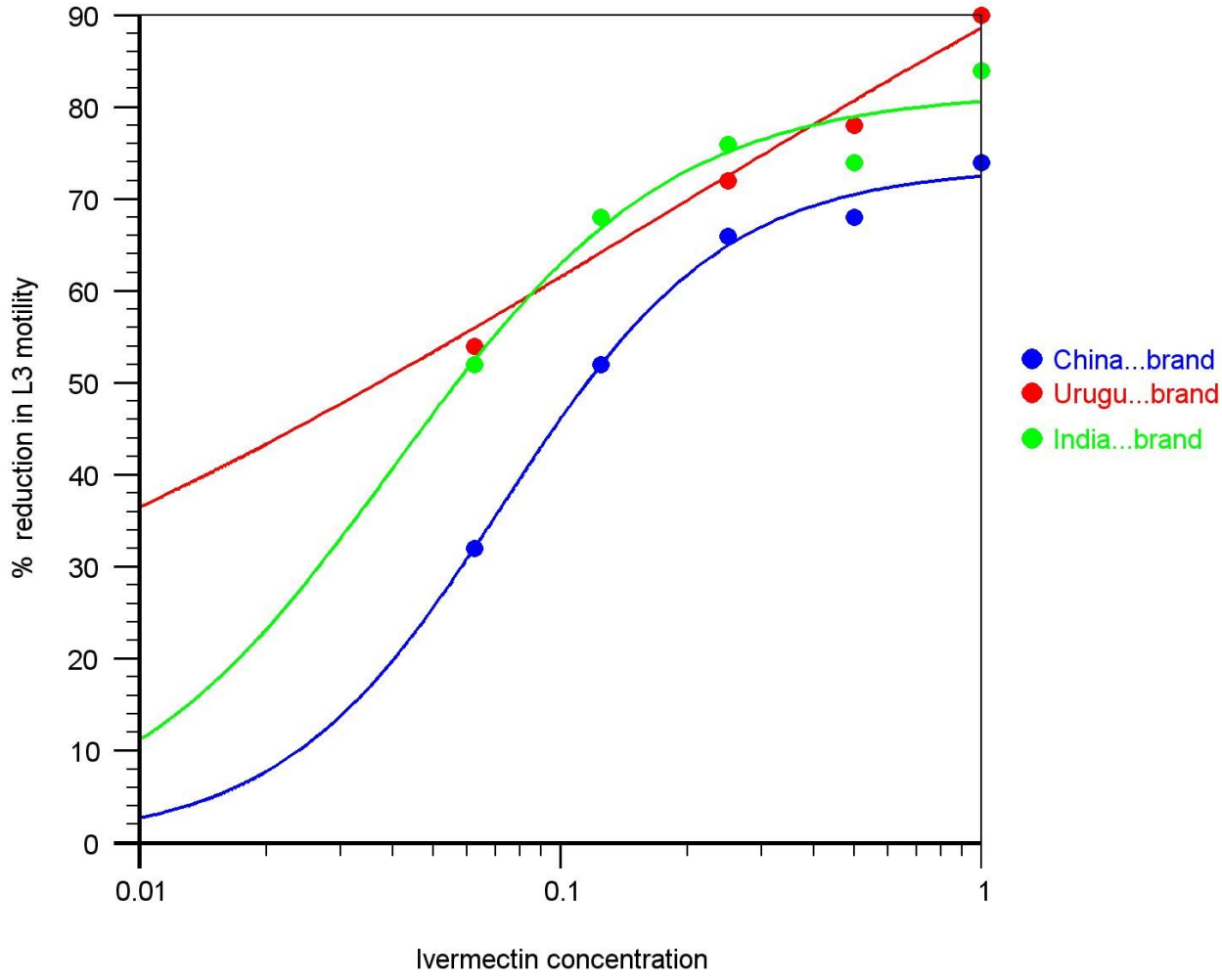

Figure 11. Larval motility test log transformed concentration-response curve for three brands of ivermectin against eggs of *T. colubriformis*

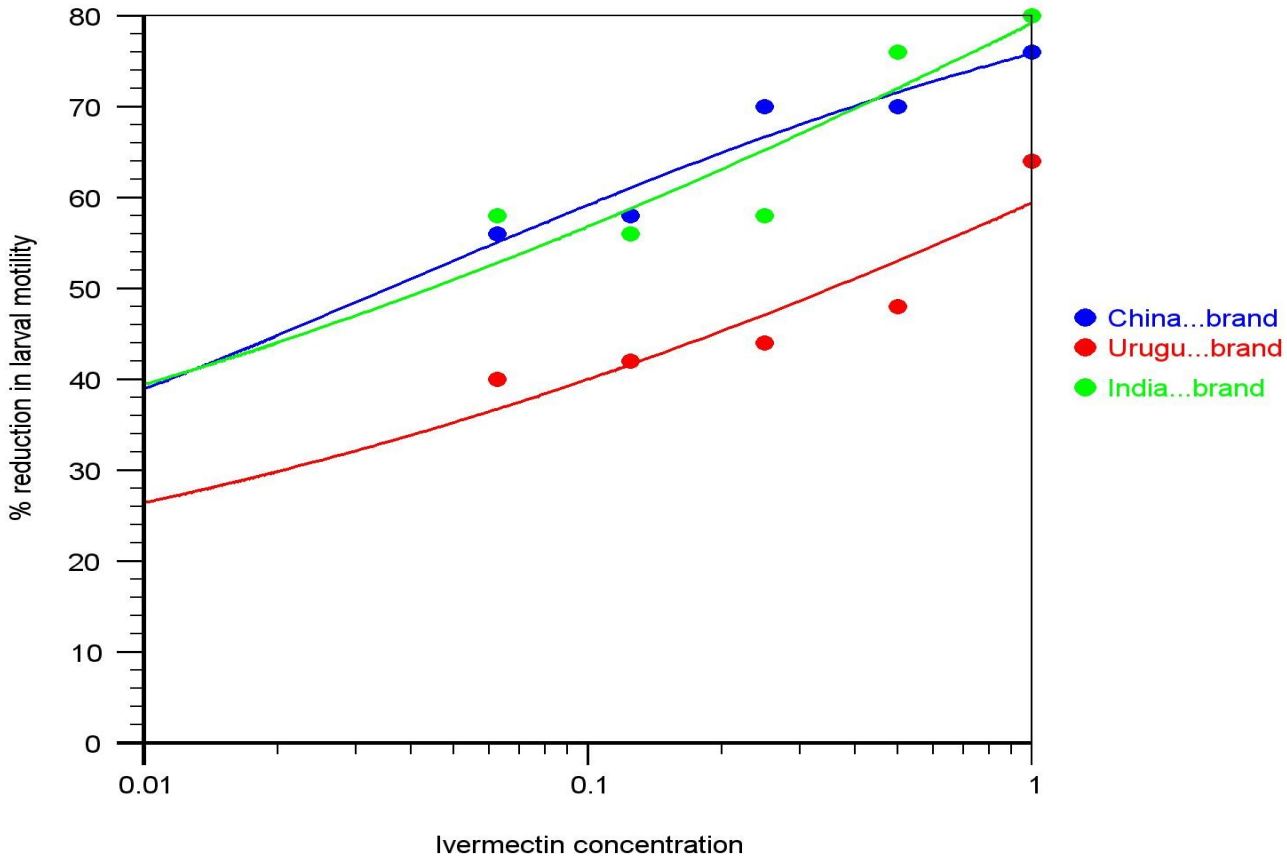

Figure 12. Larval motility test log transformed concentration-response curve for for three brands of ivermectin against eggs of *H.contortus*

4.2.3. Adult motility test (AMT)

China and India brands of tetramisole were tested against Adult *H. contortus* worms using adult motility test by incubating at 37 ^{0}c for ten hours of incubation time. Then the effect of tetramisole on adult parasite was analyzed to compare their anthelmintic activity in inhibition/killing of the parasites (Figure 13). In this regards, both brands of the drug have performed similarly excepting at 0.25µg/mL where the brand from China was significantly better at killing the adult parasite ($P< 0.05$). The log transformed treatment response indicates that the EC50 for inhibition of motility of adult *H. contortus* by tetramisole was 0.134µg and 5.576µg respectively for China and India brands (Figure 14) suggesting a marginal performance of the former and a much lower efficacy of the latter brand.

