

Anthelmintics Resistance Against Gastrointestinal Nematodes of Sheep: A Review

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Abstract: Between 1960 and 1990, the pharmaceutical industry made major progress in developing deworming compounds with excellent broad-spectrum activity and safety. This led to the discovery of major drug classes available for ruminants each with distinct modes of action: benzimidazoles, imidothiazoles- tetrahydropyrimidine and macrocyclic lactones. However, shortly after their introduction into the market, the development of resistance against all anthelmintic drug classes has been reported throughout the world. Anthelmintic resistance is one of the most serious threats to the effective control of gastrointestinal nematodes of ruminants, especially in small ruminants. Further complicating the situation today and for future parasite control program is the fact that all the economically important parasite species of sheep have developed resistance to all groups of anthelmintics. Although, the chief factors involved have long been recognized, it is evident that their importance varies greatly between situation and some are more easily addressed than others. However, the speed with which selection in a population will occur on a number of factors such as improper treatments strategies, proportion of worm population, that in refugia, parasite genetics, parasite biology and host-parasite relationship are the major factor for the development of anthelmintic resistance. Thus, requires sensitive diagnostic tests like in-vivo and in-vitro test to identify the development of anthelmintic resistance. Therefore, it is important that livestock producers and veterinarians find a balance between achieving good parasite control and sustainability of their control strategies. In this way anthelmintic resistance may be delayed and the effectiveness of anthelmintic drugs may be prolonged.

Key words: Anthelmintic resistance • Gastro intestinal nematode • Sheep

INTRODUCTION

Gastrointestinal nematode (GIN) infections remain one of the most prevalent and important issue affecting small ruminants worldwide. They are responsible for both direct and indirect major losses, causing decreased productivity, costs of control measures and deaths [1, 2].

The control of parasitic helminths in domestic animals relies largely on the use of anthelmintic drugs [3]. However, increasing reports of parasitic populations that have developed anthelmintic resistance (AR) have become increasingly common and this phenomenon severely threatens the beneficial exploitations of this control strategy [4]. In fact, AR in GIN of sheep and goats has been reported in different parts of the world [5], making it a seriously increasing problem [6]. Thus it is

become a major practical problem in many countries of Africa [7], Europe [8], Asia [9], South America [10] and Australia [11].

Resistance to anthelmintic is common among sheep and goats. In addition, adopting appropriate control strategies aimed at preventing the development of resistance is essential one for the purpose of effective worm control in livestock and promote productivity [12].

Therefore, information on the use of anthelmintics is extremely important; because drugs are the likely mainstay of nematode control in the near future [13]. Increasing numbers of reports on anthelmintic resistance further emphasize the importance of such knowledge in order to extend the effective life span of the existing anthelmintics on the market and to avoid the use of inefficient treatments. Therefore, the objectives of this paper are:

- To review the current status of anthelmintic resistance in sheep
- To review the possible causes and diagnosis anthelmintic resistance in gastrointestinal parasites of livestock.
- To highlight the strategies for managing the development of anthelmintic resistance.

Gastrointestinal Nematode of Sheep: The major GINs reported in sheep are *Haemonchus contortus* and *Teladorsagia circumcincta* in the abomasums; *Bunostomum triginocephalum*, *Strongyloides papillosus*, *Trichostrongylus species (spp.)*, *Cooperia* spp. and *Nematodirus* spp. in the small intestine. The nematode common in the large intestine are *Oesophagostomum* spp., *chabertia ovina* and *Trichuris ovis* [13, 14]. *Haemonchus contortus* is the most pathogenic and prolific nematode. *Haemonchus contortus* attaches to the wall of the abomasum in sheep and goats, feeding on the host's blood, causing anemia. Other nematodes usurp the nutrients eaten by the host, causing weight loss [15].

Clinical signs and sequelae GINs infection are dependent on the parasite fauna present and the intensity of infection. In sheep, these can range from subclinical weight loss to lethal pathologies such as anemia, diarrhea and severe protein loss. In addition, parasitism can have indirect consequences on metabolism such as mobilization of proteins for an immune-response, reduced feed intake due to anorexia or increased susceptibility to other pathogens [16, 17].

Economic losses are caused by low fertility, decreased production, condemnation of organs, cost of prevention, cost of treatment and the death of infected animals in severely infected cases [13].

