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

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**Abstract**
Anthelmintic resistance is described as a considerable improvement in the capacity of individuals within a strain of parasites to tolerate dosages of a substance that would be deadly to the vast majority of individuals in a normal population of the same species. People who have innate or acquired resistance to the medications are chosen when populations of gastrointestinal nematodes are repeatedly treated with the same anthelmintics. By passing on resistant alleles, resistance is inheritable. The target gene mutation or deletion of one or more amino acids, the reduction in the number of receptors, the lower drug affinities of the receptors, and the lack of bioactivation enzymes are examples of anthelmintic resistance mechanisms. The detection and monitoring of anthelmintic resistance have been carried out using a variety of techniques, both in vivo and in vitro. All classes of antiparasitic medications have failed to work as intended as a result of frequent and improper use, which has resulted in an international anthelmintic resistance crisis. Wherever the same drugs are overused, anthelmintic resistance (AR) is likely to emerge. If an animal's clinical state does not improve after receiving anthelmintic treatment, anthelmintic resistance in cattle is typically suspected. This frequently results from underdosing, improper application of the drenching solution, and poor estimation of body weight. Before contemplating anthelmintic resistance, some considerations must be considered. A "heritable shift" in an individual parasite's ability to withstand an anthelmintics prescribed therapeutic dose is called anthelmintic resistance. The issue of anthelmintic resistance is a major one in Ethiopia and is frequently reported from many regions of the nation; nevertheless, the rural population is unaware of these issues with anthelmintic resistance.

**Keywords:** Anthelmintic resistance, Detection**,** Ethiopia, Nematode, Ruminants

## 1. Introduction

An issue having global significance for effective parasite management is anthelminthic resistance (Cotter *et al*., 2015) [22]. When worms can withstand a medicine at a typical dose and convey this ability to their progeny, it happens. Globally, the prevalence of anthelmintic medication resistance in livestock has been measured using diagnostic techniques with varying degrees of sensitivity (Papadopoulos *et al*., 2012; Cotter *et al*., 2015) [71, 22]. Despite never having been validated against the gold standard of controlled slaughter studies, the Faecal Egg Count Reduction Test (FECRT) is the most commonly used field-based method for estimating anthelmintic efficacy and as an indicator of the presence of anthelmintic-resistant nematodes in cattle (Levecke *et al*., 2012; Lyndal-Murphy *et al*., 2014; Geurden *et al*., 2015) [63, 65, 33]. (Love *et al*., 2017) [64].

Reports of anthelmintic resistance pose a threat to the survival of the cattle sector given how heavily farms rely on drugs to manage worms. Since resistant worms are becoming a major issue, modifications to current control strategies are urgently needed. The best ways to stop the emergence of anthelmintic resistance include lowering the pressure on doctors to prescribe certain drugs and using the right timing to maximize their effectiveness (Verschave *et al*., 2016) [95].

Modern gastrointestinal nematode control is based primarily on anthelmintics. The three primary chemical families of broad-

spectrum anthelmintics previously used to treat gastrointestinal nematode infections were benzimidazoles (BZs), levamisole, and other imidazothiazoles, and macrocyclic lactones (MLS). The "white drenches," "yellow drenches," and "clear drenches" are other names for each of them (Abbott, Taylor, & Stubbings, 2004) [1]. The presence of AR is dependent on the host, the parasite, the kind of anthelmintic, animal management, and climatic conditions, which makes it more difficult to design preventive measures that should vary based on the animal production systems (Jackson *et al*., 2000) [44].

The development of anthelmintic resistance, which is mostly brought about by the use of unconscious anthelmintics, also harms the economy. According to a definition that is more technically correct, resistance is the genetically determined decline in an anthelmintic's ability to effectively combat a population of parasites that are typically sensitive to that medication. To kill 95% or more vulnerable parasite species, anthelmintics are often sold at dose rates that are many times higher than those needed. When a population has a higher percentage of people who can tolerate drug doses than would be expected in a healthy population of the same species, anthelmintic resistance is evident. The welfare and productivity of livestock are restricted globally by parasite-related infections. Anthelmintic medication is the mainstay of treatment for helminth infections (McKellar and Jackson, 2004) [67].

A short time after the medicine was introduced to the market; anthelmintic resistance emerged as a result of heavy, unintentional drug use. In 1992, methods to identify anthelmintic resistance were released by the World Association for the Advancement of Veterinary Parasitology (WAAVP) to raise awareness of this problem (Coles *et al*., 1992) [18]. An important issue today is anthelmintic resistance, particularly in sheep. Several sheep and goat farms have been shut down because of multiple medication resistance in certain nations, including Australia, the United Kingdom, New Zealand, and South Africa (Kaplan, 2004 and Geary, 2005) [49, 31].

**Therefore the objective of this seminar is**

- To review the anthelmintic resistance in nematodes in general including Ethiopia.
- To review methods of detection of drug resistance of nematodes
- Overview factors affecting the development of anthelmintic resistance

## 2. Literature Review

### 2.1 Modes of Action of Anthelmintics

The precise mechanism of action of many anthelmintics is unknown, although it relies on interfering with vital biochemical functions of the parasite, such as energy production, and paralyzing the worms (Kaplan, 2002) [48].

