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Review on Molecular Mechanism of Anthelminthics Resistance

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ABSTRACT

Parasitic helminthes cause serious infectious diseases in humans and live stocks. Control of these infections depends mostly on anthelmintics namely the Benzimidazoles (BZs), Levamisole (LEV) and other imidazothiazoles and the Macrocyclic Lactones (MLs) but resistance has developed against most of these broad-spectrum drugs in many parasite species. Therefore, the objectives of this paper are to give review on molecular mechanism and mechanism of resistance to anthelminthics and the factor that leading anthelminthics resistance and its possible management. Due to their difference I n mechanism of action and their mechanism of resistance of some anthelmintics is also different from each other. Drug resistance in general arises through drug accumulation, drug inactivation, alteration of target cells and metabolic alteration mechanism of the drugs. Some, of the most common mechanisms of drug resistance involve altered levels or altered drug specificities of ABC (ATP Binding Cassete) transporters. ABC transport protein called P-glycoprotein was the first active pump described for leading to multidrug resistance. Frequent usage of the same group of anthelmintic; use of anthelmintics in sub-optimal doses, prophylactic mass treatment of domestic animals and frequent and continuous use of a single drug have contributed to the widespread development of anthelmintic resistance. Appropriate dosage, combination of the drugs, strict quarantines, refugia and pasture management are some that should be used to minimize the problem. Finally, recommendations are forwarded regarding the mechanisms that delaying the onset of drug resistance development.

KEYWORDS

ABC transport, Anthelmintic, Helminths, Resistance

INTRODUCTION

Diseases have a great damaging effect on livestock production. Although parasitic infections, in particular with nematode infections, may not be the most important of diseases in ruminants with regard to animal mortalities, they have a high economic impact because they cause retarded growth, weight loss, disorder in fertility and loss in milk production [1].

Helminths are a diverse group of parasitic worms, encompassing nematodes, cestodes and trematodes, and constitute a major health problem for humans and animals in many parts of the world. Parasite helminthes are major causes of morbidity in animals and humans, infecting more than one billion people worldwide [2].

Parasitic nematodes burden on human health and nutrition by parasitizing livestock and cause an estimated 80 billion loss of worldwide crop production each year [3]. Besides the huge suffering, they cause tens of thousands of human deaths each year, reduce life expectancy and condemn millions to poverty. Despite the huge number of affected individuals, the market for anti-parasitic drugs for humans is not big enough to foster the development of anthelmintics because most infestations that occur are in developing countries that lack the ability to pay for the development of these drugs and lack of new developed technology [4]. With the rare exception of a vaccine for Taenia infection in pigs, no vaccines are available for these diseases and by far the major control measures in humans and animals rely on the use of anthelmintic drugs [5].

Anthelmintics drugs are currently the cornerstones of the control of veterinary helminth infections and probably remain so for the foreseeable future. The impressive efficacy, the standard being more than 95% worm reduction, the overall excellent tolerability, the broad spectrum of affected species and the low costs of the modern anthelmintics were the characteristics which led to great successes in the chemical

control of worms in livestock and companion animals during the past five decades [6]. Until recently, there have been three main chemical families of broad spectrum anthelmintics commercially available to treat parasitic infections, namely; the Benzimidazoles (BZS), Levamisole (LEV) and other imidazothiazoles and the Macrocyclic Lactones (MLs). These are also known, respectively, as the "white drenches", "yellow drenches" and "clear drenches" [7]. The most recent discovery of the Amino-Acetonitrile Derivatives (AADs) as a novel drug class, apparently using a different mode of action to those described for the commercially available anthelmintics [8].

Unfortunately, serious and often dramatic levels of Anthelmintic Resistance (AR) are found, mainly in ruminant and horse gastro-intestinal nematodes and also in others such as liver fluke [6]. The situation is now further complicated by the lack of new mode of action drugs for nematode control in livestock for more than two decades. It is therefore one of the key issues to identify the mechanisms of AR, to develop improved tools, especially molecular tools, to detect and to monitor for AR and to seek means to overcome the resistance mechanisms in order to maintain the current status of helminthes control [9].

The objectives of this paper are:

- To review the molecular mechanism and mechanism of resistance of Anthelminthics.
- To highlight the factors which leads to parasite resistance and its management.