Infections with GIN in the field are usually mixed infections and involve several different species of nematodes. Its impact on the animals is mainly influenced by the intensity of the infection and the physiological status of the host. Growing lambs and periparturient ewes are most susceptible to the infection by nematodes [18]. The parasite should not be diagnosed on the bases of fecal egg count alone. The result should be considered together with clinical signs, age of the animals, season of the year, nutritional status and grazing history [19].

Anthelmintics and Their Mechanism of Action: Anthelmintics are drugs that are used to treat infections with parasitic worms and/or expel parasites from the body by either stunning or killing them and without causing significant damage to the host [20]. Many modern

anthelmintics are effective against both adult and larval stages and an increasing number are efficacious against arrested or dormant larvae [21]. Three classes of anthelmintics are available for use in ruminants. These are Macrocycliclactones (MLs), Benzimidazoles (BZs) and the Imidazolthiazoles –Tetrahydropyrimidines(I-T). These three substance classes of anthelmintics vary in their mechanisms of action [22].

The MLs act as gamma-aminobutyric acid (GABA) antagonists and glutamate-gated chloride (GluCl) channel potentiators, acts on the same receptor as the GABA neurotransmitter in nematodes that is a ligand-gated Cl⁻ channel found on the synaptic and extrasynaptic membrane of nematode muscle membrane. Ivermectin induces release of GABA which leads to the complete paralysis and immobilization of the worms [22]. The effect is irreversible and the consequence is paralysis and death of the nematode [23].

The I/Ts (levamisole and morantel) act selectively as agonists at synaptic and extrasynaptic nicotinic acetylcholine receptors on nematode muscle cells and produce contraction and spastic paralysis. They produce the block at the narrow region of the channels, which subsequently cause muscle contractions in nematodes. This leads to worm paralysis in a contractile state and, once rendered immobile, the worms are expelled [24].

The BZs bind to helminth β -tubulin and prevent the polymerization of the microtubules and exert its effects by interfering with cell division and the glucose uptake [25, 26]. Thiabendazole was the first BZs and also the first broad spectrum anthelmintics [27]. The mechanism of action of albendazole is by blocking glucose uptake in larval and adult stages of susceptible parasites and also depleting their glycogen reserves, thus decreasing ATP formation [28]. The drugs are relatively insoluble in water and partially soluble in most organic solvents what has an impact on their bioavailability in tissues [29].

Anthelmintics are generally used in two ways, namely, therapeutically, to treat existing infections or clinical outbreaks, or prophylactically, in which the timing of treatment is based on knowledge of the epidemiology. When used therapeutically, drug must be effective against the pathogenic stage of the parasite, anthelmintics could be successfully removing parasites and resulting in cessation of clinical signs of infection such as diarrhea and respiratory distress and if the use of anthelmintics are prophylactically; cost-benefit of anthelmintic prophylaxis and interfere with the development of an acquired immunity should be considered [14]. The effect of anthelmintics treatment is manifested in many ways, such as enhanced growth rate, reproductive performance and wool production [30].

Development of Anthelmintic Resistance: Infections with parasitic nematodes restrict the welfare and productivity of livestock throughout the world. The control of these parasites relies heavily on the administration of anthelmintic drugs. Between 1960 and 1990, the pharmaceutical industry made major progress in developing deworming compounds with excellent broad-spectrum activity and safety [31]. This led to the discovery of major drug classes available for ruminants each with distinct modes of action: BZs, (I/Ts) and MLs. However, shortly after their introduction into the market, the development of resistance against all anthelmintic drug classes has been reported throughout the world [32].

Anthelmintic resistance is defined as the heritable ability of the parasite to tolerate a normally effective dose of the anthelmintic [33]. AR occurs when parasites usually eliminated by a given dose suddenly survive the treatment. Since resistance is inherited, the surviving worms will pass their resistance alleles to their progeny [34]. Today, the problem of AR is by far the most severe in the major GINs of small ruminants [35] and is escalating problem in most sheep-rearing countries worldwide which is a threat to both agricultural income and sheep welfare [6, 36].

Most of the helminthes of sheep possess the capacity to develop resistance to anthelmintics. Frequent dosing (extensive use), adoption of common management and therapeutic strategies, long-term utilization, inappropriate handling, rapid reinfection, under dosage and inefficiency against arrested or dormant larva may be some of the reasons for the reduced efficacy and for the increasing development of drug resistance [21]. The availability of fake and adulterated anthelmintics in the drug market in many of the under developed countries is another important contributing factor to the development of resistant strains [37].