**Benzimidazoles and pro-benzimidazoles**: The original benzimidazole anthelmintic was thiabendazole. The inhibition of numerous parasite metabolic enzymes, such as fumarate reductase and malate dehydrogenase, was believed to be the primary mechanism of action of benzimidazoles. In eukaryotic cells, microtubules play crucial roles in processes like intracellular trafficking, cellular uptake, and secretion, anchoring of membrane receptors at specific sites, like synapses in nerve cells, mitosis, and meiosis, cellular architecture, including the lengthening of axons, and cell migration via cilia and cell pseudopods (Caviston; Holzbaur, 2006) [15].

**Imidazothiazoles (Levamisole) and tetrahydro pyrimidines**: Tetrahydro pyrimidines and levamisole both bind to and activate nicotinic acetylcholine receptors. Resistance to these medications is thought to result from either a change in binding properties or a decrease in the number of acetylcholine receptors (Hoekstra *et al*., 1997) [41]. Levamisole activated one of these two cholinergic receptors, however mutants who did not express the - and non-subunits of the acetylcholine receptor lost their levamisole susceptibility (Richmond and Jorgensen, 1999) [75]. Other investigations also identified various acetylcholine receptor subtypes, including N-type (nicotine sensitive) and L-type (levamisole sensitive) receptors. It is hypothesized that the depletion of the L-type cholinergic receptor contributes to levamisole resistance (Martin *et al*., 2003) [99].

**Avermectin**: Regarding the mechanism of avermectin/milbemycin resistance, various theories exist. In an avermectin-susceptible and an avermectin-resistant isolate of C. oncophora, the genetic variability of two genes, GluCl-alpha3 and GluCl-beta (encoding for subunits of glutamate-gated chloride channels) was examined. The statistical analysis shows a link between avermectin resistance and glutamate-gated chloride channels (GluCl) (Njue and Prichard, 2004) [69].

**Piperazine**: These medications cause helminth paralysis by acting anticholinergically at the neuromuscular junction (Urquhart *et al*., 1996) [91].

**Organophosphates**: Inhibiting cholinesterase causes a buildup of acetylcholine, which causes the parasites' neuromuscular paralysis and ejection (Urquhart *et al*., 1996) [91].

**Table 1:** Summary of Classes of Anthelmintics with their mode of action

| Drug group | Spectrum of Activity | Mode of action | Drugs |
|---|---|---|---|
| Benzimidazoles | Broad | Disruption of microtubules | Albendazole, Fenbendazole, Thiabendazole, Oxfendazole (Probenzimidazoles) Febental, Netobimin, Triclabendazole,Mebendazole,Oxibendazole |
| Imidazothiazoles/ Tetrahydoxy-pyrimidines | Broad | Nicotinic acetylcholine receptor agonists/Cholinergic agonists | Levamisole, Tetramisole |
| Macrocyclic lactones | Broad | Glutamate-gated chloride channel agonists | Avermectins, Moxidectin |
| Substituted Salicylanides | Narrow | Uncouple oxidative phosphorylation | Oxyclonazide, Rafoxanide, Nitroxynil, Closantel |

**Source:** (Urquhart *et al*., 1996) [91]

### 2.2 History of anthelmintic resistance

Different publications have different definitions of resistance. The World Association for the Advancement of Veterinary Parasitology's (WAAVP) Guideline on Anthelmintic Combination Products Targeting Nematode Infections of Ruminants and Horses (Geary *et al*., 2012) [32] defines it as follows: "the ability of parasites to withstand doses of medications that would typically kill parasites of the same species and stage." Because resistant helminths can avoid the effects of therapy and convey their resistance to the following generation, resistance is both inherited and selected during treatment. An increase in nematode eggs, higher adult survival rates in the host, and subsequently an increase in the number of immature stages on the pasture after treatment can all be signs of anthelmintic drug resistance (Geary *et al*., 2012) [32].

Resistance genes that develop in the population through mutation are initially uncommon, but as selection progresses, their relative proportion in the population rises, and as a result, the proportion of resistant parasites rises as well. Resistance across different chemical classes is known as cross-resistance (Dargatz *et al*., 2000) [24]. When evidence of phenothiazine-resistant Haemonchus contortus was discovered, the first report of worm resistance to anthelmintic treatment was made in the USA in 1964 (Drudge *et al*., 1964) [27]. Three years following the product's introduction, Haemonchus contortus strains resistant to thiabendazole were found in sheep ten years later.

Ruminants frequently exhibit resistance to anthelmintics, which has been well documented. Some ruminant species and some nematode species are more likely to build up resistance. The development of nematode resistance to various chemical anthelmintic groups is acknowledged as a serious issue. Due to their comparable modes of action, the benzimidazole group's members frequently exhibit cross-resistance. Because of its several modes of action, levamisole is believed to be able to control parasites that are resistant to benzimidazole (Susan *et al*., 1998) [86].