LITERATURE REVIEW

Brief history of anthelmintics

The era of modern anthelmintics started in the middle of the 20th century with the introduction of phenothiazine and piperazine, products that are considered to be the first generation of the broad-spectrum drugs. The second generation of truly broad spectrum anthelmintics were released in the 1960s and included the BZs; albendazole, fenbendazole, probenzimidazoles, imidazothiazoles and tetra-hydro-pyrimidines. Following the early success of the introduction of the BZs, extensive research programs were initiated during which successful structural modification resulted in the production of a series of BZs. BZs are effective against a broad range of parasites and also have wide safety margins, working at dosages of mg/kg bodyweight [10]. Most recently, the third generation of broad spectrum anthelmintics the macrocyclic lactones, emerged in the early nineteen eighties [11].

The ML, IVM was isolated from *Streptomyces avermectinius* in 1974 and released onto the market in 1981 and is effective at doses of mg/kg bodyweight [12]. IVM was the first commercially available endectocide, being effective against helminthes, arachnids and insects [13]. IVM, the fermentation by-product of the saprophytic soil fungus *Streptomyces avermitilis* is the prototype member of the MLs class of anthelmintics and discovered in 1977, it was generally released in 1981, although the release was delayed until late 1987 in Australia [14].

A decade later, another broad spectrum anthelmintic, LEV was released onto the market. Like the BZs, the dose is also in the mg/kg bodyweight range. However, LEV acts on the host so care has to be taken with the dosage. Between 1960 and 1990, the pharmaceutical industry made major progress in developing deworming compounds with excellent broad-spectrum activity and safety [10].

Classification of Anthelmentics

Currently there are three broad spectrum classes of anthelmintic used to control helminthes in animals. These chemical classes are the BZs including albendazole, fenbendazole, mebendazole, oxfendazole, oxibendazole, ricobendazole, thiabendazole, as well as pro-benzimidazoles such as febantel, netobimin. Imidazothiazole drugs are LEV, Morantel and pyrantel. MLs comprising two sub-classes, the avermectin such as IVM, abamectin, doramectin, eprinomectin and selamectin; and the milbemycins such as moxidectin [16].

Molecular Mechanism of Anthelmenthics

Benzimidazole: The anthelmintic efficacy of BZs is due to the ability of compromising the cytoskeleton through a selective interaction with β-tubulin factor [17]. Their mode of action appears to be mediated through binding to β-tubulin within the parasite, thus inhibiting the formation of microtubules that are central to the form and function of the parasite's cells. This prevents various essential cellular processes such as the transport of secretory granules and enzymes in the cell cytoplasm, resulting in cell lysis, with knock-on detrimental effects on motility and feeding [18].

Microtubules play essential roles in eukaryotic cells such as intracellular trafficking, cellular absorption and secretion, anchoring of membrane receptors at specific locations, such as at synapses in nerve cells, mitosis, meiosis and cellular architecture [19]. Microtubules are dynamic polymers with a growing end (+ end) where additional α- ß-tubulin dimers can be added and a loss end (-end) where α-ß-tubulin dimers disassociate from the polymer. The process of adding tubulin dimers at one end and losing tubulin dimers from the other end of the microtubule is termed treadmilling. BZs act by binding to the growth end of microtubules preventing microtubules from adding new ß-tubulin dimers. As this occurs at the same time as microtubules are losing tubulin dimers from the other end, this results in the microtubules shortening and disappearing, disrupting essential cellular functions. It is therefore, perhaps, not surprising that ß-tubulin and microtubules, which are formed by polymerization of α- ß-tubulin dimers, are the targets for a large number of pharmaceuticals [20].

Typically drugs that target tubulin or microtubules either cause instability in microtubules or cause microtubules to become excessively stable. The exact dimensions of the BZs binding site have not been unequivocally determined [21].

However, BZ-susceptible nematodes have the amino acid phenylalanine at positions 167 and 200, as well as glutamate at position 198 of the ß-tubulin protein and this results in a high affinity binding site for BZs. In contrast, vertebrates commonly have Tyr at codon 200, and lack a high affinity BZ binding site and are not susceptible to significant toxicity from BZ drugs [22].