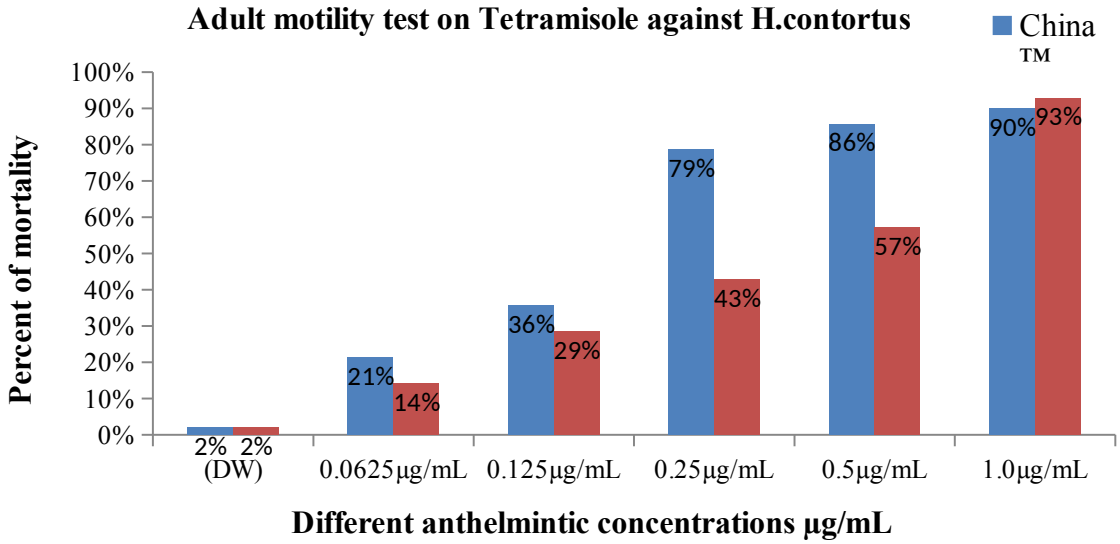

Figure 13. Effect of two brands of tetramisole on adult H.contortus motility/survival during the adult motility test

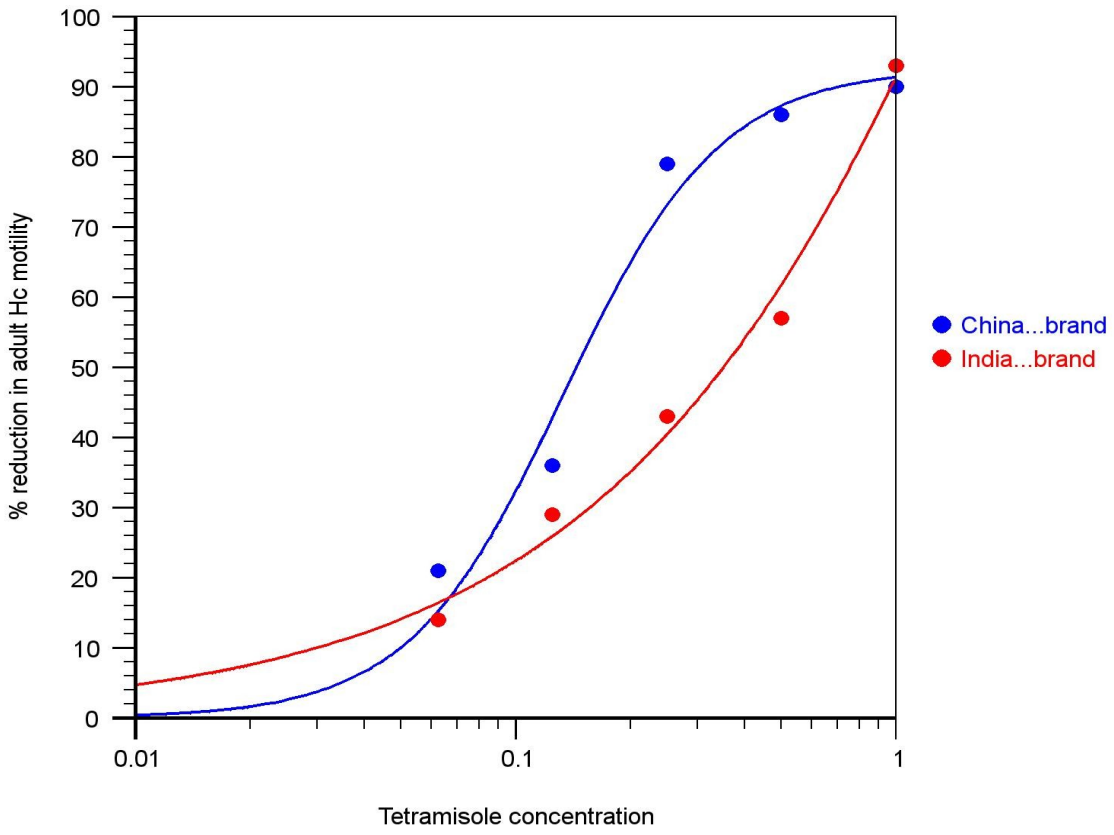

Figure 14. Larval motility test log transformed concentration-response curve for for three brands of ivermectin against eggs of *H.contortus*

5. DISCUSSION

5.1. Anthelmintic utilization practices

Anthelmintic treatment such as Albendazole, Ivermectin, Levamisole and Tetramisole are used by the farmers residing in and around Bishoftu to treat parasitic infections in ruminants. According to (Adediran *et al*.,2015) it has been observed that frequent use of the same group of anthelmintic, use of anthelmintics in suboptimal doses, prophylactic mass treatment of domestic animals, and frequent and continuous use of a single drug in a given area contribute to the widespread development of anthelmintic resistance. In this study, the result of the questionnaire survey indicated that Ivermectin was the most widely used anthelmintic followed by Albendazole, Tetramisole and Levamisole respectively. This is contrary to many other reports which have demonstrated that easily administrable anthelmintics in the form of bolli are more preferred than the injectable ivermectin (Seyoum *et al*., 2017; Terefe, 2017; Melaku *et al*., 2013; Terefe *et al*., 2013). Such variation could be attributed to access to formal veterinary services. In and around Bishoftu, there are a number of on station and mobile veterinary service providers. Ivermectin is commonly given because it attacks both internal and external parasites (Ahammed *et al*., 2016). A similar study eight years back in the same locality, reported Albendazole followed by ivermectin were drugs of choice (Datiko *et al*., 2013) suggesting that there have been shifts over time. This is supported by the fact that most of questionnaire survey respondents affirming anthelmintic drug administration by animal health personnel.