### 2.3 Anthelmintic drug resistance

A rising issue in the world is parasite resistance to anthelmintic medications (Wanyangu *et al*., 1996) [98]. The lowering of fecal egg counts, copro-antigen reduction tests, and egg hatch assays have all been used to demonstrate anthelmintic resistance (Coles *et al*., 1992; Brockwell *et al*., 2014) [18, 13]. (Fairweather *et al*., 2012) [29]. An anthelmintic is a substance that kills helminths or makes them leave the digestive tract, as well as any other organs and tissues they may inhabit in their hosts. There are several safe anthelmintics on the market right now, some of which work against a variety of helminth diseases while others have broader-spectrum efficacy. Many contemporary anthelmintics are effective against latent larvae as well as adults in the larval life cycle.

Anthelmintic resistance (AR) is the ability of the worm population or individual worms within the population to endure doses of an anthelmintic that would have otherwise killed a normal population of the same species and to pass on this "resistant" fitness to their progeny. A helminth population's accumulation of resistance genes occurs through an evolutionary process that is influenced by the genetic diversity of the parasite populations that are being selected for AR, the selection pressure (such as anthelmintic treatment), and time (Prichard, 2002) [74]. As a result, the development of resistant genes in nematode populations is a process of evolution that is influenced by the genetic diversity of the parasite populations that are being selected for anthelmintic resistance, the selection pressure (use of anthelmintics), and time (Prichard, 2002) [74].

Anthelmintic medication given as chemotherapy or chemoprophylaxis is a major component of the treatment of parasitic helminths in domestic animals. Although all domestic species utilize anthelmintics, the ruminant market, particularly cattle, accounts for the highest market share, with millions of pounds being spent there each year to lessen the impacts of parasitism. Therefore, it is expected that parasite populations treated with anthelmintic medications will evolve progressively from totally vulnerable to fully resistant and at varied speeds under diverse situations (Kaplan *et al*., 2007) [50].

There are several ways that anthelmintic resistance can develop:

**Side resistance**: A parasite strain may be resistant to a dose of medications with a similar mode of action but a different chemical structure. For example, a parasite strain that is resistant to Thiabendazole may also be resistant to Fenbendazole. A distinction between resistance and tolerance should be made, and it should be noted. Between susceptibility and total pharmacological failure, "tolerance" is described as the midpoint (Hastings and Watkins, 2006) [39].

**Cross Resistance:** It is similar to side resistance, but parasite strains can withstand therapeutic doses of drugs with different mechanisms of action or drugs with unrelated chemical structures. For example, a parasite resistant to benzimidazole will also show resistance to levamisole.

**Multiple Resistance:** When parasites are resistant to two or more chemically unrelated anthelmintic groups due to independent selection by each group or through side resistance, for example, when a parasite resistant to thiabendazole also exhibits resistance to tetramisole, rafoxanide, and avermectin. Multidrug-resistant parasites exhibit resistance to various anthelmintic classes. For instance, Haemonchus contortus isolates with multi-drug resistance to benzimidazoles and macrocyclic lactone anthelmintics (Anziani *et al*., 2004) [5].

**Single Resistance:** It involves a farm where worms that are resistant to a single anthelmintic group are present. The term "multi-generic resistance" is frequently used when more than one worm species is involved in the resistance. For example, if only Haemonchus contortus is resistant to benzimidazole on a farm, this is referred to as a single resistance case, whereas if Haemonchus contortus, Trichostrongylus species, and Oesophagostomum species are resistant to benzimidazole in the same farm,

**Dual Resistance:** When a farm raising cattle has worms that are resistant to one family of anthelmintics and another family of anthelmintics, for example, a farm where Haemonchus contortus is resistant to benzimidazole but Trichostrongylus species is resistant to levamisole.

**Reversion:** An originally resistant strain of the parasite reverts to being susceptible to an anthelmintic.

### 2.4 Mechanisms of anthelmintic resistance

Target site sensitivity, metabolic detoxification, or a change in drug transport can all lead to resistance in gastrointestinal worms (James *et al*., 2009) [45]. In general, drug resistance is linked to many genetic changes, and non-receptor-based

mechanisms frequently also play a role in the emergence of resistance (Beech *et al*., 2011) [9]. The main problem with this kind of resistance is that it may equally affect numerous types of medications with various modes of action and targets, which could undermine the efficacy of those drugs due to the altered pharmacokinetics (Lespine *et al*., 2012) [62]. Reduced sensitivity of C. elegans to LEV due to selection for IVM resistance indicates that cross-resistance to other anthelmintics to which the parasite has not been exposed may result from resistance to one anthelmintic due to non-receptor-based mechanisms (Ardelli and Prichard, 2008).