The time from introducing a new class of anthelmintic drugs until resistance has been detected seems to be less than 10 years [32].

The mechanism of AR is not completely understood. Drug resistance can happen in a limited number of ways [6]. There may be a change in the molecular target, so that the drug no longer recognizes the target or a change in metabolism that inactivates or removes the drug or that prevents its activation, or a change in the distribution of the drug in the target. Drug resistance research has revealed the importance of appropriate target identification for efficacy of the drug (Table 3).

Table 1: Introduction of anthelmintic drugs for ruminants and the development of resistance to the drug.

Anthelmintic	Mode of action	Generic drugname	Introduced on the market	Resistance reported	Reference
Benzimidazoles	Inhibiting polymerization of microtubules	Thiabendazole	1961	1964	[38]
		Albendazole	1972	1983	[39]
Imidazothiazoles and Tetrahydropyrimidines	Agonist of nicotinic acetylcholine receptors	Levamisole	1970	1979	[40]
		Pyrantel	1974	1996	[41]
Macrocyclic lactones	Allosteric modulators of the glutamate-gated chloride channels	Ivermectin	1981	1988	[42]

Table 2: The first reports of anthelmintics resistance in nematodes of sheep to drugs with different modes of action

Year	Country	Drug	Nematode
1957	USA	Phenothiazine	<i>H. contortus</i>
1964	USA	Thiabendazole	<i>H. contortus</i>
1968	USA	OP-compounds	<i>T. circumcinctus</i> .
1976	Australia	Levamisole/Morantel	<i>H. contortus</i>
1980	S. Africa	Rafoxanide	<i>H. contortus</i>
1987	S. Africa	Ivermectin	<i>H. contortus</i>

Source: [12]

Table 3: Possible mechanisms of resistance to the major anthelmintic families

Anthelmintic family	Mechanism of resistance
Benzimidazoles	Altered target structure (β -tubulin isotype 1 mutations)
Avermectins	β -tubulin isotype 2 mutations, deletion, Altered metabolism and/or uptake. Mutations in GluCl and/or GABA-R genes Overexpression of P-glycoproteins
Levamisole	Altered target (structure of GluCl channel & subunits) Changes in nicotinic acetylcholine receptors

Source: [43, 6]

Generally, the presence of AR depends upon factors associated with the host, the parasite and type of anthelmintic, animal management and climatic characteristics, thus increasing the difficulties for the establishment of preventive measures, which should vary according to the animal production systems [44].

Methods for Detecting Anthelmintic Resistance: The growing importance of AR has led to an increased need for reliable and standardized detection methods. A variety of *in vivo* [(faecal egg count reduction test (FECRT) [45] and controlled test [46] and *in vitro* tests [(egg hatch assays [47], larval motility tests [48] and bio-chemical tests [49] are now available for the detection of resistance to the main anthelmintic groups and there is an ongoing effort to refine, standardize and validate these tests. The development of molecular tests is also progressing and is trying to apply DNA probe and polymerase chain reaction (PCR) technology [50]. However, each test has some shortcomings, which may include high cost, poor reliability, reproducibility, sensitivity and ease of interpretation [51, 52].

The World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) has recommended two tests for detecting anthelmintic resistance in ruminants, horses and pigs. These are *in vivo* test (FECRT recommended for use in infected animals) and an *in vitro* test (the egg hatch test recommended for detection of benzimidazole resistance in nematodes that hatch shortly after embryonation) [45].

***In vivo* Techniques:** There are two *in vivo* tests currently in use for diagnosis anthelmintic resistance in animals. These tests are; FECRT and controlled efficacy test:

Faecal Egg Count Reduction Test: FECRT is the standard and the most common test for determining AR under field conditions [45]. This test was originally designed for sheep, but can be used also for cattle, swine and horses. The test measures faecal egg counts before and after treatment with an anthelmintic, with calculation of the post treatment reduction. For monitoring of normal fluctuation, the treated group is generally compared with non-treated (controls) [50]. The WAAVP established guidelines that give precise details and recommendations for the use of this detection method [52]. The test estimates anthelmintic efficacy in terms of nematode egg output and does not necessarily relate to worm numbers because it only measures the effect on adult female worms [3]. A major advantage of the FECRT is its easy and

straightforward use for all three available broad spectrum anthelmintic groups [45]. Despite being the standard test for AR determination, the FECRT is laborious and time-consuming [53, 54]. As a result, various alternative diagnostic tests have been suggested for the determination of anthelmintic susceptibility [52]. The FECRT may not provide sufficient information on its own for correct interpretation. Larval culture can be used to determine the species involved [55].

