



# Review on anthelmintic drug resistance nematodes and its methods of detection in Ethiopia

**\*Corresponding Author(s): Abe kebede**

Debre-libanos livestock and fishery office, Shararo,  
Ethiopia  
Email: abekebe82@gmail.com

## Abstract

Small ruminants (sheep and goats) contribute to the self-sufficiency of resource-poor farmers by providing milk, meat, skin, manure and direct cash income. In the absence of sufficient feed supplies and proper health care and management, the productivity of these animals is very low.

Helminthosis represents one of the constraints to livestock production in Ethiopia by reducing production and reproductive performance. These helminthes that infect ruminants includes; *Haemonchus*, *Trichostrongylus*, *Mecistocirrus*, *Cooperia*, and *Nematodirus*, and the *Strongyloidea* and *Ancylostomatoidea* with *Oesophagostomum* and *Bunostomum*. Control of gastrointestinal nematode parasites of livestock in smallholder farmer and pastoralist communities is done with limited anthelmintic drug use, or with traditional herbal remedies, and is performed mainly during the rainy seasons. Unfortunately drug resistance develop while we use chemical control (anthelmintic) like albendazole, avermectin, levamisoles, etc, due to some failure that is frequent treatment, under dosing, using lesser drug quality. Anthelmintic resistance is a heritable change in a population of worm that enables them to survive drug treatments that are generally effective against the same species of infection at the same dose rate.

Anthelmintic resistance in nematodes of small ruminants has been reported from different parts of the world including Ethiopia. Anthelmintic resistance is detected by using in vivo (FECRT) and in vitro test Once anthelmintic resistance is detected it is possible to manage this development of resistance by using; Quarantine newly introduced animals (Biosecurity) treating animals that only need medication by applying FAMACHA, treating animals by triple anthelmintic drugs, regulating refugia, pasture management, alternative/mixed grazing and producing vaccines that disrupt the worms ability to process the nutrients necessary to maintain proper growth. In these paper I try to review on anthelmintic drug resistance nematodes parasites, causes of anthelmintic drug resistance, the methods of anthelmintic drug resistance detection, situation of anthelmintic resistance in small ruminant in Ethiopia and possible management and control of anthelmintic drug resistance.

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## Introduction

One of the most important and immediate goals for Ethiopia is to become self-sufficient in food production, a goal that is clearly expressed in the National Food Policy and Strategy and in the Poverty Reduction Programme [1]. The country has faced critical food shortages for decades and with the rapid growth in its population, it becomes crucial to maximize agricultural production through improved management. Therefore, the country needs to prioritize and improve agricultural production in various sectors, including the livestock industry.

Sheep and goats are the most important livestock species in Ethiopia, with approximately 11.5 million and 9.6 million, respectively, found within the country. These small ruminants contribute to the self-sufficiency of resource-poor farmers by providing milk, meat, skin, manure and direct cash income. In the absence of sufficient feed supplies and proper health care and management, the productivity of these animals is very low. The productivity of this huge population however remains marginal due to prevailing diseases, poor nutrition and husbandry systems and lack of effective veterinary services [2].

Helminthosis represent one of the constraints to livestock production in Ethiopia by reducing production and reproduction performance [3,4]. Sheep and goats are usually infected with a range of different species of nematodes. Gastrointestinal nematodes are one of the major impediments to the economical benefits from small ruminants [4]. The economically most important and widely prevalent gastro-intestinal nematodes are the *Trichostrongyloidea* that include genera such as *Haemonchus*, *Trichostrongylus*, *Mecistocirrus*, *Cooperia*, and *Nematodirus*, and the *Strongyloidea* and *Ancylostomatoidea* with *Oesophagostomum* and *Bunostomum*. In modern pastoral farming systems the main emphasis for nematode control is to limit the number of infective larvae on pasture. This is commonly achieved by regular use of anthelmintics and other manipulations of grazing management such as treating and moving animals from contaminated sites to clean pasture.

Anthelmintic have a pivotal role in minimizing the negative effects of nematodes worldwide. Anthelmintic drugs remain the principal means of for therapy and prophylaxis of nematode parasitic diseases in humans and animals. Other than improvements in sanitation, there are no effective alternatives to chemical control of parasitic nematodes. In addition, more persistent anthelmintics or new delivery systems have been widely used [5]. This reduces numbers of nematodes in refugia, that means not exposed to anthelmintic [6,7] and will accelerate selection for resistance. However, resistance to anthelmintics has become a major problem in veterinary medicine, threatens both agricultural production and animal welfare, and there is increasing concern that drug resistance could arise in nematode parasites in human because of indiscriminate and frequent use of these drugs has resulted in the emergence of anthelmintic resistance against most of the major classes of anthelmintics in several countries [8-10]. Resistance in nematodes of livestock (sheep, goat, cattle) to anthelmintic resistance has become a serious problem in many parts of the world. Vatta and Lindberg [11] in their recent review stated that anthelmintic resistance has been reported in goats and sheep from at least 14 countries in Africa with most of the reports emanating from South Africa and Kenya and the majority concerning *Haemonchus contortus*. Despite the great importance and considerable time of use of anthelmintics in Ethiopia, limited numbers examining the efficacy of these drugs are reported [12-14]. Resistance has arisen

