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A Review of the Study on Anthelmintic Resistance in Small Ruminants' Intestinal Nematodes

A Review Article in

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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 include changes in the genes that make receptors less sensitive to medicines, a drop in the number of receptors, and the absence of activating enzymes. These changes can happen because of amino acid mutations or deletions in the target genes. Various techniques, including in vivo and in vitro methods, have been employed to identify and track anthelmintic resistance.

Keywords: Anthelmintic Resistance, Nematodes, Risk Factors; Small Ruminants.

Introduction:

The development of anthelmintic resistance in small ruminants' gastrointestinal nematodes is becoming worrisome in veterinary medicine and livestock production. Gastrointestinal nematodes, such as *Haemonchus contortus*, *Ostertagia* spp., and *Trichostrongylus* spp., are prevalent parasites that can induce illness and result in financial losses in sheep and goats(Wondimu and Bayu, 2022).

Anthelmintics, often known as dewormers, have traditionally been employed to manage and treat gastrointestinal nematode infections in small ruminants. Nevertheless, the persistent and frequently unselective utilization of these medications has resulted in the development of anthelmintic resistance. Consequently, the parasites have developed genetic mechanisms to endure and procreate despite the drug's presence, so nullifying the efficacy of the treatment(Olanrewaju et al., 2023).

The successful control of gastrointestinal parasites in grazing animals depends on the judicious administration of very effective chemotherapy. Farmers mostly utilize synthetic medications to manage gastrointestinal parasites, resulting in a growing reliance on anthelmintics. Nevertheless, the prolonged and unselective utilization, inaccurate administration, extensive application, and heightened frequency of therapy result in anthelmintic resistance, including multi-drug resistance, in several populations of gastrointestinal nematodes(Mohan et al., 2015).

Anthelmintic medication resistance is a significant issue that adversely affects the quality of small ruminant production. Due to the recent global development of this problem, there is a growing need for alternate anthelminthic approaches, particularly those involving the utilization of indigenous economic plants or treatments to minimize the damage (Domke et al., 2012).

Anthelmintics are widely employed medications in the field of veterinary medicine. Anthelmintics are pharmaceutical substances employed for the treatment of infections caused by parasitic worms, often known as helminths(Cortez-Maya et al., 2020). Anthelmintics are pharmaceutical substances employed to eliminate parasitic worms by either incapacitating or exterminating 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., 2022) (Table 1).

Benzimidazoles and Thiabendazole was the inaugural benzimidazole anthelmintic agent synthesised. Since its release in 1961, many benzimidazoles with enhanced effectiveness and a broader range of activity have been discovered(Al-Fatlawi, 2019).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., 2019). The benzimidazoles are now proven to have a strong and specific attraction to the parasite β-tubulin. This stops the microtubules from joining together. The outcome of this process is the disruption of cellular organization and the subsequent demise of the parasite (Yilmaz, 2019).

Imidazothiazoles such as levamisole and tetramisole function as agonists of the nicotinic acetylcholine receptor (nAChR). They attach to nicotinic acetylcholine receptors (nAChRs) located on the muscles of the body wall. This leads to spastic paralysis of the worm, resulting in its removal from the host. Tetrahydropyrimidines work in a way that is similar to imidazothiazoles, and they are often called nicotinic agonists(Macedo et al., 2023).

Examples of drugs in the anthelmintic class are Pyrantel, Oxantel, and Morantel. These drugs work by acting as agonists on nicotinic receptors, causing spastic muscular paralysis. This paralysis occurs because the drugs prolong the activation of excitatory nicotinic acetylcholine (nAch) receptors in muscle (Walker et al., 2022). Macrocyclic lactones, specifically avermectins and milbemycins, are chemical compounds that originate from soil microorganisms belonging to the genus Streptomyces. MLs were first developed in the 1980s as antiparasitic drugs that have a wide range of effectiveness against nematodes and arthropods (Imai, 2020).