Although majority of then get drugs from formal veterinary service providers or drug stores, 14% of the respondents indicated purchase of anthelmintics from open markets suggests that mishandling and misuse of the commonly used anthelmintics is possible. This observation also agrees with those from previous works by (Kumsa and Nurfeta 2010; Melaku *et al*., 2013; Terefe *et al*., 2013; Seyoum *et al*.,2017). Forty four percent of respondents stated that they have dewormed their sheep twice a year; while 51% of owners treated three times a year. This is in line with previous findings (Melaku *et al*., 2013; Teklemariam *et al*., 2016; Seyoum *et al.*, 2017) elsewhere in Ethiopia. Although this frequency is acceptable on individual animal basis, the fact that few anthelmintic groups are consistently utilized in the area indicates the parasites circulating in the area are continuously

exposed to these drugs and hence the risk of anthelmintic resistance is evident. Most the respondents indicated that their animals displayed improvement on both clinical signs and body condition after treatment. This supports the report of Datiko *et al*.(2013) who stated that 81% of the respondents indicated that their animals have shown improvement in both clinical signs and body condition after treatment.

5.2. Efficacy of anthelmintic brands circulating in Bishoftu

It is very important to monitor anthelmintic efficacy at regular intervals to detect subtle changes, as early as possible in order to avoid the establishment of anthelmintic resistance. This may be performed by accessible and sensitive *in vitro* diagnostic tests, *in vivo* tests on animals and other techniques. This ultimately enables the choice of an anthelmintic for therapeutic use in the field (Dolinská *et al.*, 2016; Zarlenga *et al*., 2016). In this study, *in vitro* egg hatch inhibition, larval motility and adult motility assays were done with different brands of albendazole, ivermectin, levamisole and tetramisole against local isolate of *H.contortus* and *T.columbriformis* and interpreted according to WAAVP recommendation (Coles *et al*., 1992; Belew *et al*., 2012).

Accordingly, all tested anthelmintics brands of the drugs showed a dose-dependent inhibition of egg hatching on both *T. colubriformis* and *H. contortus*. This result is consistent with the findings of Várady (2007) and Belew *et al.* (2012). However, based on their EC50 (the median drug concentration that produces the desired effect in 50% of the test eggs/larvae/adult and the cutoff value of 0.1µg for efficacy, the brands vary in their effectiveness. China brands (already described in Table 8) were effective against eggs of *T. colubriformis* and *H. contortus* (Albendazole), L3 of the two parasites (Ivermectin) and marginally effective against adult *H. contortus* (Tetramisole). These values are also well below the commonly accepted cut-off value for BZ-resistance of 0.1 µg TBZ/ml (Coles et al., 2006). Similar result to benzimidazole agents (Greece) has already been demonstrated on susceptible isolates of *H. contortus* obtained from Ogaden region (Ethiopia) (Bersissa and Abebe 2006). On the other hand, Albendazole from East Africa and India brands require much higher doses to produce the desired efficacy suggesting that they are of inferior quality. Ivermectin from China brand and levamisole from East Africa brand are both effective against L3 of the two parasites in vitro whereas Indian brands of Levamisole and ivermectin were

respectively effective on *H.controtus* L3 and *T. colubriformis* indicating the need for such information while selecting the best anthelmintic of choice.

Generally, better efficacy of anthelmintic brands could be attributed to the high quality of the drugs or the low frequency of anthelmintic treatment practice in the area as similarly reported with some *in vivo* studies made on the susceptibility of *H.contortus* by (Keyyu *et al*., 2002) and (Chaka *et al*.,2009). However, Kumsa and Abebe (2009) reported resistance of *H. contortus* to different anthelmintics. The variation in efficacy of anthelmintic agents may indicate the spectrum of treatment practice or use of anthelmintic agents, introduction of resistant worms with livestock from other farms, or selection of resistant strains in some localities.

6. CONCLUSIONS AND RECOMMENDATIONS

This study was initiated to assess farmers' anthelmintic utilization practices on sheep and goats in Ada'a district and evaluate the efficacy of locally available anthelmintic brands using three in vitro tests. Ivermectin followed albendazole; tetramisole and levamisole were the most commonly used anthelmintics in the study area. There are practices which potentially predispose the available anthelmintics to drug resistance. A dose-dependent inhibition of egg hatching, larval and adult motility were observed in all *in vitro* tests performed. The Egg hatch assay showed higher performance of albendazole brand from China compared to those from East Africa and Indian manufacturers. Similarly, the larval motility test suggests that based on their EC50, both levamisole and ivermectin from china have good efficacy against L3 of *T. colubriformis* and *H. contortus* whereas only levamisole of East African origin was effective against L3 of both parasites. Levamisole of Uruguay, Levamisole, Ivermectin and Tetramisole of Indian origin were not effective or only partially effective against the two species of parasites as per the different assay type. The lower efficacy of some of the brands could be attributed to their quality or other factors that are worth investigating in the future.

Based on the above conclusion, the following recommendations are forwarded:

- Farmers should continuously be educated on the risks of anthelmintic resistance and the need to utilize formal veterinary services in their vicinity
- Veterinary service delivery should be further improved to address all farmers at their convenient location
- Further research is needed on testing the quality of anthelmintic drugs being imported and circulating in the area and beyond
- Those brands that have been proven effective in vitro should be further tested in vivo
- The few brands that were tested and proven to be good should be used strategically and carefully so as to minimize the risk of development of resistance

7. REFERENCES

Abongwa, M., Martin, R. J. and Robertson, A. (2017) 'A brief review on the mode of action of antinematodal drugs', (June). doi: 10.1515/acve-2017-0013.

Adedipe, O. D (2014) 'Gastrointestinal Helminths in Slaughtered Cattle in Ibadan , South-Western Nigeria', 2014.

Adediran, O. A. and Uwalaka, E. C. (2015) 'and Vernonia amygdalina in West African Dwarf Goats', 2015.

Ahire, S. and Lahane-ahire, S. (2014) 'Review on Anthelmintic Drugs. *International Journal of Pharmaceutical Research*; 6(3):1-10

Albonico, M. M,.(2008) 'Monitoring Anthelmintic Efficacy for Soil Transmitted Helminths (STH).

Alessandro,F.R. (2011) 'Why is it important to correctly identify Haemonchus species .*Rev. Bras. Parasitol. Vet., Jaboticabal,* 20(4):263-268

Avinash, B,. (2017) 'Evaluation of the Anthelmintic Activity (Iin vitro) of Neem Leaf Extract-Mediated Silver Nanoparticles against Haemonchus contortus', 5(2):118–128.

Barragry, T. B. and Gaba, G. (1987) 'A Review of the Pharmacology and Clinical Uses of Ivermectin *Canine Vet erinary Journal,* 28(8): 512-517.

Belina, D. (2017) 'Gastrointestinal Nematodes in Ruminants : The Parasite Burden , Associated Risk Factors and Anthelmintic Utilization Practices in Selected Districts of East and Western Hararghe , Ethiopia,10(16): 2157-7579.