to all of the major families of broad-spectrum anthelmintics [15], the benzimidazoles (BZD), levamisole (LEV) and the other nicotinic agonists, and the avermectins and milbemycins (AM), which include ivermectin, doramectin, and moxidectin. Nematodes resistant to other narrow-spectrum anthelmintics such as closantel have also been reported. Resistance is present when there is a greater frequency of individuals with in a population able to tolerate doses of compound than in normal susceptible population of the same species. One member usually confers resistance to the each chemical class of anthelmintics, resistance to other members it is possible, and increasingly common, to have multiple resistances where nematodes develop resistance sequentially and independently to several anthelmintic classes. Once resistance is present in a nematode population, reversion or loss of resistance occurs very slowly [16]. Drug resistance can arise in a limited number of ways: a change in the drug receptor so that the drug no longer binds with high affinity and is thus ineffective at safe low concentrations change in metabolism that inactivates or removes the drug; or a change in the distribution of the drug in the target organism, which prevents it accessing its site of action. Smallholder farmer's pastoralists of Ethiopia practice varying degree of parasite control in their livestock. These practices ranges from the use of traditional medicine to anthelmintic drug [17].

The development of cost-effective and sustainable control programme to control helminth infections requires a thorough knowledge of the species of parasites present, the flock/herd structures, grazing management, seasonal availability of parasites and weather conditions in a particular area [18]. Therefore, the objectives this paper

- To review the prevalence of anthelmintic resistance and the factors contributing to the development of resistance on sheep and goat in Ethiopia.
- To summarize some detection methods of anthelmintic resistance.
- To review risk factors for anthelmintic resistance in small ruminant in Ethiopia

## Review on anthelmintic drug resistance

### Description of anthelmintic drug resistance

Resistance is defined by the World Health Organization (WHO) as development of an ability in a strain of some organism to tolerate doses of a toxicant that would prove lethal to a majority of individuals in a normal population of the same species. Anthelmintic resistance is defined as a decrease in the efficiency of anthelmintics against a population of parasites that were originally susceptible [15]. This decrease in susceptibility is caused by an increase in the frequencies of resistance gene alleles that result by selection through repeated use of anthelmintic drugs [19,20]. Frequent use of anthelmintics to control helminthes poses the risk of resistance populations' development [21-23]. Resistance in the field is usually suspected when there is an apparent poor clinical response to treatment with anthelmintic [24]. Anthelmintic resistance is defined as a heritable change in a population of worm that enables them to survive drug treatments that are generally effective against the same species of infection at the same dose rate. In practical terms, resistance is present in a population of parasites when the efficacy of the drug falls below that which is historically expected (when all other factors are the same). Such changes occur slowly, usually over many years, and are the direct result

of natural selection on parasite populations in response to drug treatments. This stands in contrast to drug tolerance, where the drug is not highly effective against a particular parasitic stage or worm species at the first exposure to the drug [25].

Many parasitic nematodes have biological and genetic features that favor the development of anthelmintic resistance. Of considerable importance is the exceptionally high level of genetic diversity seen in most parasites that reproduce sexually and that parasitize mobile vertebrate hosts. Short life cycles, high reproductive rates, rapid rates of nucleotide sequence evolution, and extremely large effective population sizes combine to give many parasitic worms an extremely high level of genetic diversity [26]. In addition, most nematode species demonstrate a population structure consistent with high levels of gene flow, suggesting that host movement is an important determinant of nematode population genetic structure. Thus, many parasitic nematodes possess both the genetic potential to respond successfully to chemical assault, and the means to assure dissemination of their resistance alleles via host movement [27].

Why resistance develops more slowly in some hosts and parasites than others is a complex question which is dependent on many factors. These factors relate to the parasite biology and epidemiology, the dynamics of the host-parasite relationship, and the pharmacokinetics of the drugs. Some factors relating directly to the parasite biology include: life history (generation time, direct or indirect life cycle), fecundity of female worms, lifespan of mature worms, survival of free-living stages in the environment, level of genetic diversity, manner of inheritance of resistance traits, number of genes involved, actual dose level required to kill susceptible worms of a particular species as compared to label dose level, and worm pathogenicity (and therefore need for treatment). Host factors include: levels of innate and acquired immunity, behavioral differences affecting exposure rates and differences in anthelmintic pharmacokinetics between host species. In livestock species, anthelmintic drugs generally demonstrate highest bioavailability in cattle, and lowest bioavailability in goats. It is frequently suggested that the extremely high prevalence of anthelmintic resistance in nematodes of goats is associated with this unique pharmacokinetic profile. All of these factors combined with treatment frequency, means of drug delivery (affecting pharmacodynamics and kinetics), dose rate, drug persistence, quality of drug used (e.g. expired drug) and levels of *refugia* at time of treatment interact to influence the rate of resistance development. It is difficult to know with precision or certainty how large a role each of these different factors play in the development of resistance, and most likely they change with each host/parasite relationship. However, the fact that the important nematodes of cattle and sheep/goats are extremely closely related (both phylogenetically and biologically), but resistance is much slower to evolve in nematodes of cattle, gives strong evidence that many factors other than the genetics of the worms are involved in the dynamic process of resistance selection [16].

