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Study on Anthelmintic Resistance in Small Ruminants' Intestinal Nematodes

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Abstract

Globally, gastrointestinal nematode parasitism significantly reduces sheep and goat production. Farmers mostly rely on synthetic medications to manage gastrointestinal parasites, resulting in a growing reliance on anthelmintics. Still, long-term and inappropriate use, incorrect administration, heavy use, and higher treatment frequencies have led to the development of anthelmintic resistance, including multidrug resistance, in several groups of gastrointestinal nematodes. If a parasite is genetically modified to be less able to handle the therapeutic dose of an anthelmintic drug, this is called anthelmintic resistance. The issue of anthelmintic resistance in Ethiopia is a significant concern, with frequent reports from various regions of the country. Unfortunately, the rural population lacks awareness regarding this problem of anthelmintic resistance. The objective of this research was to provide a comprehensive analysis and contextual information on anthelmintic resistance. Anthelmintic resistance mechanisms involve gene changes that reduce medicine sensitivity, decreased receptor numbers, and the absence of activating enzymes. Amino acid mutations or deletions in target genes can cause changes, and various techniques, including in vivo and in vitro methods, have been used to identify and track anthelmintic resistance.

Keywords:

Anthelmintic Resistance, Nematodes, Risk Factors, Small Ruminants.

Introduction:

Anthelmintic resistance in small ruminants' gastrointestinal nematodes is posing a significant challenge in veterinary medicine and livestock production. Sheep and goats frequently suffer from gastrointestinal nematodes like *Haemonchus contortus*, *Ostertagia* spp., and *Trichostrongylus* spp., which can cause disease and potentially lead to financial losses. (Wondimu and Bayu, ٢٠٢٢).

As a result, the parasites have evolved genetic mechanisms to survive and reproduce despite the presence of the medicine, so rendering the therapy ineffective.(Olanrewaju et al., ٢٠٢٣).

Resistance to anthelmintic drugs is an enormous issue that affects the standard of production for small ruminants. Since this problem has become more prevalent around the world, there is a greater need for alternative anti-helminthic methods, especially those that employ native economic species or treatments to lessen the damage. (Domke et al., ٢٠١٢).

Anthelmintics are medicines that are used to get parasitic worms by either making them incapable of doing their task or killing them. They are alternatively referred to as vermifuges or vermicides. A diverse array of medications exists that can be categorized into several classes according to their similar chemical structures and methods of action(Walker et al., ٢٠٢٢) (Table ١).

Benzimidazoles and Thiabendazole was the inaugural benzimidazole anthelmintic agent synthesised. Since its release in ١٩٦١, many benzimidazoles with enhanced effectiveness and a broader range of activity have been discovered(Al-Fatlawi, ٢٠١٩).These medications consist of mebendazole, albendazole, and febendazole. At first, it was thought that benzimidazoles worked by stopping parasite metabolic enzymes like fumarate reductase and malate dehydrogenase from doing their jobs(de Andrade Picanço et al., ٢٠١٩).

Imidazothiazoles such as levamisole and tetramisole function as agonists of the nicotinic acetylcholine receptor (nAChR). They bind to nicotinic acetylcholine receptors (nAChRs) present on the skeletal muscles of the body wall. As a consequence, the worm experiences spastic paralysis, which ultimately leads to its expulsion from the host. Tetra-hydro-pyrimidines function in a manner similar to imidazothiazoles, and they are often referred to as nicotinic agonists.(Macedo et al., ٢٠٢٣).

Pyrantel, Oxantel, and Morantel are examples of medications belonging to the anthelmintic class. These medications function by acting as agonists on nicotinic receptors, resulting in

spastic muscular paralysis. This paralysis occurs due to the medicines' ability to extend the activation of excitatory nicotinic acetylcholine (nAch) receptors in muscle. (Walker et al., ٢٠٢٢). Macrocyclic lactones, namely avermectins, and milbemycins, are chemical substances derived from soil bacteria of the Streptomyces species. MLs were first developed in the ١٩٨٠s as antiparasitic drugs with broad efficacy against nematodes and arthropods. (Imai, ٢٠٢٠).