Modern broad spectrum anthelmintics are highly efficacious and treatment should normally result in a reduction of faecal egg counts by more than 95 percent [50]. Resistance is suspected when either of the criteria are met: 1) the percentage reduction in egg count is less than 95% and, 2) the lower limit for its 95% confidence interval is equal or below 90% [45].

The Controlled Test: Controlled test is considered the gold standard in measuring efficacy of anthelmintics [56], which is the most reliable method of assessing anthelmintic efficacy against mixed nematode infections [57]. This tests the efficacy of an anthelmintic by comparing parasite populations in groups of treated and untreated animals. Basically, the procedure compares worm burdens of animals artificially infected with suspected resistant isolates of nematodes. The parasitized animals are randomly separated into medicated and non-medicated groups and the animals are necropsied after a treatment interval (10 to 15 days) and the parasites are recovered to be identified and counted. This test must be compulsorily done before the registration of a new drug [46] and is not extensively used except in cases of special interest or when confirmation of resistance is required at species level and for evaluation of the effect on larval stages [58]. In an attempt to reduce the cost and labor required for this test, laboratory animal models have been used and guidelines for evaluating anthelmintic efficacy using the controlled test have been published [55, 58].

***In vitro* Techniques:** The test is based on the action of different concentrations of anthelmintics on embryonation, development and motility of parasites. These include egg hatch assay, larval development assay [57].

Egg Hatch Assay: Egg hatch assay has been developed to differentiate between resistant and susceptible strains of gastrointestinal nematodes for the BZs and for the levamisoles that used to calculate the 50% of lethal dose of the drug on freshly collected nematodes eggs.

It provides an accurate method for assessing the susceptibility of mixed nematode populations and is comparatively more rapid and economic to conduct than the FECRT [59]. 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 BZs decreases as embryonation proceeds. The test has only been shown to work on nematode species in which eggs hatch rapidly. There are several variations of the egg hatch assay, but the essential aim is to incubate undeveloped eggs in serial concentrations of the anthelmintic. Due to difficulties in the interpretation of the results, this assay is not widely used for field surveys [57, 60].

Larval Development Assay: Larval Development Assay (LDA) is based on culturing a known number of GIN eggs in the presence of different anthelmintics. It is reported to be relatively easy to perform, more sensitive than the FECRT and allows for the identification of parasite larvae to the genus level [49]. LDA is the only one that allows the detection of resistance against all the drugs irrespective of their mode of action. In this test, nematode eggs isolated from fecal samples are applied to the wells of a micro-titer plate and larvae hatch and develop to the L3 stage in the presence of anthelmintic. The concentration of anthelmintic required to block development is related to an anticipated *in vivo* efficacy [61].

Prevention of Anthelmintic Resistance and Control of Nematode Parasites: Resistance is a genetic trait that only becomes expressed phenotypically once allele frequencies of resistance genes reach fairly high levels [62]. Therefore, prevention of resistance must be aimed at reducing the rate with which resistance alleles accumulate and strategies designed to slow the development of resistance must be integrated early on in the process of resistance evolution, before there is any clinical evidence of reduced drug effect. This is accomplished best by following practices that ensure maintenance of an adequate level of refugia (a term used to describe the proportion of a parasite population that is not exposed to a particular drug, thereby escaping selection for resistance) and maximize the likelihood that drug treatment will kill partially resistant parasites [63]. Worms in refugia

provide a pool of genes susceptible to anthelmintics, thus diluting the frequency of resistant genes. As the relative size of the refugia increases, the rate of evolution toward resistance decreases. In GINs of small ruminants, which have a direct life cycle, refugia are supplied by: stages of parasites in the host that are not affected by the drug treatment, parasites residing in animals that are left untreated with a particular drug and free-living stages in the environment at the time of treatment [64]. Treatment of animals with low worm burdens does little to control parasites, but removes an important source of refugia, thereby accelerating the evolution of resistance [65]. Drugs should be used in ways that maintain refugia [58].

Treating simultaneously with two drugs from different anthelmintic classes is one method of preventing the development of anthelmintic resistance. Compared with individual drug effects, anthelmintics of different chemical classes administered together induce a synergistic effect, resulting in clinically relevant increases in the efficacy of treatment. This synergistic effect is most pronounced when the level of resistance is low [66].