Mahlatse,A.B,.(2011) Biological Control of Gastrointestinal Nematodes of Small Ruminants Using Bacillus Thuringiensis (Berliner) and Clonostachys rosea (schroers), Un published

Brik, K., Elkharrim, K. and Belghyti, D,.(2019) 'A survey of Haemonchus contortus parasite of sheep from Gharb plain, *Parasite Epidemiology and Control*, 3(3): 0–5. doi: 10.1016/j.parepi.2019.e00094.

Campbell, W. C. and Benz, G. W. (2000) 'Ivermectin : a review of efficacy and safety', pp. 1–16.

Castagna, F.(2019) ‘Use of Complementary Natural Feed for Gastrointestinal Nematodes Control in Sheep : E ff ectiveness and Benefits for Animals’.

Chaka, H.,Tadesse E,. Desalegn L,.& Daniel Gizaw (2009) ‘Evaluation of the efficacy of anthelmintics sold on Ethiopian markets against Haemonchus contortus in experimentally infected Sheep’, *Trop Anim Health Prod* , 13(41) :973–978. doi: 10.1007/s11250-008-9287-2.

Charlier, J., Bartley, S. M. T. D. J. and Kenyon, P. J. S. F. (2018) ‘Mind the gaps in research on the control of gastrointestinal nematodes of farmed ruminants and pigs’, 65(2), pp. 217–234.

Coles, G. C,.F. Jackson.B,. W.E. Pomroy C,. R.K. Prichard., D, G. von Samson-Himmelstjerna E,. A. Silvestre F,. M.A. Taylor G,. J. Vercruysse. H,. (2006) The detection of anthelmintic resistance in nematodes of veterinary importance, Veterinary Parasitology 136 (2006): 167–185.

Demessie.Y,. Seyoum.Z,. Getnet.K,.& Yitbarek.D,. (2016) A Review on ‘Anthelmintics Resistance Against Gastrointestinal Nematodes of Sheep, *World Journal of Agricultural Sciences* 12 (4): 245-253,doi: 10.5829/idosi.wjas.2016.12.4.23761.

Afras.A., Alilo.A.B.,and Meseret.M.,(2018) ‘Advances in Dairy Research Assessment of Sheep and Goat (Small Ruminants) Production System in’, 6(4). doi: 10.4172/2329-888X.1000215.

Anteneh.W.,& Sagni.G., (2017) Gastrointestinal nematodes of small ruminants in Guto Gida District, East Wolloega, Ethiopia,*J. Vet. Med. Anim. Health,* 9(5), pp. 83-87

Muhammad. H.,Avais.M,.Zameer.A.,Arif Khan.M.,Hussain. A.,(2016) Dynamic Dispersal of Haemonchosis , its Treatment and Effect on Blood Profile of Small Ruminants of Lodhran District, Punjab, Pakistan, 48(3), pp. 755–761.

Dolinská, M. U., Königová,A., Babják, M., Várady ,M.,(2016) Comparison of two in vitro methods for the detection of ivermectin resistance in Haemonchus contortus in sheep’, pp. 120–125. doi: 10.1515/helmin-2015-0002.

Dyary, H. (2016) ‘Veterinary Anthelmintics and Anthelmintic Drug Resistance Veterinary Anthelmintics Resistance and Anthelmintic Drug’, (January). doi: 10.17656/jzs.10463.

Dinaol,B., (2017) ‘Gastrointestinal Nematodes in Ruminants : The Parasite Burden , Associated Risk Factors and Anthelmintic Utilization Practices in Selected’, 8(2). doi: 10.4262/2157-7579.1000433.

Enejoh,O.S and Suleiman ,M.M.,(2017) ‘Anthelmintics and their Application in Veterinary Medicine’, pp. 1–10. doi: 10.31031/RMES.2017.02.000536.

Ferreira, L. E ., Castro, P .M. N.,Chagas, A C ., França, S C., Beleboni, R O.,(2013) ‘Experimental Parasitology In vitro anthelmintic activity of aqueous leaf extract of Annona muricata L . (Annonaceae) against Haemonchus contortus from sheep, *Experimental Parasitology*. *134(3)*, pp. 327–332. doi: 10.1016/j.exppara.2013.03.032.

Francisco, A. (2014) ‘Differentiation of Haemonchus placei from Haemonchus contortus by PCR and by morphometrics of adult parasites and third stage larvae , pp. 495–500.

Furgasa W , Abunna F , Yimer L , Haile G (2018) Review on Anthelmintic Resistance against Gastrointestinal Nematodes of Small Ruminants：Its Status and Future Perscpective in Ethiopia. *Journal of Veterinary Science & Animal Husbandry*,6(4): 2348-9790

Gharbi, M., Darghouth, A. and Kumsa, B. (2013) ‘New sublinguiform vulvar fl ap of Haemonchus species in naturally infected domestic ruminants in Béja Abattoir , North Tunisia’, *83(3)*, pp. 281–291.

Hoberg, E. P. and Zarlenga, D. S. (2016) *Evolution and Biogeography of Haemonchus contortus：Linking Faunal Dynamics in Space and Time*, *Advances in Parasitology*. Elsevier Ltd. doi: 10.1016/ bs.apar.2016.02.021.

Holden-dye, L., Walker, R. J. and Sciences, B. (2011) ‘Anthelmintic drugs, 44(0), pp. 1–13. doi: 10.1895/wormbook.1.143.1.

Hong, S. (2018) ‘Albendazole and Praziquantel：Review and Safety Monitoring in Korea’, 50(1), pp. 1–10.

https://www.aatbio.com/tools/ec50-calculator, Quest Graph™ EC50 Calculator." *AAT Bioquest, Inc*, retrieved on 18 Jun. 2020,

Jambre, L., J. A. Mckenzie.,R. A. Stones (1988) ‘The Inheritance of Thiabendazole Resistance in *Trichostrongylus.colubriformis International Journal for Parasitology:*8(6):703-70

Jegede, O. C., Adejoh.A., Obeta, S.S., Olayemi, O.D.,(2015) Gastrointestinal Parasites of Sheep and Goats in Gwagwalada Area Council , Federal Capital Territory , Abuja , Nigeria；with a Special

Reference to sex , breed and age', *Alexandria Journal of Veterinary Sciences* 2015 (46): 170-176, doi: 10.5455/ajvs.177135

Belew.S,.Hussein.J.,Regassa.F.,(2012) Susceptibility Assay of Haemonchus contortus to commonly used Susceptibility assay of Haemonchus contortus to commonly used anthelmintics in Jimma , southwest Ethiopia, (February 2014). doi: 10.1007/s11250-012-0113-5.

Jose, M., Vega, M. S. and Garcı, J. J. (2009) 'The Veterinary Journal', 179, pp. 25–37. doi: 10.1016/j.tvjl.2007.07.011.

Kaplan, R. M. (2014) 'Recommendations for control of gastrointestinal nematode parasites in small ruminants : These ain ' t your father ' s parasites.

Karshima,S.N.,Maikai,B.V.,Paghi Kwaga,J.K.,(2018) Helminths of veterinary and zoonotic importance in Nigerian ruminants : a 46- year meta-analysis (1970 – 2016) of their prevalence and distribution'. *Infectious Diseases of Poverty,*7(52):1–15.

Kassahun, C., Adem,A,. Zemene,M,. Getaneh,G.,Berrie,K.,(2016) Identification of Commonly Used Anthelmintic Drugs and Evaluation of their Utilization in University of Gondar Veterinary Clinic, *Journal of Veterinary V eterinary Science & T echnology* 7(6) : 7–10. doi: 10.4172/2157-7579.1000.