Ivermectin, abamectin, doramectin, and selamectin are commercially available avermectins. However, milbemycin oxime and moxidectin are milbemycins that may be purchased commercially. MLs are selective agonists that specifically bind to glutamate-gated chloride channels (GluCls). These channels are present in the neurons and pharyngeal muscles of worms and arthropods, but they are absent in humans. (Table ١).

Classes of Anthelmintic resistance	Mode of action
Benzimidazoles: Albendazole, Fenbendazole, Thiabendazole, Oxfendazole (Probenzimidazoles) Febental, Netobimin	Disruption of microtubules
Imidazothiazoles: Levamisole, Tetramisole	Nicotinic acetylcholine receptor agonists
Tetrahydropyrimidines Morantel, Pyrantel	
Macrocyclic lactones: Avermectins: Ivermectin, Doramectin, Eprinomectin, Abamectin Milbemycins:- Milbemycin, Moxidectin	Glutamate-gated chloride channel agonists

Table ١: Summary of Classes of Anthelmintics with their mode of action (Harder, ٢٠٠٣)

Anthelmintic Resistance and its Historical Background

The earliest evidence of AR was reported in the United States, involving the administration of the medication phenothiazine to sheep (Kaplan, ٢٠٢٠). Within the categories of anthelmintic treatments being utilized, benzimidazoles were the initial medications to exhibit a decline in efficacy against nematodes found in small ruminants. In sheep, thiabendazole was the first

compound to be documented as losing its effectiveness. The initial documented occurrence of resistance to benzimidazoles in goats took place in the ١٩٨٠s (Batista et al., ٢٠٢٣).

Anthelmintic resistance refers to the reduction in the effectiveness of a medicine used to treat parasitic infections, specifically in a population of parasites that were previously susceptible to the drug. Given that anthelmintics within each drug class function comparably, the development of resistance to one anthelmintic in a particular drug class is expected to be accompanied by resistance to other anthelmintics within the same class (cross-resistance) (Mondragón-Ancelmo et al., ٢٠١٩).

Resistance, in clinical terms, is defined as a test result showing a reduction of ٩٥٪ or less in a "fecal egg count(Kaplan et al., ٢٠٢٣).. The potential to enhance productivity in ruminants by managing helminth parasites relies on the presence of affordable and efficient anthelmintic treatments(Kotze and Hunt, ٢٠٢٣).Variable degrees of resistance have been observed in different species of gastrointestinal nematodes for all major categories of anthelmintic medications. Several things have been linked to the widespread development of anthelmintic resistance in helminths, such as using the same group of drugs too often, not giving the right number of drugs, treating large groups of small ruminants for prevention, and using the same drug over and over again (Raza, ٢٠١٩).

Anthelminthic	**Locality**	**Host**	**Nematodes population involved**
Albendazole	Shashemene CE	Goat	*Haemonchus spp*
	Mojo CE	Sheep	
	Chacha CE	Sheep	
	Sheno CE	Sheep	
	Haramaya university EE	Goat	
	DSBIC CE	Sheep	
	Zeway goats CE	Goat	
	Sidama - WE	Sheep	
	Sidama - WE	Goat	
	Hawasa - SE	Sheep	
	Hawasa University - SE	Goat	
	Hawasa University - SE	Goat	*Haemonchus, Oesophagostomunan Trichostroglus*

	Haramaya university- EE	Goat	*Haemonchus spp*
Tetramisole	Ziway goats - CE	Goat	*Nematodes & Trichuris*
	Gondar – NE	Sheep	*Trichuris, Haemonchus, Oesophagostomun*
	Hawasa University - SE	Goat	*Haemonchus,Oesophagostomun and Trichostrongylus*
	Haramaya university -EE	Goat	*Haemonchus spp.*
Levamisole	Zeway – CE	Goat	*Haemonchus spp.*
	Haramaya university-EE	Goat	*Haemonchus spp.*
	Gondar – NE	Sheep	*Trichuris, Haemonchus, Oesoph-agostomun spp.*
Ivermectin	Hawasa University - SE	Goat	*Heamonchus and trichostrongylus spp.*
	Haramaya university - EE	Goat	*Haemonchus spp.*

Table ٢: Anthelmintic drugs for ruminants and the development of resistance to the drug (Harder, ٢٠٠٣).