There are several ways resistance is supposed to arise (Wolstenholme *et al*., 2004). First off, a medication target that has been altered in a resistant nematode may make it impossible for the drug to attach or cause it to bind less strongly. Second, a change in the drug's metabolism could prevent it from being converted into its active form or cause it to be eliminated from its target sites. Thirdly, the drug's distribution within the parasite can alter, preventing it from reaching its intended spot. Fourth, the drug's effects can be blocked by a change in the target gene's expression. Alternately, there might be non-specific resistance mechanisms (i.e., a mechanism unrelated to the specific drug's mode of action), like alterations in the expression level of non-target proteins that the parasite uses to manage poisons and medications (Wolstenholme *et al*., 2004).

These mechanisms must be discernible as both quantitative and qualitative genomic alterations. Modification of the receptors that serve as the medications' target sites causes specific mechanisms related to AR, also known as targeted resistance, and this alters the drug's mode of action. Targeted resistance can result from several factors, including I single nucleotide polymorphisms (SNPs) or other genetic changes that affect the amino acid sequence of drug receptors and their affinity to bind drugs, (ii) altered ancillary proteins or other substances that affect receptor functionality, and (iii) modifications to regulatory elements that alter the expression level of receptors or ancillary proteins (Lespine *et al*., 2012) [62].

The mechanisms of resistance in worms are better known as a result of developments in molecular technology. According to James *et al*., (2007) and Westenholm *et al*., (2004), resistance in worms can result from a variety of mechanisms and can be roughly categorized as genetic changes in the drug target, changes in drug transport (for example, ATP-binding Cassette (ABC) transporters), or changes in the parasite's internal drug metabolism.

Different helminth species have different relationships between the aforementioned alterations and the occurrence of resistance. A mutation in the target site gene can cause benzimidazole resistance in nematodes, but it does not appear to cause triclabendazole resistance in the trematode Fasciola hepatica (Wilkinson *et al*., 2012) [100]. Additionally, many changes within a single worm species can result in resistance to a single anthelmintic. For instance, the phenylalanine to tyrosine substitution at amino acid position 200 of the isotype 1-tubulin gene has been implicated in the development of benzimidazole resistance in the worm Haemonchus contortus (Kwa *et al*., 1994) [57]. The incidence of this resistance point mutation (single nucleotide polymorphism, SNP) varies greatly, and it can even be low in populations of benzimidazole (BZ)-resistant individuals who also possess other mutations (James *et al*., 2007, Ghisi *et al*., 2007) [34].

Although genetic selection plays a role in resistance, various resistance mechanisms to the same anthelmintic can also be attributed to differences in drug transport pathways or drug metabolism within a worm species (Blackhall *et al*., 2008, Vokral *et al*., 2013) [10]. Avermectin, benzimidazoles, and derivatives of imidazothiazoles are only a few examples of the numerous medications that the P-glycoprotein, a cell membrane transport protein, can transport. This may increase the active transport of pharmaceuticals, which may promote the development of multi-drug resistance (James *et al*., 2007, Kerboeuf *et al*., 2003, Xu *et al*., 1995) [52]. The conclusion that can be drawn from this is that additional research is required to comprehend the mechanisms and create appropriate assays for the identification of resistance.

### 2.5 Risk Factors for Development of Anthelmintic Resistance

The contribution that the worms that survive treatment given to the subsequent generation are the most crucial element in the development of resistance in veterinary helminths to anthelmintics. The amount of worms in refugia, or the number of worms that are not exposed to the medications, is what determines this in turn. Once a particular level of resistance genes has been generated, additional treatments cause exponential growth in these resistance genes to the point where treatment failure occurs. Initially, the development rate of AR appears to be slow (Barnes *et al*., 1995; Sangster, 1999) [8, 79].

Drug resistance is more likely to develop the more aggressively parasites are treated with medications. There is no indication of reversion or loss of resistance once resistance is present in a parasite population (Andronicos *et al*., 2010) [4]. There has been extensive research on the dynamics of parasite selection for AR in sheep (Leathwick *et al*., 2009). These elements, which may be related to the parasite species, the infected host, pharmacological therapy, on-farm management, or the environment, function either independently or additively.

**Parasitic factor:** A population of parasites does not respond to treatment consistently due to their genetic variety (Vercruysse and Rew, 2002) [103]. Before the first administration of a medicine, it is assumed that resistance alleles already exist in the parasite population (Wolstenholme *et al*., 2004). A competing theory, however, proposes many sources of resistance via recurrent and spontaneous mutations (Skuce *et al*., 2010) [83]. Resistance develops more quickly if only one gene is implicated than if numerous genes are involved, even though the genetics of resistance are still poorly understood. Additionally, resistance emerges more quickly if the genes are dominant as opposed to recessive: both heterozygote and homozygote worms will endure the treatment and contribute to the following generation (Le Jambre *et al*., 2000 and Coles, 2004) [60, 5]. Additionally, some parasites have biological traits, such as direct life cycles, rapid generation times, and high fecundity that encourage resistance alleles to accumulate in the population more quickly. The spread of resistance in the population is predicted to rise if resistant parasites are more fit or if resistance is connected to other fitness genes. Fitness includes all traits that allow more worms to complete their life cycles, such as the rate of egg production, the worms' ability to remain in the host for an extended period, their ability to migrate on plants, and their ability to spread disease when ingested.