### History of anthelmintic resistance

The problems posed by anthelmintic resistance in helminth parasites of veterinary importance are not new. Resistance is probably an inevitable consequence of the use of anthelmintics, and the history of parasite resistance to anthelmintics starts with the first report on phenothiazine

Resistance in 1957, then to the albendazole and followed by levamisole [28].

*Haemonchus contortus* was the first nematode to develop resistance against the different anthelmintics. In Ethiopia Kasahun Asmare [29] observed the susceptibility of nematode to albendazole, tetramisole and ivermectin in eastern and southern Ethiopia respectively. Whereas, Bersisa Kumsa and Ajebu Nurfeta [30], Bersisa Kumsa and Abebe Wossene [31] reported the presence of anthelmintic resistance in small ruminant nematodes in the country.

**Table 1:** The first reports of anthelmintic resistance in nematodes of sheep to drugs with different modes of action [32].

Year	Country	Drug	Nematodes
1957	USA	Phenothiazine	H. Contortus
1964	USA	Thiabendazole	H. Contortus
1968	USA	Op. compounds	T. Circumcinctus
1976	Australia	Levamisole/morentel	H. Contortus
1987	S. Africa	Invermectin	H. Contortus

### Mechanisms of anthelmintic resistance

Resistance is present when there is a greater frequency of individuals with in a population able to tolerate doses of a compound than normal susceptible population of the same species. For each chemical class of anthelmintics resistance to one member usually confers resistance to the other members (side resistance). Example resistance albendazole confers side resistance to the other members of benzimidazole family because of same mode of action.

Drug resistance can arise in a limited number of ways: a change in the drug receptor so that the drug no longer binds with high affinity and is thus ineffective at safe low concentrations change in metabolism that inactivates or removes the drug; or a change in the distribution of the drug in the target organism, which prevents it accessing its site of action [33]. Parasites have a number of strategies to become resistant, including;

1. Molecular change affecting the capacity of drug to accumulate at intracellular site action (reduce uptake, enhanced active efflux and metabolism). E.g. Benzimidazole(BZM) resistance in *haemonchus contortus* has been associated with the loss of high affinity binding receptors and an alteration of the  $\beta$ -tubulin isoform pattern based well conserved mutation at amino acids 200 or 167 (phenylalanine to tyrosine) in both  $\beta$ -tubulin isotype 1 and 2;
2. Modified activity of parasite enzymatic system;
3. Changes of number, structure and or affinity of cellular drug receptor and
4. Amplification of target genes to overcome the effect of anthelmintic drug.

The mechanism of action determines the time of appearance of the antiparasites effect and the potential risk for the development of resistance to a give drug chemical class.

### Cause of anthelmintic resistance

It is very important to make a distinction between reduced efficacy and anthelmintic resistance, though in practice it is not at all easy to do so. Many potential confounding factors may af-

fect the efficacy of an anthelmintic, and should first be excluded before anthelmintic resistance can be assumed. Such issues have been extensively investigated in veterinary intestinal nematode infections, but less so among species infecting humans. An important host-related factor is the significant variation in the pharmacokinetics of anthelmintics. A greater understanding of the pharmacokinetics of anthelmintic drugs such as the BZM in livestock in the last few years has contributed significantly to improving parasite control in livestock [34]. In contrast there is a paucity of pharmacokinetic and -dynamic data for anthelmintics in humans [35]. Since the broad-spectrum anthelmintic activity of BZM compounds relies on the extended presence of effective drug concentrations at the location of the parasite in its precise niche in the host [36], it implies that in humans increased drug concentration at this site and extending the exposure period of the parasite to the drug should result in enhanced clinical efficacy [34].

If so, manipulation of the formulation and dose regimen may result in an improved pharmacokinetic profile, thereby improving drug efficacy. For example, reduction of feed intake resulted in increased plasma availability of albendazole in animals [35], Pharmacogenetic variation in drug handling, age-related changes in drug distribution, drug interactions due to concomitant therapy (anti-inflammatory drugs or antibiotics) and co-morbidities (e.g. gastrointestinal diseases, malnutrition and immunodeficiency) may also affect anthelmintic efficacy. In addition, many drugs require a competent immune system to achieve optimal efficacy. Some food types and drugs such as grapefruit and the antacid cimetidine have an effect on cytochrome P450-mediated drug handling at the intestinal luminal interface thereby modifying ALB pharmacokinetics [38]. Four factors have been identified as contributing to the development of anthelmintic resistance: 1. initial resistance allele frequency, 2. treatment frequency, 3. *refugia* and 4. possibly under dosing.