Mechanism of Anthelmintic Resistance

An anthelmintic is a medicine or drug utilized to treat or prevent disease caused by parasitic worms. Resistance mechanisms include genetic alterations such as deletions or mutations in the target genes, reduced receptor abundance, receptors with less affinity for medications, and the absence of bioactivating enzymes. (Choudhary et al., ٢٠٢٢).

The development in molecular technology has resulted in a more profound comprehension of the mechanisms of resistance in worms. Resistance in worms may originate from many mechanisms and can be categorized as genetic modifications in the drug target, drug transport, or drug metabolism. (von Samson-Himmelstjerna et al., ٢٠٢١). The cause of resistance in worms is often complex. The resistance of nematodes to ben-zimidazoles may be ascribed to a mutation in the gene that governs the target site, as shown by the aforementioned mutation. (Beesley et al., ٢٠٢٣). Even among individuals of the same worm species, several mutations can confer resistance to the same anthelmintic.

In *Haemonchus contortus*, benzimidazole resistance is often caused by a change at amino acid position ٢٠٠ of the isotype one be/a-tubulin gene. More specifically, phenylalanine is swapped for tyrosine. However, the frequency of this significant mutation that leads to resistance can vary greatly and may even be low in populations that are resistant to benzimidazole. In

addition to point mutations, populations resistant to benzimidazoles (BZ) can possess other mutations that confer BZ resistance (Kalule et al., ٢٠٢٣).

Moreover, variations in drug transportation or drug metabolism within a particular species of worm are responsible for distinct resistance mechanisms against the same anthelmintic. Conversely, as P-glycoprotein can transport other medications such as ivermectin, benzimidazoles, and imidazothiazole derivatives, alterations in this protein could potentially result in resistance to multiple additional treatments. Passive diffusion is the primary method by which BZs enter parasites, distinguishing them from other anthelmintics. The ability of these molecules to dissolve in lipids plays a crucial role in determining their diffusion through the parasite's outer covering, known as the tegument (Lalthanpuii and Lalchhandama, ٢٠٢٠).

Benzimidazoles (BZs) work by attaching strongly and specifically to the beta subunit of tubules, a microtubule protein found in helminths. This attachment results in a disturbance of the balance between tubules and microtubules. Benzodiazepines (BZs) attach to empty beta tubules and stop the formation of alpha and beta tubule molecules. They also stop glucose absorption, which depends on microtubules. This leads to immobility and demise. The parasite's beta tubules have undergone molecular changes that are the cause of the resistance to BZs. Some types of tubules were destroyed during the process of selecting for resistance, which led to a drop in the number of high-affinity BZ-binding sites(Fairweather et al., ٢٠٢٠). Helminths mostly develop drug resistance through receptor loss or a reduction in the target site's affinity for the medication. There are GluCl channels on the membranes of the pharynx, somatic muscle, and some neurons of helminths that are controlled by MLs (Hedtke et al., ٢٠٢٠).

IVM enters the nematode through sensory (amphidial) neurons found in the cephalic region of the nematode [٤٧]. Upon entering the cuticle, it selectively focuses on three groups of alpha subunits of GluCl channels [٤٨]. The resistance is attributed to the drug efflux pump found in P-glycoprotein. GluCl channels, which are present in insects, worms, and crustaceans but absent in vertebrates, share a similar sequence. They are likely homologous to the subunit A of gamma-aminobutyric acid (GABAA) receptors(Gao et al., ٢٠٢٢).

Risk Factors for the Development of Anthelmintic Resistance

The main thing that determines how resistant veterinary helminths become to anthelmintic drugs is how many worms that survive treatment are passed on to the next generation. This, in turn, relies on the quantity of worms in refugia, which refers to the number of worms that are not subjected to the medications (dos Santos et al., ٢٠٢٢).

The parasites have a restricted range of genetic variation. When a population of parasites has little genetic variety, there is a higher probability that certain individuals will have genetic features that provide resistance to anthelmintics. This phenomenon might arise due to kinship, the introduction of a limited number of resilient parasites through migration, or other causative reasons.(Ralaingita et al., ٢٠٢٢). Specific geographical areas may demonstrate elevated levels of resistance as a result of local circumstances, including climate, agricultural methods, and the presence of particular parasites(Ma et al., ٢٠٢١).