**Treatment Frequency**: The development of AR has been

seen to occur when the same class of anthelmintics is used frequently. There is proof that areas, where animals are regularly dewormed, see a faster development of resistance. In some humid tropical regions where 10 to 15 treatments per year were employed to control this parasite in small ruminants, anthelmintic resistance in Haemonchus contortus has been recorded. However, when the same medication is used repeatedly over a long time, drug resistance can also develop at lower treatment frequencies. Even when only two or three treatments were administered each year, Coles documented the emergence of AR.

**Mass Treatment**: The widespread emergence of AR in helminths has been facilitated by preventative mass treatments of domestic animals. Computer simulations suggest that when 20% of the flock is not treated, the emergence of resistance is postponed, but experimental verification is required. This strategy would guarantee that the offspring of the worms that survive the treatment will not only be resistant worms. Leaving a portion of the group untreated, particularly those with the lowest worm burdens shouldn't necessarily lessen the treatment's total effectiveness. Regularly transferring flocks to clean pastures following mass treatment and/or scheduling treatment during the dry seasons are frequent practices in livestock worm control to minimize rapid reinfection. The following generation of helminths produced by these acts, however, is nearly entirely made up of worms that have endured therapy and may therefore have a role in the emergence of AR.

**Management systems**: The epidemiology of gastrointestinal nematodes is significantly influenced by it. High stocking density raises the level of nematode eggs in the environment, which makes the infectious stages more accessible to susceptible animals. The traditional husbandry systems prevent a build-up of high worm burdens due to low stocking rates and extensive management systems. AR is primarily brought on by how frequently anthelmintics are used and how much is under-dosed (Van Wyk, 2001) [92]. Treatment and pasture management must be carried out to lessen the selection pressure.

Due to worms surviving short interval treatments, which puts selection pressure on AR, pasture contamination results. Summer drought is a changeable factor that clears out the free-living stages on pasture, and farmers should be mindful of this (Saeed *et al*., 2010) [78]. Additionally, to effectively dilute the progeny of survivors of the quarantine treatment, the bought-in fresh entry of the herd animals should be quarantined before they are placed on pasture (Pomroy, 2006) [72].

## 2.6 Methods of Detecting Anthelmintic Resistance

The most popular technique for identifying and tracking the presence of anthelmintic resistance in nematodes is the fecal egg count reduction test (FECRT), which is appropriate for all anthelmintics, even those that are metabolized by the host. Alternative methods of detection have also been developed, including several in vitro assays that track how anthelmintics affect the growth, development, or migration of worm stages. Both in vivo and in vitro methods can be used to detect the resistance to anthelmintics (Taylor *et al*., 2002) [89].

Detecting resistance at an early stage is just as crucial as creating new anthelmintics and modifying alternative tactics to halt the spread of resistance. For the detection of AR in different nematodes, numerous in vitro and in vivo assays have been created; however, each technique has drawbacks in terms of its usefulness in real-world settings, adaptability, repeatability, or sensitivity. Additionally, the majority of tests used to identify AR are for GINs in horses and livestock (Coles *et al*., 2006; Jabbar *et al*., 2006) [19, 43], while only a small number of in vitro tests have been created and modified for GINs in other species (Kotze *et al*., 2004; Kotze *et al*., 2005; Kopp *et al*., 2008) [54, 55, 53].

### 2.6.1 in Vivo Methods

**Faecal egg count reduction test**

One of the earliest techniques for determining anthelmintic efficacy involved comparing the number of worm eggs in a group of animals' feces before and after treatment (Presidente, 1985) [73]. The World Association for Advancement of Veterinary Parasitology (WAAVP) has advised using this test to track animal nematode resistance (Coles *et al*., 1992). Even now, anthelmintic resistance is determined using this technique (Coles *et al*., 1992) [92].

According to Waller (1986) [26] and Jackson *et al*., (2000), the FECRT is the most widely used technique for field or research projects (Coles *et al*., 1992; Wood *et al*., 1995) [91, 103]. This test is simple to conduct and appropriate for all anthelmintic kinds as well as ruminants, horses, and pigs. Additionally, it can be used on any nematode species whose eggs are shed in feces (Coles *et al*., 1992) [92]. The FECRT is a popular approach for evaluating anthelmintic efficacy; however, Palcy *et al*., (2010) [70] found that it had a low sensitivity for detecting AR in one nematode species in the setting of multi-species worm infections.

The FECRT's key benefit is that it is relatively inexpensive because it does not require highly qualified employees, significant resources, or advanced facilities and equipment. It can be used to identify resistance to all classes of anthelmintics in any kind of animal and can be carried out on a farm without involving the movement or slaughter of cattle (Presidente, 1985) [73]. The interval between the initial and subsequent collections of fecal samples varies depending on the type of anthelmintic.