#### Initial resistance allele frequency

Genetic data suggest that alleles of genes that confer resistance exist in worm populations prior to the introduction of the drug. The same allele that is linked to benzimidazole resistance is found in a wide variety of resistant lines, implying that resistance arose once and then spread as a neutral allele [39]. Since new mutations are not required, selection for resistance is most accurately viewed as the loss of susceptibility, rather than the gain of resistance [40]. At this stage, there is almost no information on the frequency of putative resistance alleles, based on veterinary parasites, in human soil transmitted helminthes (STH), although the tools are now available for assessing this for BZD in treatment naive and treated populations infected with Soil transmitted helminthes (STH).

#### Treatment frequency

This is an important determinant of the speed of selection of anthelmintic resistance: the greater the drug pressure (related to treatment frequency, relative fitness of resistant compared with susceptible worms, dose regimens, survival of free-living stages, *refugia* and other factors), the faster the selection of resistant nematode strains. Treatment frequencies of five or more times a year (up to 10 treatments/year) are not common in livestock [41]. In humans, the frequency of treatments is usually limited to 1–3 per year for *A. lumbricoides*/hookworms [42]. However, even at these lower treatment frequencies, selection of anthelmintic resistance has been repeatedly reported

in sheep and goat nematodes [43]. This is especially the case when the same drug has been used over prolonged periods, as is the case with BZM in the control of human STH, and this combined with lower treatment frequencies might be enough to select for resistance. This has been clearly shown in nematodes of livestock, where farmers tend to use a single drug until it fail [44]. The phenomenon of *refugia*, i.e. the proportion of the parasite population that is not exposed to drugs and thus escapes selection for resistance, is a very important factor in the selection pressure for development of resistance, and one whose impact on the development of anthelmintic resistance is too often overlooked [6].

#### Under dosing

Under dosing may constitute another risk factor for the development of anthelmintic resistance, As was shown in the models developed by, the impact depends on the initial (before exposure to a given anthelmintic) and the resultant (after treatment) frequency of resistance alleles in the helminth population. Depending on their ability to kill all or part of the susceptible homozygote, heterozygote and/or homozygote resistant helminthes, and on the initial frequency of resistant alleles, specific dose regimens may select for anthelmintic resistance in different ways. Assuming that resistance is determined by a single major gene comprising two alleles at a single autosomal locus and low initial frequency of the allele for resistance, the most dangerous dose is the one that kills all susceptible homozygotes but none of the heterozygous or homozygous resistant genotypes. In contrast, when the initial frequency of the allele for resistance is high, the dose that most strongly promotes resistance is the one that kills all susceptible homozygotes and all heterozygotes, but none of the resistant homozygotes [45].

#### Refugia

The phenomenon of *refugia*, i.e. the proportion of the parasite population that is not exposed to drugs and thus escapes selection for resistance, is a very important factor in the selection pressure for development of resistance, and one whose impact on the development of anthelmintic resistance is too often overlooked [6]. The size of *refugia* will be mainly determined by

- ✓ The fraction of the population treated (i.e. mass treatments versus selective or targeted selective treatments) and
- ✓ The proportion of the worm population present in the environment where it is not subject to drug action (e.g. in the soil). This is influenced in turn by a range of factors including climate, resilience of the transmission stages in the face of environmental stressors and longevity of the free-living stages.

This is influenced in turn by a range of factors including climate, resilience of the transmission stages in the face of environmental stressors and longevity of the free-living stages [46] showed that leaving some sheep untreated worked best in situations where animals were already grazing or were moved onto pastures with low populations of infective larvae. In those cases, anthelmintic resistance was delayed and nematode control was maintained when 1–4% of adult stock remained untreated. The size of *refugia* is also largely determined by factors such as the timing of the treatment and the climatic conditions immediately prior to treatment as both will influence selection pressure on the parasites.

## Detection of anthelmintic resistance

The growing importance of anthelmintic resistance has led to increased need for reliable and standardize detection methods [47]. Most of methods described have draw backs either in terms of cost, applicability and interpretation or reproduction of finding. Different *in vivo* and *in vitro* tests are now available and there is an ongoing effort to refine, standardize and validate these tests from time to time. The development of molecular tests [48] is also progressing and is trying to apply DNA-probe and polymerase chain reaction (PCR) technology. There are a number of *in vivo* and *in vitro* assays that measure the effects of anthelmintics on development, growth or movement of nematodes stages have been developed as alternative methods of detection [49].