Administering treatment to asymptomatic animals can lead to the development of resistance. Animals without symptoms may have fewer parasites, and providing them with treatment that is not needed can provide a selective force on the parasite population, promoting the survival of individuals who are resistant to treatment (Deguine et al., ٢٠٢١) (Figure ١).

Figure ١: Improper administration of drug (Robinson, ٢٠٢٣).

Methods of Detecting Anthelmintic Resistance

The Fecal Egg Count Reduction Test (FECRT) involves comparing the number of parasite eggs in fecal samples collected before and after treatment. A decrease in egg number can help you figure out how well an anthelmintic treatment is working. An egg count under a certain level is a significant effective therapy, whereas a small decrease suggests the existence of resistance.

The Fecal Egg Count Reduction Test (FECRT) is a widely used and practical method for detecting resistance at the population level in a group of animals, such as a herd or brood.(Kholik et al., ٢٠١٩).The larval Development Assay (LDA) creates parasite eggs or larvae from feces at various anthelmintic doses. This test assesses parasites 'ability to change into transmissible larvae despite the use of drugs. Latent Dirichlet Allocation (LDA) can indicate parasites' anthelmintic sensitivity and early resistance..(Feyera et al., ٢٠٢٢).

It is possible to find genetic markers that are linked to protection against drugs used to treat parasites using molecular techniques and methods. PCR and DNA sequencing are two ways to find specific genetic changes or mutations in target genes of parasites that make them resistant to some medicines. It is possible to find resistance at the level of the individual parasite with these genetic methods. They may give us important information about how often and why resistance happens..(Kotze et al., ٢٠٢٠).

Figure ٢: McMaster egg counting slide for FECRT.

Studying molecular techniques and anthelmintic resistance mechanisms

Initially, molecular techniques for studying AR were limited to BZ-susceptible and resistant genotypes. The tests employed the resistance-associated single nucleotide polymorphism (SNP) located in codon ٢٠٠ of the beta-tubulin isotpye ١, as mentioned in the preceding section. These tests were originally designed for the detection of benzimidazole (BZ) resistance in small ruminant *Trichostrongyles* sp.. Allele-specific PCR-based tests were described to determine the genotype of *Haemonchus contortus* . (Avramenko et al., ٢٠١٩).

Molecular tools provide the chance to address some limitations. The polymerase chain reaction (PCR) technique has provided new opportunities for research in the field of veterinary parasitology, as it does in many other areas. Multiple PCR techniques have been devised that offer exceptional precision and sensitivity for studying individual worms. However, obtaining relevant results relies on testing a sufficient number of individuals (e.g. ١٠٠) from the specific isolate being studied. Due to their high cost, these tests have primarily been utilised in epidemiological surveys or foundational investigations. This signifies a significant limitation of the existing technology. A crucial problem is to create molecular tests that are appropriate for regular usage and can evaluate the resistance level of a parasite population using a combined DNA sample.(Scare et al., ٢٠٢٠).

Development monitoring and molecular technologies like RNA sequencing (RNA-seq) or quantitative real-time PCR can measure gene expression in immune and susceptible parasites. Comparing gene expression in resistant parasites may help scientists identify which genes are up or down. Knowing this helps uncover drug resistance genes like drug release pumps or cleaning enzymes.(Xue et al., ٢٠٢٠).Functionality of resistance-linked potential genes is assessed molecularly. Functional testing may confirm their resistance. Gene knockdown or overexpression studies can use RNA interference (RNAi) or gene editing. (CRISPR-Cas٩) (Nemati, ٢٠١٩).

Conclusion

Anthelmitic-resistant intestinal worms in small ruminants complicate veterinary therapy and livestock rearing. They increased treatment resistance in *Oatertagia*, *Haemonchus contortus*, and *Trichostrongylus* spp. Improves disease spread, farm losses, and the productivity of livestock. Parasite treatment and other methods must be conducted immediately to keep small livestock farms healthy and competitive**.**

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