**Table 2:** Collection time of faecal samples for FECRT anthelmintics.

| Anthelmintic group | Time before treatment (day 0) and 2nd egg count |
|---|---|
| Benzimidazoles | 8 – 10 days |
| Levamisole / Tetrahydropyrimidines | 3 – 7 days |
| Macrocyclic lactones | 14 – 17 days |

**Source:** (Coles *et al.*, 2006)

**Controlled efficacy test (CET)**

Animals are divided into two groups for this test; one group receives an infection with nematode larvae at the infective stage (L3) and is given an anthelmintic after 21 days, while the other group is kept as a control. 10-14 days following treatment, animals in the control and treatment groups are put to death, and their worm burdens are measured. If a series of increasing dose rates is applied across various treatment groups, it is possible to estimate the $LD_{50}$ or $ED_{50}$ of the medicine for the parasite. The WAAVP has provided instructions for conducting this test (Wood *et al*., 1995) [103]. The most accurate test for assessing anthelmintic effectiveness, but also the most expensive (Boersema, 1987) [12].

The test entails treating groups of worm-free animals at predetermined intervals, typically after 21 days, with single species third-stage infective larvae (when the parasite has reached the adult stage). 10 to 14 days after treatment, the animals are killed, including the control group, and their worm burdens are measured. The worm burdens are measured, and if a variety of dosages are used, the dose-response parameters ED 50 and ED 90 (concentration of drug that kills 50% and 90% of the nematode population, respectively) may be calculated (Hazelby *et al*., 1994).
The percentage efficacy of the treatment can be calculated using:

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

Although this is the most accurate technique for determining anthelmintics' effectiveness against mixed nematode infection, it is expensive in terms of the use of animals, lab time, and labor costs. As a result, it is only occasionally used for resistance testing (Taylor and Hunt, 1989) [88].
The most accurate test for evaluating AR against any kind of anthracycline is the CET. After sacrificing animals that had previously received anthelmintic treatment, it is based on the measurement of the intestinal nematode burden (Johansen, 1989) [46]. Through the slaughter of animals at various points after infection, CET is also beneficial for assessing the anthelmintic efficacy at various parasite development stages. Before the registration of a new medicine, this test must be performed without exception (Wood *et al*., 1995). The results of other tests, such as FECRT or other in vitro tests, have demonstrated a strong association with CET (Presidente, 1985) [73], hence it is used to confirm such results. When appropriate anthelmintic doses are used, it makes perfect sense (Johansen and Waller, 1989) [46], but the labor and animal costs make it impractical for routine AR detection (Boersema, 1983) [11].

### 2.6.2 In vitro Methods

**Egg hatch assay (EHA)**

The benzimidazole resistance can be routinely diagnosed using the egg hatch assay, which depends on the ovicidal activity of the medications. In contrast to susceptible strains, which were unable to survive and did not hatch the resistant strains of GI nematodes embryonated and hatched in higher concentrations of the medicines. It is advised to incubate nematode eggs in serial dilutions of the medications for 24 hours at 26°C temperature, add Lugol's iodine solution as a stain afterward, and then watch and count the number of eggs that hatch or die at various drug concentrations (Le Jambre, 1976; Coles and Simpkin, 1977; Hall *et al*., 1978; Johansen, 1989; Whitlock *et al*., 1980 and Folz, 1984) [59, 17, 37, 46, 99, 30].
The term "egg hatch assay" refers to a group of tests created to identify BZ resistance. They are predicated on the fact that BZs have an ovicide effect and those resistant strains' eggs can develop and hatch at higher BZ concentrations than susceptible strains' eggs can (Le Jambre, 1976; Coles and Simpkins, 1977) [59, 17].

**Egg hatch paralysis assay (EHPA)**

Levamiso1e (LEV) and morantel tartrate (MT) will be utilized with the EHPA, a revision of the earlier EHA (Dobson *et al*., 1986) [26]. Eggs are incubated as in EHA, but an anthelmintic is added right before hatching instead. Plates are read and an $ED_{50}$ is calculated following a 6-hour incubation period with the anthelmintic. Similar to the EHA, different $ED_{50}$ values have been noted based on the period of infection (Varady and Corba, 1999).

**Larval development assay (LDA)**

The larval development assay (LDA) is an in vitro test, and its basic premise is to incubate strongylid nematode eggs with media in serial dilutions of different anthelmintic drugs for 6-7 days at 20-26°C, after which the number of eggs and different larval stages (L1, L2, and L3) are counted after being stained with Lugol's iodine, and their proportion is calculated. The LD50 and dose-response curve can be calculated by comparing this to the number of controls in the wells. Ibarra and Jerkins (1984), Coles *et al*., (1988) [21], Taylor (1990) [87], Lacey *et al*., (1990) [58], Hubert and Kerboeuf (1992), and Gill *et al*., (1993) discuss the application of this test in real-world settings to identify anthelmintic resistance to various medications in nematode species (1995).
The incubation of nematode eggs to third-stage larvae in the presence of various anthelmintic concentrations forms the basis of the LDA. Either a liquid or solid (agar) nutritive media can be used for incubation. To detect AR against the major anthelmintic families, this approach is employed. The $LD_{50}$ (larval 50% death) has also been shown to vary for this test depending on the timing of infection, especially when macrocyclic lactones (ML) are utilized. When the day of infection was uncertain, there were no LD50 differences between ML-susceptible and ML-resistant bacteria (Gill *et al*., 1995; Amarante *et al*., 1997).