### *In vivo* methods

*In vivo* tests are available for the detection of resistance to group 1; the benzimidazoles (BZD) and group 2; the imidazothiazoles (levamisole, LEV) anthelmintics Faecal egg count reduction test (FECRT) is the most widely used *in vivo* test. This is the most common test to study anthelmintic resistance. This test was originally designed for sheep, but can be used also for cattle, swine and horses. Modern broad spectrum anthelmintics are highly efficacious, and treatment should normally result in a reduction of faecal egg counts by more than 95 percent. Thus this test provides an estimation of anthelmintic efficacy by comparing faecal egg counts of animals before and ten days after treatment [50]. For monitoring of normal fluctuation, the treated group is generally compared with non-treated controls. This test is particularly suitable for field surveys and it has the advantage that the number of groups can be increased if appropriate, to test the efficacy of a range of broad or narrow spectrum anthelmintics at one time.

In this test, the efficacy of an anthelmintic is determined by comparing parasite populations in-group of treated and non-treated animals. The procedure compares worm burdens of animals artificially infected with susceptible or suspected resistant isolates of nematodes. The parasitized animals are randomly separated into medicated and non-medicated groups and at a suitable interval after treatment (10 to 15 days), a necropsy is carried out and the parasites are recovered, identified and counted. This test 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 [51]. In an attempt to reduce the cost and labor required for this test, laboratory animal model have been used and guidelines for evaluating anthelmintic efficacy using the controlled test have been published [52,53].

### *In Vitro*

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 because there can be difficulties in the interpretation of results with field infections, which usually consist of multiple parasite species. The maintenance of standard laboratory strains, both drug susceptible and resistant is necessary for Comparative purposes [54].

### Larval Development Assay (LDA)

The larval development tests are the only ones that allow the detection of resistance against all the drugs, irrespective of their mode of action. Several methods have been described, but reproducibility, linearity of the dose-response and susceptibility differ. The LDA is an *in vitro* assay for the detection of resistance to benzimidazole, levamisole, combinations of benzimidazole and levamisole, and avermectin and milbemycin drenches in the major gastro intestinal nematode parasites of sheep, *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Ostertagia circumcincta*. In this test, nematode eggs, isolated from faecal samples submitted by producers, are applied to the wells of amicro-titre plate and larvae hatch and develop to the L3 stage in the presence of anthelmintic. The concentration of anthelmintic required to block development related to an anticipated *in vivo* efficacy [55].

### The egg hatch assay

The egg-hatch test has been developed to differentiate between resistant and susceptible strains of gastro-intestinal nematodes for the benzimidazoles and for the levamisoles. It provides an accurate method for assessing the susceptibility of mixed nematode populations, and it is comparatively more rapid and economic to conduct than the FECRT. It is based on the determination of the proportion of eggs that fail to hatch in solutions 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. 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 benzimidazoles decreases as embryonation proceeds. The test has only been shown to work on nematode species in which eggs hatch rapidly. Due to difficulties in the interpretation of the results this assay is not widely used for field surveys.

### Adult development assay

The adult development assay for detecting benzimidazole resistance in trichostrongylid nematodes has advanced significantly and *Haemonchus contortus* has been cultured through to the adult egg-laying stages, although this test is mainly for research purposes. Of all the available tests, the larval development test is the most sensitive for quantitatively measuring thiabendazole and levamisole resistance. The egg hatch assay is also sensitive and accurate in determining benzimidazole resistance. It was concluded that the other methods were unsuitable for use in field monitoring of resistance [49].

### Larval paralysis and motility assay

The test is used for levamisole and morantel resistance. This assay discriminates between resistant and susceptible strains of parasites, by estimating the proportion of third stage larvae in tonic paralysis after incubation with a range of levamisole and morantel drug concentrations. It is relatively easy to carry out, stocks of infective larvae are readily obtained and it is reported that there is a fairly good reproducibility of the test, any differences in repeatability being attributed to the age of larvae. However, the interpretation is complicated by the fact that if the anthelmintic is added to the egg suspension too early, the development has not proceeded far enough; if it is added too late the drug has no effect. A modification of the technique was developed using the micro-motility meter, an instrument for measuring the motility of larval and adult nematodes after incubation with benzimidazole and levamisole. A further modi-

fication of the larval paralysis assay has been made in order to apply it for the detection of thiabendazole resistance. Some lack of repeatability in this method has been attributed to the reversibility of paralysis [56].