It is important to avoid under dosing and ensure that treatments are fully efficacious. If practical, the access of free-living stages to the next host should be reduced by measures such as removal of faces and alternate grazing of different hosts [67]. Rotate chemical group annually, to ensure that worms are exposed to the compounds with a different mode of action each year, is another way to made slow the onset of resistance [19].

The prevalence of anthelmintic resistance of GIN in small ruminants continues to increase. The lack of new classes of anthelmintics focuses on management of parasite burdens with the use the following supportive and alternative methods;

Proper Nutrition: The strongest link between nutrition and parasitism has been illustrated between protein intake and resistance to GINs infection. The most dramatic has been the abolishment of the peri-parturient egg rise in lambing ewes by providing protein at 130% of requirements. Supplementation with phosphorus has been shown to prevent worm establishment [68]. Adequate copper levels are necessary for development of immunity to GIN. Surprisingly, addition of molybdenum at 6-10 mg/day decreases worm burdens in lambs by increasing jejunal mast cells and blood eosinophil numbers [69].

Pasture Management: A safe pasture is one that had no sheep or goats grazed on it for 6 months during cold weather or 3 months during hot/dry weather. Weaning

sheep and goats at 2 months of age and rotating them through pastures ahead of the adults will minimize the exposure to large numbers of infective larvae. Pastures should be rotated following any administration of anthelmintics to the animals [70]. It has been advocated to keep dewormed animals in a holding pen for 24 hours following deworming and then move them to a safe pasture [69].

Selection of Appropriate Weather for Treatment: Anthelmintics administration should be coordinated with the weather. During hot (dry) weather, there will be little or no exposure to infective larvae. As soon as there is significant rainfall, larvae exposure goes up exponentially as previously inactive larvae become active and new larvae are hatched. The producers should be trained to plan deworming within three weeks of significant rain after a dry spell [71].

Ethno-Veterinary Preparations: Ethno-veterinary medicine covers people's knowledge, skills, methods, practices and beliefs about the care of their animals. It provides valuable alternatives to and complements for veterinary medicine. Ethno-veterinary medicine is of specific value in developing countries where allopathic veterinary medicines are often beyond the reach of livestock producers. It can play an important role in grassroots development which seeks to empower people by enhancing the use of their own knowledge and resources. Many indigenous veterinary beliefs and practices persist in a wide majority of stock raisers and farmers, particularly in the developing countries [69].

Condensed Tannins: Condensed tannins containing forages increases weight gains, wool growth and milk production while decreasing the effects of GIN in sheep. The direct parasitic effects include: decreased fecal egg counts and decreased L3 viability. The indirect effect of condensed tannins is by binding to dietary protein, which allows it to bypass rumen and thus increases protein availability in the small intestine. It can be used as a drench or incorporated into pelleted feeds [72].

Nematophagous Fungi: Act as a biological control agent. *Nematophagous* fungi are micro-fungi which utilize nematode larvae as their main source of nutrients. The fungi are ingested by ruminants pass through the digestive tract and colonize fecal material. Three predaceous fungi have been identified but only one is

suitable for including in ruminant diets. *Duddingtonia flagrans* has thick-walled spores that can be fed to ruminants and passes safely through to the feces. The spores must be fed daily to maintain the reduction in L3 numbers [72].

CONCLUSION AND RECOMMENDATIONS

Sheep live with a burden of parasites. Parasitism compromises animal health and productivity and parasite control imposes considerable cost and time burdens on farmers. The main form of parasite control for most farmers is small number of anthelmintic compounds. However; the inevitable development of anthelmintic resistance is generating an increasing challenge that has made it virtually uneconomic to keep livestock in some regions. Misuses of drugs to treat helminthes of livestock such as under-dosing, treatment of all animals at the same time on the same farm, continued administration of anthelmintics of substandard quality and frequent use of anthelmintics of the same family are the likely cause for the development of resistance. Thus, anthelmintics resistance can be diagnosis through in vivo and in vitro techniques. But fecal egg count reduction test is the best at farm level in the field even though controlled efficacy test is the gold test. But now a day's livestock producers in every corner of the world are dependent on anthelmintics for the prevention and treatment of anthelmintics.

Therefore, based on the above conclusion, the following recommendations are forwarded:

- ▶ Using proper treatment strategies; the right dose in the right way at the right time.
- ▶ Reducing dependence on anthelmintic treatment rather, using alternative worm control
- ▶ Avoid frequent and unnecessary treatment with anthelmintics.

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