**Larval motility assay (LMA)**

To plot a dose-response line, the LMA calculates the proportion of infected third-stage larvae that are paralyzed when incubated in anthelmintic serial dilutions. Results must be compared to known susceptible strains because there is no threshold yet. Detecting Pyrantel resistance in dog hookworms has also been proposed as a potential application of the larval motility assay (Kopp *et al*., 2008) [53].
An in vitro test exhibiting the effects of BZ and IVM on human hookworms (*N. americanus*), dog hookworms (*A. caninum and A. ceylanicum*), and Strongyloides species has been developed (Kotze *et al*., 2004) [54]. The motility of each parasite species in response to anthelmintics varied significantly depending on the dose. The essay must be correlated with clinical responses among people infected with the same parasite strains but with diverse drug sensitivity for it to be useful in detecting resistance. The use of this test for the detection of AR is not widespread.

**Adult development test (ADT)**

One such in vitro procedure that has been evaluated is the cultivation of L3 on a nutritive medium for adult worms (Stringfellow, 1988; Small and Coles, 1993) [84]. Haemonchus contortus strains that are resistant to BZs have developed differently than those that are vulnerable to them (Stringfellow, 1988) [85]. Small and Coles (1993) [84] also found results that were comparable for BZs, but not for LM and closantel. This method is not appropriate for routine testing of AR because of the intricacy of the culture techniques and the requirement for a waiting period of about 21 days (time for reaching parasite maturity).

**Table 3:** In vivo and in vitro assays used in the detection of anthelmintic resistance

| No | Bioassays | Spectrum | Type of method detection |
|---|---|---|---|
| 1 | Controlled Test | All Drugs | In vivo BA |
| 2 | Larval Development | BZ, IV, LEV | In vivo BA |
| 3 | Larval paralysis | LEV, IV, | In vivo BA |
| 4 | Esterase Activity | BZ | In vivo BA |
| 5 | Tubulin Binding | BZ | In vivo BA |
| 6 | Egg Hatch Assay | BZ | In vivo BA |
| 7 | Tubulin Probe | BZ | In vitro G |
| 8 | Egg Hatch Assay (larval paralysis) | LEV | In vivo BA |
| 9 | Egg Count Reduction | All Drugs | In vivo BA |

BA-Bioassay; BC-Biochemical assay; G-Genetic assay; BZ-Benzimidazole; LEV- Levamisole; IV-Ivermectin
**Source**: Jackson, and Coop, 2000

### 6.3 Strategies to Prevent and Control of Anthelmintic Resistance

The creation and implementation of measures to stop the spread of anthelmintic resistance, particularly in nematodes of sheep and goats, and stop it from becoming a problem in cattle, are urgently needed (Waller, 1997) [97]. The following methods will assist maintain anthelmintic effectiveness and reduce the issue of drug resistance.

**Using full anthelmintic dosage:** To avoid overdosing on some animals, it is preferable to adjust the dosage for the animal that weighs the most rather than for the average animal in the group. Worms with partial resistance are likely to survive at lower dosages (heterozygotes). They might then mate with worms that are similar to them, giving rise to highly resistant progeny (homozygotes) (Hazelby *et al*., 1994) [40].

**Rotation of anthelmintics**: Because the frequent switching of anthelmintic types has historically resulted in the selection of multiple drug resistance, it is advised to rotate drugs every year from different chemical families (for example, avermectin, levamisole, and benzimidazoles).

**Avoiding high frequency of anthelmintic use**: treatment for depression By giving sheep a dose every two to four weeks, susceptible worms are eradicated, leaving only resistant worms in the pastures (Howard, 1993).

**Taking care in selecting the anthelmintics**: It would be a waste of time and resources to use the medicine if worms have already become resistant to it. This is when the fecal egg reduction test comes in handy. It's crucial to bear in mind that some drugs with interconnected effects that have similar results can also have negative side effects, such as resistance (Howard, 1993).

**Developing strategic treatment programs**: Fewer, epidemiologically based treatments will control worms just as effectively, be more cost-effective than ongoing treatments, and have less of a selection for drug resistance (Radostis *et al*., 1994).

**Synergism of anthelmintics**: Sometimes combining medications can synergistically boost their efficacy. For instance, experimental Mebendazole plus levamisole treatment in sheep in Australia boosted efficacy against benzimidazole-resistant worms. In the future, drug efficacy may be increased through the chemical alteration of already existing pharmaceuticals and new delivery technologies (Cabaret, 2000) [14].

**Genetically resistant hosts:** The selection of flocks with parasite resistance genes could be accelerated with the use of new embryo splitting and transfer techniques. Worm resistance appears to be highly heritable, and links between acquired resistance and specific lymphocyte antigen markers have been discovered (Eady *et al*., 1998) [28].