**Table 2:** Bioassays for the diagnosis of anthelmintic resistance.

Test	Diagnosis resistance to
FECRT	Benzimidazole, levamisole, anvermectin, consontel
Egg hatch	Benzimidazole
Larval paralysis	Levamisole/morentel
Tubulin binding	Benzimidazole
Larval developmental	All drugs
Adult developmental	Benzimidazole

### Research and development of new tests (DNA probes)

Lately, gene probes, allele frequencies, trans-membrane functional analysis, PCR and flow cytometry have been investigated as tools for the determination of anthelmintic resistance. Currently, these procedures are exclusively for research purposes. Gene probes have been used to analyse restriction fragment length polymorphism between susceptible isolates and isolates of *Haemonchus* resistant to benzimidazole; levamisole and benzimidazole; or benzimidazole, ivermectin and closantel. A P-glycoprotein gene probe was also isolated from *Onchocerca volvulus* and an *Onchocerca*-specific PCR was developed for detection of resistance strains [57]. Analyses of allele frequencies showed significant differences between the unselected and the drug-selected derived strains. In all three drug-selected strains, an apparent selection for the same allele was observed. It is suggested that P-glycoprotein (Pgp) may be involved in resistance to both ivermectin and moxidectin in *Haemonchus contortus* [58]. A functional analysis of trans-membrane transport of drugs in drug-resistant helminths was undertaken using a flow cytometry method on two isolates of *Haemonchus contortus* that were susceptible or resistant to benzimidazoles and ivermectin.

The results confirm those obtained with biological drug assays, using both anthelmintics and verapamil, which suggest the involvement of EPG in drug resistance, and provide a quantitative and effective methodology for the functional study of multi-drug resistance in nematodes [59]. A very sensitive PCR test was developed that can detect benzimidazole resistance in the sheep parasite *Haemonchus contortus*. With this assay, the population genetics of benzimidazole susceptible and resistant worms can be studied in more detail under different conditions of selection. This may lead to a better control and a delay in the development of anthelmintic resistance [40]. Flow cytometry could be applied to the analysis of nematode populations. Forward-scatter emission can be used as a discriminating parameter for egg size. The hatching rate and side scatter emission have a significantly positive relationship. The rate of resistance to the anthelmintic can be observed as a significant regression on the native green-fluorescence pulses that might reflect the state of oxidation of associated flavin molecules [59].

### Situation of anthelmintic drug resistance nematodes in small ruminant in Ethiopia

Helminthosis represents one of the constraints to livestock

production in Ethiopia [3,4] by reducing production and reproductive performance. Smallholder farmers may not easily detect the effects of internal parasites on performance of their animals because of the generally sub clinical, or chronic nature of the helminth infections [60,61]. Control of gastrointestinal nematode parasites of livestock in smallholder farmer and pastoralist communities is done with limited anthelmintic drug use, or with traditional herbal remedies, and is performed mainly during the rainy seasons [17]. However, for smallholder farmers and stock owners in pastoralist communities, drugs are relatively expensive and are often not easily accessible, while frequent and indiscriminate use of different classes of anthelmintics has been reported in institutional and large commercial farms in Ethiopia [62]. With the advent of helminth parasite populations that have developed resistance to anthelmintics over the last decade or so, especially in small ruminants, livestock productivity has been threatened worldwide [63].

Anthelmintics have a pivotal role in minimizing the negative effects of nematodes worldwide. However, indiscriminate and frequent use of these drugs has resulted in the emergence of anthelmintic resistance against most of the major classes of anthelmintics in several countries [64]. Several previous studies conducted in different parts of Ethiopia indicate that gastrointestinal nematodes in goats are very common and widespread in all livestock systems in the country [12,65]. Large scale studies, however, are needed to assess the current status of anthelmintic resistance against the most commonly used anthelmintics in different agro ecology, species of animals and management systems in Ethiopia. Despite the great importance and considerable time of use of anthelmintics in Ethiopia, limited numbers examining the efficacy of these drugs are reported [12,13].

The first report in Ethiopia on the presence of anthelmintic drug resistance helminthes of goats and sheep where reported in mid-rift valley of Ethiopia [66]. The anthelmintic such as Albendazole (albenol) , levamisole , benzimidazoles and imidazothiazoles where tested and the result shows there are presence of anthelmintic resistance of nematodes in sheep and goats in 4 out of 22 or 9.99% smallholder farms and on one institutional farm, where as *Haemonchus contortus* was predominant around the areas of East and North Shewa. Resistance to levamisole was also detected on one smallholder farm and one institutional farm. The development of anthelmintic resistance in mid- rift valley of Ethiopia because of the low frequency of anthelmintic use, other practices that might cause the development of anthelmintic resistance exist [67].

Multiple anthelmintic resistance on a goat was reported in southern Ethiopia around Hawassa (Hawassa University goat farm). According to the report 180 goats were selected to treatment groups of anthelmintics (albendazole,tetramisole and ivermectin ); multiple anthelmintic resistance in *Haemonchus* species; against albendazole, tetramisole and ivermectin was recorded in all age categories of the goat. Likewise, *Trichostrongylus/Teladorsagia* species showed resistance against ivermectin. Resistance against anthelmintics is attributed to the high frequency of treatment and low dosage of treatment practices on the farm [68].