Khokra, S. L. and Choudhary, D. (2014) 'Benzimidazole An Important Scaffold In Drug Discovery.

Kotze, A. C.,Hunt,P.W., Skuce,P.,Himmelstjerna,G.S., Richard J. Martin,J.M.,Sager, H., Jürgen Krücken,J., Hodgkinson,J., Lespine,A.L.,Jexi,A.R., Prichard,R.K., (2014) Drugs and Drug Resistance Recent advances in candidate-gene and whole-genome approaches to the discovery of anthelmintic resistance markers and the description of drug / receptor interactions, *International Journal for Parasitology* 4(3) :164–184.

Kumsa, B. (2006) 'Efficacy of Albendazole and Tetramisole Anthelmintics Against Haemonchus contortus in Experimentally Infected Lambs Efficacy of Albendazole and Tetramisole Anthelmintics Against Haemonchus contortus in Experimentally Infected Lambs.

Kumsa, B., Tolera, A. and Abebe, R. (2008) 'Vulvar morphology and sympatry of Haemonchus species in naturally infected sheep and goats of Ogaden region , eastern Ethiopia', 78(4), pp. 331–342.

Macon, D. (2018) ‘Managing Internal Parasites in Small Ruminants’.

Magzoub, A., Elaziz ,A ., Abdul,S.,(2009) Detection of Anthelmintic Resistance to Gastrointestinal Nematodes in sheep : Laboratory and Survey Investigation in Khartoum State.

Martin, R. J. and Robertson, A. (1997) Target sites of anthelmintics, Parasitology (1997).

Manter, H., Lichtenfels,W., Ralph,J.,Hoberg, E.P.,(1994) New Morphological Characters for Identifying Individual Specimens of Haemonchus spp . (Nematoda : Trichostrongyloidea) and a Key to Species in Ruminants of North America.

Mcintyre, J., Hamer, K.N., Morrison, A.A., Bartley, D.J., Sargison, N., Devaney, E., Laing, R., (2018) ‘Veterinary Parasitology Hidden in plain sight - Multiple resistant species within a strongyle community’, *Veterinary Parasitology,* volume 258(1)*:*79–87. doi: 10.1016/j.vetpar.2018.06.012.

Mengist, Z., Abebe.N.,Gugsa.G.,and Kumar.N.,(2014) Assessment of Small Ruminant Haemonchosis and Its Associated Risk Factors in and Around Finoteselam , Ethiopia, *Journal of Agriculture and Veterinary Science* (IOSR-JAVS) 7(12), pp. 36–41.

Nega, D. and Seyum, Z. (2018) ‘A Review on Anthelmintic Resistance and Potential Risk Factors in Domestic Ruminants’, 8(2), pp. 58–67. doi: 10.5829/idosi.apg.2017.58.67.

Oluwole, A. S., Ogudo,U.S., Mogaji,H.O., Adeniran , A.A., Alabi ,O.M & Ekpo,U.F.,(2015) Gastrointestinal Helminth Infections in a Ruminant Livestock Farm in Gastrointestinal Helminth Infections in a Ruminant Livestock Farm in Abeokuta , South Western Nigeria, *Annual Research & Review in Biology* 8(4): 1-8. doi: 10.9734/ARRB/2015/18812.

Ouellette, M & Ouellette,B.,(2001) Biochemical and Molecular Mechanisms of Drug Resistance in Parasites biochemical and molecular mechanisms of drug resistance in parasites, 6(11):1-105

Papadopoulos, E.(2011). Anthelmintic Resistance in Parasites of Small Ruminants : Sheep versus Goats. *Parasitological Institute of SAS, Košice*, 48 (3): 137 – 144

Paul,F,and Stavros,Mavrot.(2016).Livestock Nematode Infection in a Changing World : Investigating the European Situation Livestock Nematode Infection in a Changing World, *Braz. J. Vet. Parasitol,* 25 (1):3-17

Qadri,K., Subha,G., Rajesh,W., Subhash,S, Avinash ,K, and Praveen,K.(2016). A review on Resistance to Anthelmintic Medications in Animals ,3 (9):144-147.

Shalaby,H.A.,(2013) Anthelmintics Resistance; How to Overcome it? Department of Parasitology and Animal Diseases, National Research Center, Giza, Egypt. *Iranian Journal of Parasitol* 8(1) : 18-32,

Qamar, M. F., Maqbool.A., Khan .*S.M.,*Ahmad*,N.,*Munee*,A.M.,*(2009) Epidemiology of Haemonchosis in sheep and Goats under different managemental conditions, *Veterinary World* 2(11):413-417

Radostits, O.M, Blood, D.C. and Gay, C.C. (1994): Veterinary Medicine. A Text Book of the Diseases of Cattle, Sheep, Pig, Goats and Horses 8th ed., Bailliere Tindall, pp. 1228.

Rege, J.E. O. (1992): Indigenous African Small Ruminants: a case for Characterization and Improvement. Small Ruminants Research and Development inafrica. Proceedings of the 2nd Biennial Conference of the African Small Ruminant. Research Network AICC, Arusha, Tanzania.7-11 Dec. 1992 pp 205-211.

Reinecke, R.K. (1983): Veterinary Helminthology 1st Ed., Bulterworth Durban pp 55-66.

Rajagopal, A., Sabu.M. Gleeja, V. L.,(2017) 'ASSESSMENT OF BENZIMIDAZOLE RESISTANCE STATUS, 6(3) :2112–2117.

Rayes, D., Rosa,D., Mariana, B., Cecilia,B. (2004). Molecular Basis of the Differential Sensitivity of Nematode and Mammalian Muscle to the Anthelmintic Agent Levamisole. 279 (35): 36372–363781.

Raza.M, Ayaz.M, Murtaza.S and Akhtar. M.(2013). *Prevalence of GIT Helminths in Cattle at the vicinities of Tehsil Jatoi , punjab , pakistan*.25(2): 305-309

Roeber, F., Jex, A. R. and Gasser, R. B. (2013) 'Impact of gastrointestinal parasitic nematodes of sheep , and the role of advanced molecular tools for exploring epidemiology and drug resistance - an Australian perspective', *Parasites & Vectors*. Parasites & Vectors, 6(1), p. 1. doi: 10.1186/1756-3305-6-153.

Sakthikarthikeyan, S.,Ahmad,A.,Neetu,H.,Kamini,B.,Kannan,K., (2016) Pharmacokinetics of ivermectin (ivermic super) following single dose subcutaneous administration in sheep, *International Journal of Science, Environment and Technology*, 5(6), pp. 4130–4140.

Salgado, J. A. (2016) 'Overview of anthelmintic resistance of gastrointestinal nematodes of small ruminants in Brazil', 2961.

Sangster, N. C. and Gill, J. (1999) 'Pharmacology of Anthelmintic Resistance', 4758(99), pp. 141–146.

Sargison, N. D. (2016) 'Edinburgh Research Explorer Keys to solving health problems in small ruminants', *Small Ruminant Research*. Elsevier B.V. doi: 10.1016/j.smallrumres.2016.02.021.

Seifu,A.,Kebede,E.,Belachew, B.,Melaku,A.,Setegn,T.,(2019) Quality of albendazole tablets legally circulating in the pharmaceutical market of Addis Ababa , Ethiopia : physicochemical evaluation'. BMC Pharmacology and Toxicology, 5(1) 1–7.