Ivermectin, abamectin, doramectin, and selamectin are commercially accessible avermectins. On the other hand, milbemycin oxime and moxidectin are commercially available milbemycins. MLs are specific agonists that target glutamate-gated chloride channels (GluCls). These channels are found in the neurons and pharyngeal muscles of nematodes and arthropods, but they are not present in humans (Table 1). The activation of GluCls by ML suppresses both movement and pharyngeal

pumping. Avermectins not only stop GluCl from working, but they also stop nicotinic and 4-aminobutyric acid (GABA) receptors in the muscle cells of parasitic worms(Wolstenholme and Neveu, 2022).

Table 1: Summary of Classes of Anthelmintics with their mode of action (Harder, 2003)

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

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, 2020). Within the categories of anthelmintic treatments being utilized, benzimidazoles were the initial medications to exhibit a decline in efficacy against nematodes found in small ruminantutilizeds. 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 1980s (Batista et al., 2023) .

The initial documentation of the diminished efficacy of ivermectin in sheep was published in South Africa, and subsequent findings emerged in Brazil shortly after that. With time and due to the utilization of novel pharmaceuticals, there have been multiple documented instances of resistance on a global scale, particularly in nations that have a history of selectively breeding small ruminant animals. Due to the lack of new drug releases and the growing prevalence of AR in small

ruminants, the practice of combining medicines with distinct modes of action has been employed to postpone the emergence of resistance (Geurden et al., 2022).

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 (Mondragón-Ancelmo et al., 2019). 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).

There is a possibility that anthelmintics belonging to one drug class may develop cross-resistance to those of another drug class if the two drug classes have comparable targets(Zekarias and Toka, 2019). Anthelmintic resistance refers to the capacity of worms to withstand therapies that are often successful when administered at the correct dosage. This phenomenon is seen as a significant risk to the future management of worm parasites in small ruminants. The primary approach to mitigating the negative consequences of these nematode parasites is through the use of anthelmintics. However, the effectiveness of these drugs is limited due to the advent of anthelmintic resistance (Ahmad et al., 2021).Resistance, in clinical terms, is defined as a test result showing a reduction of 95% or less in a "fecal egg count(Kaplan et al., 2023).. The potential to enhance productivity in ruminants by managing helminth parasites relies on the presence of affordable and efficient anthelmintic treatments(Kotze and Hunt, 2023).

However, the emergence of anthelmintic resistance poses a significant global challenge to livestock production. 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, 2019).

Anthelminthic	Locality	Host	Nematodes population involved	Source
Albendazole	Shashemene CE	Goat	*Haemonchus spp*	[82]
	Mojo CE	Sheep		
	Chacha CE	Sheep		
	Sheno CE	Sheep		
	Haramaya university EE	Goat		
	DSBIC CE	Sheep		
	Zeway goats CE	Goat		[83]
	Sidama - WE	Sheep		[13]
	Sidama - WE	Goat		
	Hawasa - SE	sheep		[84]
	Hawasa University - SE	Goat		[85]
	Hawasa University - SE	Goat	*Haemonchus, Oesophagostomun and Trichostrongylus*	
	Haramaya university- EE	Goat	*Haemonchus spp*	[86]
Tetramisole	Ziway goats - CE	Goat	*Nematodes & Trichuris*	[83]
	Gondar – NE	Sheep	*Trichuris, Haemonchus, Oesophagostomun*	[87]
	Hawasa University - SE	goat	*Haemonchus, Oesophagostomun and Trichostrongylus*	[85]
	Haramaya university -EE	goat	*Haemonchus spp.*	[86]
Levamisole	Zeway – CE	goat	*Haemonchus spp.*	[83]
	Haramaya university-EE	goat	*Haemonchus spp.*	[82]
	Gondar – NE	sheep	*Trichuris, Haemonchus, Oesophagostomun spp.*	[87]

Ivermectin	Hawasa University - SE	goat	*Heamonchus and trichostrongylus spp.*	[81]
	Haramaya university - EE	goat	*Haemonchus spp.*	[86]

Table 2: Anthelmintic drugs for ruminants and the development of resistance to the drug (Harder, 2003).