Efficacy of selected anthelmintics (albendazole, tetramisole and ivermectin) against Gastrointestinal nematodes of sheep where reported in smallholder farmers in Wolaita, Southern Ethiopia. Eighty three sheep were selected and treated with Albendazole,tetramisole and ivermectin. The efficacy for each anthelmintic was measured using the faecal egg count reduc-

tion test. The result showed that the anthelmintic resistance was not present for any of the tested anthelmintic drugs. Therefore, there is a good state of efficacy for all tested anthelmintics against gastrointestinal nematodes of sheep in wolaita, shouther Ethiopia [69].

Anthelmintic resistance in nematode parasites also reported in eastern Ethiopia at Haramaya University. According to the report the status of anthelmintics (albendazole (ABZ), tetramisole (TET), a combination (ABZ + TET) and ivermectin (IVM), at the manufacturers' recommended dose rates) where tested. Results showed that there was no evidence of anthelmintic resistance in nematode parasites of either sheep and goats in any community. Whereas no resistance was observed in parasites found in the University sheep flock, a high of level multiple anthelmintic resistance was recorded in the goat flock [70].

Getachew [71] reports the anthelmintic efficacy and risk factors for anthelmintic drug resistance in sheep at Bedelle District of Oromia Region, Ethiopia. According to the study the status of anthelmintic efficacy and associated risk factors for anthelmintic resistance in sheep was conducted. Four hundred fourteen sheep were sampled to assess the efficacy of albendazole, tetramisole and ivermectin against gastrointestinal nematode parasites prevailing in sheep. The result shows the three tested anthelmintic were effective with egg count reduction levels of 96%, 99% and 97% respectively for albendazole, tetramisole and ivermectin. However, post-treatment fecal cultures and postmortem adult worm recovery showed that some *Haemonchus contortus* worms have escaped the treatments was reported in the area.

The study that done in Ziway, Oromia Regional states (southern Ethiopia) reports the efficacy of the albendazole, tetramisole and ivermectin against gastro intestinal nematodes. Sixty goats treated with albendazole, tetramidazole and ivermectin; the result shows that 100% efficacy against strongyle and trichuris species was reported in goat treated with albendazole and ivermectin. On contrary, low efficacy of 90.1% and 63% against strongyle and trichuris species was observed respectively in goat treated with albendazole [72].

Recently anthelmintic resistance nematodes were reported in Horro sheep breed in Western Oromiya. Efficacy tests showed a suspected resistance against albendazole by *Haemonchus contortus* and *Trichostrongylus* species, whereas tetraclozan and ivermectin demonstrated high efficacy against all nematode genera isolated on the farms. In this area, a suspected resistance against albendazole was observed in sheep nematodes, particularly *Haemonchus contortus* on farms where drugs were indiscriminately used for worm management. Some worm control practices which are thought to enhance the selection of nematodes resistant to anthelmintics have been evident. Among the major drawbacks, risks of underdosing and continued use of one class of anthelmintics, irrespective of its efficacy status were widely practiced which may accelerate selection dynamics in horro, Western Ethiopia [73].

### Control and management of anthelmintic drug resistance

From a clinical stand of point, it is important to appreciate that resistance is a genetic trait that only becomes expressed phenotypically once allele frequencies of resistance genes reach fairly high levels therefore, prevention of drug resistance must be aimed at reducing the rate with which resistance alleles accumulate, and strategies designed to slow the develop-

ment of resistance must be in integrated early in the process of resistance evaluation, before there is any clinical evidence of reduce drug effect. Treating simultaneously with two drugs from different anthelmintic class is one method of preventing the development of anthelmintic resistance [74].

The most important single requirement for the successful implementation of rational and Sustainable helminth parasite control programmes in grazing animals, is a sound knowledge of the epidemiology of the parasite as it interacts with the host in a specific climatic, management and production environment [75]. The epidemiological knowledge base has been established through extensive studies and field trials in many developed countries, and mostly in the context of industrialized livestock production. The epidemiological knowledge base has been established through extensive studies and field trials in many developed countries, and mostly in the context of industrialized livestock production. This is not the case for the majority of developing countries and countries in transition, and if information is available it rarely covers the diversity of their production systems. The reasons for this are often the obvious lack of human, economic and infra-structural resources. However, it is also often wrongly assumed that the epidemiological work conducted in one climatic region or production system can be extrapolated to another, or that the availability of modern broad spectrum anthelmintics eliminates the need for epidemiological knowledge. The currently available tools for gastrointestinal nematode control consist of chemical and nonchemical technologies. The chemical technology relies entirely on treatment with different formulations of anthelmintics used in different control strategies according to whether epidemiological knowledge is absent or available. The non-chemical technology is based on, among other things, pasture and breeding management and nutritional interventions.