Silvestre,A. & Humbert, J.F. (2002). Diversity of benzimidazole-resistance alleles in populations of small ruminant parasites. *International Journal for Parasitology,* 32, 921-928

Sissay, M.M., Asefa, A., Uggla, A., Waller, P.J. (2006). Anthelmintic resistance of nematode parasites of small ruminants in eastern Ethiopia: exploitation of refugia to restore anthelmintics efficacy. *Veterinary Parasitology* 135, 337-346.

Selemon, M.,(2018) Review on Control of Haemonchus Contortus in Sheep and Goat,*Journal of Veterinary Medicine and Research,* 5(5): 1139.

Seyoum, Z., Demessie,Y., Bogale,B., Melaku,M.,(2017) Field evaluation of the efficacy of common anthelmintics used in the control of gastrointestinal nematodes of sheep in Dabat district , Northwest Ethiopia'. *Irish Veterinary Journal,* pp. 1–8. doi: 10.1186/s13620-017-0097-6.

Shahbazi, A ., Fallah, E., Hassan, M Kohansal, Mohammad,H., (2012) Morphological characterization of the Trichostrongylus species isolated from *Research Opinions in Animal & Veterinary Sciences 9(2)* :117-136

Storey, B., Marcellino, C., Melissa, M., Mustafa, M., Howel,E.,(2014) Drugs and Drug Resistance Utilization of computer processed high definition video imaging for measuring motility of microscopic nematode stages on a quantitative scale. *International Journal for Parasitology:* 4(3): 233–243.

Swan, G. E. (1999) 'The pharmacology of halogenated salicylanilides and their anthelmintic use', 70(December 1998), pp. 61–70.

Tan,T.K ., Chandrawathani ,P., Van ,L.L ., Soo,C L., Romano,N., Reuben ,S K.Sh., and Yvonne ,A L L. (2014). Co-Infection of Haemonchus Contortus and Trichostrongylus Spp . *BMC Veterinary Research* 10 (1): 1–7.

Tehrani, A.,Javanbakht,J.,Jani,M.,Sasani , F., Solati, A.,Rajabian,. Khadiva,F.,Akbari,H and Mohammadian,M., (2012) Histopathological Study of Haemonchus contortus in Herrik Sheep, J*ournal of Bacteriology and Parasitology* 3(5):1-5

Tibebu, A., Tamiru, Y. and Abdeta, D. (2018) 'Prevalence of Major Gastrointestinal Nematode and Degree of Parasite Infestation in Sheep of Bako Agricultural Research Center Community Based Breeding Program Project Small Holder Farms at Horro District', 8(3). doi: 10.19080/JDVS.2018.08.555740.

Teklemariam T, Fantahun T, Alemayehu B and Beyene A (2016). Efficacy of selected anthelmintics against gastrointestinal nematodes on naturally infected small ruminants in Gimbo district, Kaffa Zone, South West Ethiopia. *Livestock Research for Rural Development. Volume 28, Article #220.* Retrieved June 19, 2020, from http://www.lrrd.org/lrrd28/12/tseg28220.html

Terefe,G., Datiko,J.,Bekele ,J., (2013) Anthelmintic utilisation practices and prevalence of gastrointestinal helminth infections in sheep kept in the urban and peri-urban areas of Bishoftu Town, *Tropical Animal Health and Production* 45(2013): 633–639.

Udhavrao, B. A., Kaka, J. R. and Mohan, A. (2017) 'Successful Therapeutic Management of Ivermectin Toxicity in Deoni Calf', 2(4), pp. 10–12. doi: 10.19080/JDVS.2017.02.555594.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M. and Jennings, F.W. (1996): Veterinary Parasitology 2nd. Longman Scientific and Technical pp 261-264.

Vadlejch, J., Lukešová,D.,Vašek, J.,Langrová,I., I. Jankovská,I.,Salaba,O., (2014) Comparative morphological and molecular identification of Haemonchus species in sheep, *Institute of Parasitology, SAS, Košice*,51(2): 130 – 140.

Van,Wyk., Estelle ,M .,. n.d. 'Morphological Identification of Parasitic Nematode Infective Larvae of Small Ruminants and Cattle : A Practical Lab Guide. *Journal of Veterinary Research*. 80(1) ;1–14

Van Wyk, J.A., Malan, F.S., Randles, J.L. (1997). How long before resistance makes it impossible to control some field strains of *Haemonchus contortus* in South Africa with any of the modern anthelmintics? *Veterinary Para sitology* 70, 111-122.

Várady, M., Corba, J, (1999). Comparison of six in vitro tests in determining benzimidazole and levamizole resistance in Haemonchus contortus and Ostertagia circumcincta of sheep. Veterinary Parasitology, 80: 239-249

Várady,M.,Papadopoulos,E.,Dolinská,M.,Königová,A.(2011). Review Article Anthelmintic Resistance in Parasites of Small Ruminants : Sheep versus Goats, *Parasitological Institute of SAS*, 48 (3):137–144.

Verma, R. and Das, G. (2018) 'livestock and its management : India perspectives An overview of anthelmintic resistance in gastrointestinal nematodes of livestock and its management : India perspectives.

Yacob, H.T., Mistre, Ch., Adem, A.H., Basu, A.K. (2008). Parasitological and clinical responses of lambs experimentally infected with Haemonchus contortus (L3) with and without ivermectin treatment. Veterinary parasitology, in press.

Waruiru, R.M. (1997). Efficacy of closantel, albendazole and levamisole on an ivermectin resistant strain of *Haemonchus contortus* in sheep. *Veterinary Parasitology,* 73, 65-71.

Weingartz, C. R. (2017) 'A Study of the Prevalence of Gastrointestinal Nematodes in Goats Obtained from Northwest Arkansas'.

Welay, G. M.., Tedla,D.G., Teklu,G.G., Weldearegay,S.K., Shibeshi,M.B ., Kidane,H.K., Gebrezgiabher,B.B., Abraha,H.T.,(2018) A preliminary survey of major diseases of ruminants and management practices in Western Tigray province , northern Ethiopia'. *BMC Veterinary Research*, 14(293):1–9.

Williamson R L, Metcalf R L 1967 Salicylanilides: A new group of active uncouplers of oxidative phosphorylation. Science 58: 1694–1695.

Worku,E.,Kiros.A.,Asgedom,H and Tadesse,B.(2017). Alternative Control Methods of Gastrointestinal Nematode Infections in Small Ruminants : Biological Method and Use of Medicinal Plant Extracts. 3 (2): 11–27.

Worku, Y. and Bedanie, T. (2019) 'Identification of Helminths Parasites , Species Richness and their Effects on Hematological Components in Chicken Kept Under Scavenging Chicken Production System in and Around Bishoftu , Ethiopia', 10(2):90-117

Viviane, A., Wanderley, DF, Rossiane, C.V., (2017) Assay to Access Anthelmintic Activity of Small Molecule Drugs Using *Caenohabidtis elegans* as a Model 7(2): 1–8

Van Wyk, J. A. and Mayhew, E. (2013) 'Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide', *Onderstepoort J Vet Res*, 80(1) :1–14.