Mechanism of Anthelmintic Resistance

An anthelmintic is a substance or medication used to treat or prevent infections brought on by parasitic worms. Genetic changes like deletions or mutations in the target genes, fewer receptors, receptors that don't bind as well to medicines, and the lack of bioactivating enzymes are all examples of resistance mechanisms (Choudhary et al., 2022).

Advancements in molecular technology have led to a deeper understanding of the processes of resistance in worms. Resistance in worms can arise from several pathways and can be classified as genetic alterations in the drug target, drug transport, or drug metabolism (von Samson-Himmelstjerna et al., 2021). The etiology of resistance in worms is frequently intricate. The resistance of nematodes to benzimidazoles can be attributed to a mutation in the gene responsible for the target site, as mentioned in the same mutation (Beesley et al., 2023). 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 200 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., 2023).

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, 2020).

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., 2020).

There are several aspects to the problem of LEV and imidazothiazole/tetrahydropyrimidines like pyrantel and morantel not working as well as they should. The specific location that these nicotinic agonists target is a pharmacologically unique channel known as the nAChR channel in nicotinic receptors. When the drug efflux pump P-glycoprotein is overexpressed in midazothiazole, it actively flushes out drugs, which makes the cancer resistant to treatment(McHugh et al., 2020). 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., 2020).

IVM enters the nematode through sensory (amphidial) neurons found in the cephalic region of the nematode [47]. Upon entering the cuticle, it selectively focuses on three groups of alpha subunits of GluCl channels [48]. 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., 2022).

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., 2022).

Frequency of treatment and repetitive use of a certain category of anthelmintic drugs have been found to lead to the emergence of anthelmintic resistance. The ability of parasitic worms, especially nematodes, to live and reproduce even when anthelmintic drugs are used is called anthelmintic resistance. Multiple risk factors contribute to the emergence and dissemination of anthelmintic resistance, including Excessive and improper utilization of anthelmintics(Timi and Buchmann, 2023).

The excessive or incorrect utilization of anthelmintic medications, such as delivering them too frequently or utilizing inadequate doses, can elevate the likelihood of developing resistance. This occurs because the parasites that can survive treatment as a result of genetic resistance qualities can transmit these traits to their progeny, resulting in the emergence of resistant populations(Doyle et al., 2022).

Administering anthelmintic medications at doses below the prescribed levels can contribute to the emergence of drug resistance. Inadequate dosage may fail to completely eradicate all parasites completely, enabling the survival and reproduction of resistant individuals(Fissiha and Kinde, 2021).

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., 2022) Need for more availability of diagnostic tools and monitoring equipment: Inadequate utilization of diagnostic methods for identifying and monitoring anthelmintic resistance can impede prompt action. Implementing effective control tactics becomes problematic without knowledge of the prevalence and extent of resistance. Cross-resistance refers to the

phenomenon where an organism that has developed resistance to one substance or treatment also exhibits resistance to other substances or treatments with similar mechanisms(Loya, 2022).

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., 2021). Regions characterized by elevated parasite loads and regular administration of anthelmintic drugs are more susceptible to the emergence of resistance—management of animals without symptoms.

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(Packard, 2021) (Figure 1).

Genetic predisposition refers to the inherent tendency of some animal species or breeds to possess resistance to or tolerance to specific parasites(Pal and Chakravarty, 2019). In such instances, the likelihood of resistance development may be elevated if anthelmintics are administered without taking hereditary variables into account. To reduce the likelihood of anthelmintic resistance, it is essential to establish strategic parasite control programs that encompass appropriate administration of medication, focused treatment, effective management of pastures, and consistent monitoring of parasite populations.

Hence, it is imperative to adopt comprehensive parasite control methods that take into account these aspects and advocate for sustainable management techniques to minimize the emergence of resistance and maintain the effectiveness of anthelmintic medications. It is crucial to emphasize that biosecurity measures must be customized to suit the particular circumstances and hazards of each farm or facility. It is creating an all-encompassing biosecurity strategy in collaboration with a veterinarian or animal expert(Deguine et al., 2021).