### Biosecurity

Effective management strategies to prevent development of anthelmintic resistance are worth less if producers purchase resistant worms residing in breeding stock therefore, strict quarantine producers should be instituted for all new additions there is no faster way to spread resistance than to bring GI nematodes to a farm. The current recommendation is to quarantine (on dry lot where faeces can be removed) every new addition, dose with triple-class anthelmintic therapy, and perform faecal egg count reduction test after receiving this treatment animals should be on a contaminated pasture. Never should the animals be placed on the clean pasture after the triple anthelmintic class treatment regimen is administered, because any surviving worms will be triple resistant and there will be no refugia on pasture to dilute the future transmission of any egg that are shed [76].

### Famacha-Rethinking strategy

The typical strategy used by small ruminant producers for controlling *haemonchus contortus* involves the treatment of all animals at fixed frequent intervals during peak transmission period or treating the entire group when one or more animals manifest clinical sign suggestive of worm infection or both. The major limitation developed in south Africa for identifying sheep that are anemic in this method, called FAMACHA, the ocular mucous membranes of sheep and goat are categorized by comparison with a limited color chart of sheep conjunctiva because anemia is the principal pathological effect from infection with *haemonchus contortus*, this system can be an effective tool for

identifying animals that require treatment [77].

### Pasture management

Reducing exposure of susceptible hosts in control program is paramount. The goal of pasture management is to provide save pasture for grazing. The thorough knowledge of epidemiology, including the seasonal variations in the pattern of larval development and availability on pasture, can form the basis for control of gastro-intestinal nematodes through pasture management. A number of different grazing systems have proved helpful in the control of these parasites [75].

#### Rapid rotational grazing:

Recently there has been increased interest in using rotational grazing of pastures for the optimization of pasture growth and productivity. This is an excellent tool, from the productivity point of view, as animals will consume a higher proportion of the available forage, which stimulates pasture re-growth [75].

#### Alternate grazing

Using alternate grazing for parasite control is based on different age groups of the same species, or different species grazing the pastures in sequence. In cases where different age groups are used it is common practice to graze calves followed by older cattle, taking advantage of higher resistance in the older animals. If the system is based on alternating between species (sheep – cattle) it utilizes the fact that many parasites show little cross-infectivity between adult cattle and sheep and/or the reduced susceptibility of different host species. It should be kept in mind that cool moist weather prolongs larval survival, and it is likely that alternate grazing systems will be less efficient in controlling parasites in temperate climates compared to tropical and subtropical regions [76].

### Vaccines

Considerable resources have been and still are being allocated to research into the effector mechanisms of naturally acquired immunity to gastrointestinal helminth infections of sheep [78] and cattle, with the aim of facilitating the development of vaccines. However, the situation is complex, involving a combination of local hypersensitivity, in addition to cell mediated, antibody and inflammatory responses, and is complicated further by the natural unresponsiveness, which exists, in the young lamb or calf, and in the dam around parturition. Using the successful development of the irradiated larval vaccine against the bovine lungworm, *Dictyocaulus viviparus* as a model, attempts have been made to produce vaccines against gut parasites in ruminants, but they have all been disappointing. The most promising vaccines for small ruminant worms is based on a hidden gut antigen and specifically targets *haemaphysalis contortus*, this antigen is derived from the gut of the worm and when administered to the animal, antibodies are produced when the worm ingest blood during feeding, it also ingest antibodies, the antibodies then attack the target gut cells of the worm and disrupted the worms ability to process the nutrients necessary to maintain the proper growth and maintenance [79].

### Conclusion and recommendation

Helminthes are the main constraints in reducing production and reproduction of livestock. Nematode parasites remain one of the most prevalent and important diseases affecting small ruminants worldwide. They are responsible for both direct and indirect major losses, Losses occur through mortalities, reduced

production due to subclinical parasitism and direct costs associated with control. It is possible to control those using chemical and non-chemical treatments especially by anthelmintics. To maximize the efficacy of anthelmintic compounds against parasites difficult to control in human and veterinary medicine while preserving an adequate margin of safety, a complete understanding of their pharmacokinetic and metabolic patterns in the host, is necessary, but it is true that anthelmintic resistance develop due to certain reasons which are ;under dosing ,frequent treatment, the extensive and indiscriminate use of the drugs has resulted in the development of resistance. To control anthelmintic resistance and to sustain food sufficiency strategy of the country via improving the health and production of small ruminants the following points are recommended.

- Use triple-anthelmintic treatment and keep them in contaminated pasture after treatment because any parasite that survives may develop triple drug resistance.
- Teaching to the small holders and pastoralist on: when and how animals should be to prevent under dosing.
- Development of FAMACHA; only animal that need treatment should be treated.
- Farmers and Veterinary professionals should start to consider anthelmintic resistance as a serious problem and the routine diagnosis of infections by helminths should be complemented by efficacy evaluation techniques.
- Proper veterinary extension and services should be implemented and correct parasite control programmes.
- Pasture management and alternate grazing with other species of livestock.

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