**Avoiding prolonged drug encounters**: This can happen when using devices like licks, blocks, or small-dose sustained-release rumen retention systems that gradually "tail off" the medication concentration. Due to its persistence at low quantities for several weeks following therapy, it might also happen with avermectin (Susan *et al*., 1998) [86].

### 6.4 Current Status of Anthelmintic Resistance in Ethiopia

In different regions of Ethiopia, different anthelmintics have been used to treat helminth parasites in sheep and goats. Anthelmintics have been used for a very long period, and they account for a sizable portion of the country's costs associated with helminthiasis control. Also common in the nation is the misuse and trafficking of veterinary medications that contain anthelmintics. Some of these medications, most notably albendazole and tetramisole, have been consistently imported and supplied across the nation under various trade names and by various producers (Amante, 2020) [2].

Regarding the status of anthelmintic resistance in agricultural animals, very few and inconsistent reports are known. Oesophagostomum, Bunostomum, and Trichuris parasites of goats in Adami Tullu have developed anthelmintic resistance to tetramisole, despite the study's constrained and confined scope (Nessru *et al*., 1997) [68]. In a study conducted in the Southern region of Ethiopia, Kassahun (1997) [51] found suspicion of resistance in small ruminant nematodes.

In addition, Daniel, 1998 noted the prevalence of albendazole resistance in the nematodes of Stella State Farm crossbreed animals and moderate resistance in native Zebu cattle raised in large numbers in the Sebeta cities. For the treatment and management of helminth parasites in farm animals, anthelmintics are widely utilized across the nation. Due to the large number of illegal dealers and the non-professionals that sell anthelmintics as common drugs on the open market, drug smuggling and incorrect use of anthelmintics are quite frequent throughout the nation (Nessru *et al*., 1997) [68].

**Table 4**: Summary of some of anthelmintic suspected for resistance in Ethiopia

| Study area | Authors | Anthelmintics suspected for resistance | Anthelmintics used | Nematode parasites reported |
|---|---|---|---|---|
| Bedelle zone | Terefe *et al.*, 2013 | None | Albendazole, Tetramisole, Ivermectin | *Haemonchus* spp in sheep |
| Sidama Zone, Dale District | Desie, *et al.*2013 | Albendazole | Albendazole, Tetramisole, Ivermectin | Mixed GIT Parasite |
| Woliata sodo | Sheferaw, *et al.*2010 | None | Albendazole, Tetramisole And ivermectin | |
| Hawassa | Kumsa and Abebe,(2009) | Albendazole, Tetramisole and Ivermectin | Albendazole, Tetramisole and Ivermectin | *Haemonchus* in Goat |
| | | Ivermectin | Albendazole, Tetramisole and Ivermectin | *Teladorsagia* in Goats |
| Haramaya | Wondimu, 2022 | Albendazole, Tetracloza, Ivermectin | Albendazole, Tetracloza, Ivermectin | *Trichostrongylus* spp, *Teladorsagia* spp, *Haemonchus* spp in Goat |
| Dabat district, North west Ethiopia | Seyoum *et al.*, 2017 | Albendazole, Ivermectin | Albendazole, Tetramisole, and Ivermectin | *Haemonchus and Trichostrongylus* in sheep |
| Gondar, North West Ethiopia | Seyoum, Zewdu, *et al.*, 2017 | Febendazole,Ivermactin | Febendazole,Ivermectin | *Strongyle* Spp in Horses |
| Sebeta, Central Ethiopia | Bahiru *et al.*, 2017 | Ivermectin | Albendazole,Tetramisole,Ivermectin | *Haemonchus, Trichuris and Ostertagia* |

**Source:** Compiled by Author from Publication

## 3. Conclusion and Recommendations

The lack of clear information on anthelmintic efficacy, susceptibility, or resistance on a regional and national basis, the lack of livestock owners and public awareness about the impact of anthelmintic resistance on the economy of the country, the lack of a functional drug use policy, the lack of effective drug quality control, and drug smuggling are just a few of the reasons why anthelmintic resistance warrants urgent attention in the current Ethiopian context. Therefore, everyone from the producer to the last person giving medication to animals could be to blame for the development of anthelmintic resistance.

Studies on drug resistance can help to lessen its impact, and there are methods to improve the effectiveness or longevity of some medicines. When determining the prevalence of resistance in particular geographic areas or when treating individuals who have parasitic infections with resistant organisms, the ability to identify resistance can be helpful.

The following suggestions are therefore made in light of the aforementioned conclusion:

- To avoid seasonal contamination buildup from parasites, treatment schedules should be planned.
- Avoiding Underdosing and making sure that therapies are completely effective are critical.
- To properly administer anthelmintics, veterinarians should be assigned to each veterinary facility.
- At all levels, there should be a focus on the issues of helminthiasis and anthelmintic resistance.
- Raising public awareness of the issue of helminthiasis and the possibility of anthelmintic resistance.
- It is necessary to create a practical drug policy.
- Anthelmintics should be taken on a yearly rotational basis.
- Animals should receive a precise dosage of their medications.

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