8. LIST OF ANNEXES

Annex 1. Collections of Abomasumal parasites to local isolate of Haemonchus.contortus from sheep

1) The sheep were first selected based on the result of fecal egg count prior to slaughtering for recovery of adult parasite.
2) Then two sheep were slaughtered and their abomasums was ligated with string and separate it from Omasum and duodenum.
3) The abomasum was placed on the tray on the tray prepared for accumulation and washing of its contents.
4) Along the greater curvature, the abomasum was opened so that its contents fall into the tray and washed repeatedly until the abomasal contents clear by using pressurized tape water.
5) The content of the abomasum was washed by tape water by using the sieve with different size and transferred into different Petridish.
6) The parasites with red color and barber pole appearance were a lot and they selected for local isolate of Haemonchus.contortus in other sheep for consecutive anthelmintic efficacy trial.
7) Number of adult haemonchus parasites were then identified and collected in separate bottles by using blunt forceps to examine under microscope.
8) The male and female were identified based on their morphological features.
9) Then only female adult H.contortus were selected and crashed to liberate eggs.
10) The eggs were then cultured in sterile cattle feces to collect the infective stage of local isolate of H.contortus.

Annex 2. Collections of Small intestinal parasites to local isolate of Trichostrongylus.columbriformis

1. Single sheep was purchased based on fecal egg count (FEC) for presence of helminth parasites to collect trichostrongylus.columbriformis adult parasites.
2. The sheep was slaughtered at postmortem room and small intestine was selected to recovery of adult parasites in step by step.
3. When examining small intestine it is important to run the intestines out, free from mesentery into one tray.
4. Initially, the gut is washed by pouring water into one end of the gut and flushing it out into the total volume jar.
5. The small intestine was opened for further washing and scraping of its mucus membrane and content
6. After the content of small intestine was washed and sieved in appropriate way, in manner to recover *Trichostrongylus* columbriformis.
7. The washed part of the intestine was cleared and added into beaker and put to settle out the needed parasites for certain time.
8. The clear content of the intestine was then added into separate petridishes and number of Trichostronglyus.columbriforms species was collected and identified under microscope.
9. After the male and female parasites are identified, only female parasites were selected to culture the isolate of Trichostrongylus.columbriformis in separate jar.
10. The collected female parasites were crashed to liberate eggs to culture into infective stage in order to develop the isolate of Trichostrongylus.columbriformis purchased from local farmers of the district.
11. The liberated eggs were cultured in sterile fecal samples collected from cattle into jar according guidelines for fecal culture recommended by (Van *et al*.,2013).
12. Infective stage (L3) was then harvested in universal bottle and stored until artificially infected into sheep purchased from local of market.

Annex 3. Preparations of serial dilution concentrations for Albendazole

A. The stock solutions of albendazole of Chinese, East Africa and Indian brands was prepared at concentrations of 1000µg/ml by dissolving 100mg of albendazole in 20 ml of Dimethyl sulfoxide solution and 80 ml of distilled water.
B. The second mother solution of dissolved albendazole was prepared at concentrations of 500µg/ ml by mixing 50 mg of mother solutions with 50 ml of distilled water.
C. The third concentration of albendazole solution was prepared at concentrations of 250µg/ml by mixing 25 mg of first mother solution with 75 ml of distilled water.
D. The fourth concentration of albendazole solution was prepared by dissolving 12.5 mg of mother solutions in 87.5 ml of distilled water.
E. The fifth final solution of albendazole was prepared by dissolving 6.25 mg of albendaazole solutions in 93.75 ml of distilled water.
F. From prepared anthelmintic serial concentrations,10µl of Anthelmintic solutions were then added to the micro dilution plates at final dilutions of (1 µg, 0.5 µg, 0.25 µg 0.125 µg and 0.0625 µg/mL) according to (Sileshi *et al.*,2012) to use directly for the in vitro cultivation of isolated parasites eggs.

Annex 4. Preparations of serial dilution concentrations for Ivermectin solutions

1) The stock solutions of **Ivermectin** of Chinese, Uruguay and Indian brands was prepared at concentrations of 1000µg/ml by dissolving 10mlof 1% **Ivermectin** in 20 ml of Dimethyl sulfoxide solution and 80 ml of distilled water.
2) The second mother solution of dissolved **Ivermectin** was prepared at concentrations of 500µg/ml by mixing 50 mg of mother solutions with 50 ml of distilled water.
3) The third concentration of albendazole solution was prepared at concentrations of 250µg/ml by mixing 25 mg of first mother solution with 75 ml of distilled water.
4) The fourth concentration of **Ivermectin** solution was prepared by dissolving 12.5 mg of mother solutions in 87.5 ml of distilled water.
5) The fifth final solution of **Ivermectin** was prepared by dissolving 6.25 mg of **Ivermectin** solutions in 93.75 ml of distilled water.
6) From prepared anthelmintic serial concentrations,10µl of **Ivermectin** solutions were then added to the micro dilution plates at final dilutions of (1 µg, 0.5 µg, 0.25 µg 0.125 µg and 0.0625 µg/mL) according to (Sileshi *et al.*,2012) to use directly for the in vitro cultivation of isolated parasites eggs.

Annex 5. Preparations of serial dilution concentrations for Levamisole solutions

A. The stock solutions of **Levamisole** of East Africa and Indian brands was prepared at concentrations of 1000µg/ml by dissolving 100mg of **Levamisole** in 20 ml of Dimethyl sulfoxide solution and 80 ml of distilled water.
B. The second mother solution of dissolved **Levamisole** was prepared at concentrations of 500µg/ ml by mixing 50 mg of mother solutions with 50 ml of distilled water.
C. The third concentration of **Levamisole** solution was prepared at concentrations of 250µg/ml by mixing 25 mg of first mother solution with 75 ml of distilled water.
D. The fourth concentration of **Levamisole** solution was prepared by dissolving 12.5 mg of mother solutions in 87.5 ml of distilled water.
E. The fifth final solution of **Levamisole** was prepared by dissolving 6.25 mg of **Levamisole** solutions in 93.75 ml of distilled water.
F. From prepared anthelmintic serial concentrations,10µl of Anthelmintic solutions were then added to the micro dilution plates at final dilutions of (1 µg, 0.5 µg, 0.25 µg 0.125 µg and 0.0625 µg/mL) according to (Sileshi *et al.*,2012) to use directly for the in vitro cultivation of isolated parasites eggs.

Annex 6. Preparations of serial dilution concentrations for Tetramisole solutions

For adult motility test, tetramisole of Indian and Chinese brands were tested against adult parasites of Haemonchus.contortus and each tested concentrations prepared according to the following procedure.

1) The stock solutions of tetramisole of Chinese and Indian brands was prepared at concentrations of 1000µg/ml by dissolving 100mg of tetramisole in 20 ml of Dimethyl sulfoxide solution and 80 ml of distilled water.
2) The second mother solution of dissolved tetramisole was prepared at concentrations of 500µg/ml by mixing 50 mg of mother solutions with 50 ml of distilled water.
3) The third concentration of albendazole solution was prepared at concentrations of 250µg/ml by mixing 25 mg of first mother solution with 75 ml of distilled water.
4) The fourth concentration of albendazole solution was prepared by dissolving 12.5 mg of mother solutions in 87.5 ml of distilled water.
5) The fifth final solution of albendazole was prepared by dissolving 6.25 mg of tetramisole solutions in 93.75 ml of distilled water.
6) The prepared anthelmintic serial concentrations were then added to the micro dilution plates at final dilutions of (1 µg, 0.5 µg, 0.25 µg 0.125 µg and 0.0625 µg/mL) according to (Sileshi *et al.*,2012) to use directly for the in vitro cultivation of isolated parasites eggs.