Figure 1: Improper administration of drug (Robinson, 2023).

Methods of Detecting Anthelmintic Resistance

Detecting anthelmintic resistance is crucial for implementing appropriate control measures and preserving the effectiveness of anthelmintic drugs. Several methods are available to assess and monitor anthelmintic resistance. Here are some commonly used approaches:

The fecal Egg Count Reduction Test (FECRT) involves comparing the number of parasite eggs in pre- and post-treatment fecal samples. It helps determine the efficacy of an anthelmintic treatment by assessing the reduction in egg counts. A decrease in egg counts below a certain threshold indicates effective treatment, while a minimal reduction suggests resistance. FECRT is a practical and widely used method for detecting resistance at the herd or flock level(Kholik et al., 2019).

Larval Development Assay (LDA) involves incubating parasite eggs or larvae from fecal samples in the presence of different concentrations of anthelmintic drugs. It assesses the ability of the parasites to develop into infective larvae despite exposure to the drug. LDA can provide insights into the susceptibility of parasites to specific anthelmintics and detect resistance at an early stage(Feyera et al., 2022).

Molecular techniques and methods can detect genetic markers associated with anthelmintic resistance. Polymerase Chain Reaction (PCR) and DNA sequencing techniques are used to identify

specific genetic mutations or changes in target genes of parasites that confer resistance to particular drugs. These molecular techniques enable the identification of resistance at the individual parasite level and can provide valuable information on the prevalence and mechanisms of resistance(Kotze et al., 2020).

Figure 2: McMaster egg counting slide for FECRT.

Studying molecular techniques and anthelmintic resistance mechanisms

Molecular techniques play a crucial role in understanding the mechanisms of anthelmintic resistance by providing insights into the genetic and molecular changes that contribute to resistance.Molecular techniques such as polymerase chain reaction (PCR) and DNA sequencing allow for the identification and characterization of specific genetic markers associated with anthelmintic resistance(Avramenko et al., 2019). By comparing the genetic profiles of resistant and susceptible parasites, researchers can pinpoint the genetic mutations or alterations that confer resistance. These markers can be located in various genes, including those encoding drug targets, drug transporters, or detoxification enzymes(Kaur et al., 2020).

Target gene analysis and molecular techniques help analyze the target genes of anthelmintic drugs in parasites. By sequencing and comparing these genes in resistant and susceptible parasites, researchers can identify specific mutations or changes that impair the binding or action of the drug. This information provides insights into how the parasite's target site has been altered to reduce the drug's effectiveness (Scare et al., 2020).

Expression profiling and molecular techniques such as gene expression analysis, including quantitative real-time PCR (qPCR) or RNA sequencing (RNA-seq), allow researchers to assess the expression levels of various genes in resistant and susceptible parasites. By comparing the gene expression profiles, researchers can identify genes that are upregulated or downregulated in resistant parasites. This information helps identify genes involved in resistance mechanisms, such as drug efflux pumps or detoxification enzymes(Xue et al., 2020).

Functional validation of candidate genes associated with resistance is identified through molecular techniques; researchers can conduct functional studies to validate their role in conferring resistance. This can involve techniques like gene knockout or overexpression studies using molecular tools like RNA interference (RNAi) or gene editing technologies (e.g., CRISPR-Cas9)(Nemati, 2019).

conclusion

The emergence and spread of anthelmintic resistance among gastrointestinal nematodes in small ruminants pose significant challenges to veterinary medicine and livestock production. With parasites like *Haemonchus contortus*, *Ostertagia spp*., and *Trichostrongylus* spp. becoming increasingly resistant to available treatments, there is a heightened risk of disease transmission, reduced productivity, and economic losses for sheep and goat farmers. Urgent interventions, such as sustainable parasite management strategies and alternative treatment approaches, are essential to mitigate these threats and safeguard the health and profitability of small ruminant farming operations.

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