Annex 7. Egg hatch test (EHT) and Egg recovery procedures for evaluation albendazole efficacy

In egg hatch assay, benzimidazole group of anthelmelmintic was tested to evaluate the percentage inhibition of eggs on eggs recovered from *Haemonchus.contortus* and *Trichostrongylus.columbriformis* in sheep according to the following procedures.

1. Fresh fecal sample was collected from donor sheep and mixed very well before two (2hrs) of collection to recover eggs.
2. Then collected fecal sample was labeled for each test performed to evaluate efficacy each tested brands of albendazole in egg hatch test techniques.
3. Fresh fecal samples collected in less than two hours were then homogenized with mortar and pestle in tape water to have good mixed suspension of eggs in its the sediment.
4. The mixed faeces in water were then centrifuged at 2000 rpm for 3 to recover eggs respectively. The sediment was loosened and mixed with saturated saline (saturated salt solution) and centrifuged at 2000 rpm for 2 minutes.
5. Then tube was left undisturbed in a stand for 3 minutes after which 1 ml of the supernatant was collected. The nematode eggs from the supernatant were washed twice by sedimentation in water.
6. Then serial dilution of different concentrations of albendazole was prepared in solutions of Dimethyl sulfoxide (DMSO) and distilled water.
7. The final concentration of the egg suspension was then adjusted to 100µl containing 100 eggs per 10µg using automatic pipette.
8. The adjusted numbers of eggs in 100µl of water by automatic pipette were then incubated for 48 hours at 23oc in serial concentrations of Albendazole dissolved in 1% DMSO.
9. Lugol's iodine was added to stop further hatching and all eggs and larvae at each Albendazole concentration.
10. Then 100µL of agitated sample was transferred into a petridish marked with grid and hatched or embryonated, unhatched eggs and dead eggs were counted.

Annex 8. In vitro larval motility test for evaluation of Ivermectin and Levamisole against local isolate of T.columbriformis and H.contortus larvae.

Efficacy evaluation of Ivermectin and Levamisole using larval motility test against infective stage L3) of Haemonchus.contortus and Trichostrongylus.columbriformis were performed according to the following procedures.

A. After slaughtering of sheep with sufficient gastrointestinal parasites eggs, pure adult Haemonchus.contortus and Trichostrongylus.columbriformis parasites were crashed to liberate eggs.
B. The librated eggs were cultured with sterile fecal sample for harvesting of pure infective stage of isolated parasites.
C. The donor animals were infected orally with 2500 infective larvae (L3) of Haemonchus.contortus and Trichostrongylus.columbriformis respectively.
D. The infected sheep were then checked for the establishment of the infection as of day 21 post-infection using faecal examinations. The sample was collected directly from rectum of the sheep.
E. Then 15 gram of feces with nematode eggs were crashed and homogenized with sterile pestle and mortar to culture it into infective larvae of the parasites.
F. The culture material was moistened daily by spraying distilled water to provide the appropriate humidity and air to grow the larvae.
G. After incubation for 10 days at room temperature, L3 were recovered by spontaneous migration by downward inverting the culture material and adding warm water.
H. The concentrated larvae were washed three times and counted three times to implement the larval motility test after stored in separate tubes.
I. The storage of counted larvae was finally diluted and counted three times to only distribute the required amount of larvae in well plates.
J. Finally, dilutions of counted larvae were adjusted at 50 µL of suspensions containing 50 L3 per 10µL of serial concentrations of ivermectin and levamisole solutions according to guidelines of (Dolinská *et al*., 2016) .

Annex 9. In vitro Adult motility test on tetramisole of different brands against local isolate of H.contortus.

Adult motility assay was conducted on mature *H. contortus* worms, collected from abomasums of freshly slaughtered sheep, following the technique of (Avinash *et al*.,2017).

1. Serial dilution of different concentrations of tetramisole was prepared at first step prior to slaughtering of the animal.

1) The sheep infected with sufficient number of adult *Haemonchus.contortus* parasites was sacrificed in order to collect fresh adult parasites from abomasums. Accordingly, immediately after animal´s death, the abomasum was removed, opened and washed for the collection of adult worms.
2. Then fresh adult *Haemonchus.contortus* was collected from abomasum of the sheep and washed with distilled water.
3. Then fifteen actively moving adult stages of *Haemonchus.contortus* were manually picked up from the mucosal surface and the contents of the abomasums, collected and washed in distilled water were placed in petridishes containing tetramisole dissolved in DMSO and diluted in distilled water at final concentrations of (1.0, 0.5, 0.25, 0.125, and 0.0625 µg/ml) and distilled alone for negative control.
4. Then parasites in petridish were incubated in five different Anthelmintic concentrations (1.0, 0.5, 0.25, 0.125, and 0.0625 µg/ml) and distilled water at 37°C for 2hr interval and checked their motility.
5. Then After 8hours, the tetramisole were washed away and the parasites suspended in distilled water for thirty minutes for possible recovery of parasite motility.
6. Finally, the numbers of motile and immotile worms were counted by gently prodding the worms using a pointed syringe for checking of their motility and mortality.

Annex 10 . Fecal flotation procedure for isolation of parasites

Before in vitro detection of Anthelmintic resistance, in small ruminants, the presences of gastro-intestinal nematode parasites were surveyed by examinations of fecal samples following flotation techniques of (Hansen and Perry 19994). Fresh faecal samples for examination of parasite eggs was collected from the rectum of the animal and processed according to the following procedure.

1) Put approximately measured 3 g of faeces collected from sheep into first container1.

2) After preparation of flotation fluid, pour 50 ml of flotation fluid was added into container 1.

3) Then the faeces was mixed with flotation fluid thoroughly with a stirring device (tongue blade, fork).

4) In order to remove the debris from fecal material, faecal suspension was sieved through a tea strainer or a double-layer of cheesecloth into Container 2.

5) The filtered faecal suspension was again added into a test tube from Container2.

6) The test tube containing filtered faecal suspension was put in test tube rack.

7) Gently top up the test tube with the suspension, leaving a convex meniscus at the top of the tube and carefully a coverslip was then placed on top of the test tube.

8) Finally after standing of the test tube for 20 minutes, then carefully lift off the coverslip from the tube, together with the drop of fluid adhering to it, and immediately place the coverslip on a microscope slide.

9) Then final result was considered to identify the sheep with high number of the eggs.

Annex 11. **Faecal cultures and harvesting of infective larvae**.

For evaluation of efficacy of the tested anthelmintics,donor animals were firstly infected and checked for establishment of infection. Then fecal cultures and harvesting of infective stage of infected parasites were done following the techniques of (Van Wyk & Mayhew, (2013) as follows.

A. Pure infective stage of Haemonchus.contortus and Trichostrongylus.columbriformis were initially infected into donor sheep for harvesting of infective stage of both parasites.
B. Then infected sheep were checked for the establishment of the infection as of day 21 post-infection using faecal examinations according to (Chaka *et al*., 2009).
C. Then the feces with nematode eggs were crashed and homogenized with sterile pestle and mortar or using other a stirring device.
D. The culture material was moistened daily by spraying distilled water to provide the appropriate humidity and air to grow the larvae.
E. Finally after incubation for 10 days at room temperature, larvae were recovered by downward inverting after filling the jar with water.