Imidazothiazoles: These anthelmintics are anthelmintic that target nicotinic acetylcholine receptors. LEV and tetramisole are imidazothiazoles that act by interfering with parasite nerve transmission causing muscular spasm and rapid expulsion. The cholinergic agonist at the nicotinic neuromuscular junctions that works by first opening and then blocking the acetylcholine receptor-mediated cation channels. This causes a sustained neuromuscular depolarization, leading to a rapid tonic paralysis of the parasite's somatic musculature, resulting in expulsion from the host [10]. The molecular mechanisms of LEV activity remain largely unknown in parasitic nematodes [23].

Macrocyclic lactones: The unique mode of action of MLs provides valuable efficacy against parasites resistant to other compounds [24]. The glutamate-gated chloride channels (GluCl) appear to be the most sensitive site of action of the ML anthelmintics. In nematodes, GABA receptors are found on muscle cells. Piperazine is a specific GABA receptor agonist and causes a flaccid paralysis [25]. They act by binding glutamate chloride channel causing paralysis and bind to GABA gated chloride channel which normally blocks reaction in some nerves causing excessive stimulation of central nerves system. The MLs activate chloride channels causing an inhibitory effect, irreversibly open, leading to a permanent hyper polarization of the cells and paralysis and death of parasite [26].

Molecular Mechanisms of Anthelmintics Resistance

Anthelmintic resistance is defined as the ability of parasites to survive a dose of drug that would normally kill them; it is heritable and nonreversible [27].

Drug resistance in general arises through one of four mechanisms

Drug accumulation: Drug accumulation may be lessened by diminished import, through alteration of pores which drugs enter the cell, or by increased export of the drug via an efflux pump [28].

Drug inactivation: Mechanisms that inactivate drugs can diminish the amount of free drug available to bind to its intracellular target. More of the anti-metabolite 5-fluorouracil is normally catabolized by dihydro pyrimidine dehydrogenase, primarily in the liver [29]. The formation of conjugates between the thiol glutathione and platinum drugs such as cisplatin, carboplatin, and oxaliplatin is a key step in the inactivation of the drugs [30].

Alteration of target: Alterations in expression levels or mutation of a chemotherapeutic drug target can have a major impact on drug resistance. Microtubules in eukaryotic cells play essential roles, such as intracellular trafficking, cellular absorption and secretion. Drugs that target tubulin or microtubules either cause instability in microtubules or cause microtubules to become excessively stable [31].

Alteration of metabolic pathways: There could be a change in the metabolism of the drug causing not to be metabolized into its active form, or to be removed from its target sites [6].

Development of resistance to the sparse catalog of anthelmintic is an area of concern. The phenomena of drug resistance is essentially a change in gene frequency of worm populations produced by drug selection which renders the minimal effect of dosage previously used to kill the parasite. It is the ability of worms to survive the lethal effect of compounds known to have anthelmintic potential [32].

Resistance to benzimidazoles: The resistance mechanism to BZs anthelmintics has been shown to be associated with changes in ß-tubulin [33]. The sequences in the majority of organisms susceptible to BZs present a high conservation of phenylalanine and changes to a tyrosine that reduces the affinity of BZs β-tubulin. The changes of phenylalanine for a Tyr, methionine or glutamine were present themselves at position 200 in unsusceptible and resistant organisms [34]. Also, the position 167, very similar to 200, exhibits a high conservation of the amino acid phenylalanine in the susceptible group and the change to Tyr has been observed in some resistant organisms. The hydroxyl group on the Tyr allows it to function as a hydrogen bond donor/acceptor and increases the polarity and hydrophilicity of the binding site, characteristic unseen with the non-polar and hydrophobic phenylalanine. However, the presence of this Tyr at position 167 in the sequence changes may not be relevant in all β-tubulins. This could be an indication that amino acids at position 198 might play a major role in the binding of BZs toβ-tubulin [35]. The differences present at position 165 may only affect the stabilization of the BZs in the binding site. In one case a single amino acid substitution was identified as the cause of resistance. The most common single nucleotide polymorphism that causes BZ resistance is change at codon 200 in ß-tubulin [6].

The multiple sequence alignment showed that the main differences in susceptibility are presented at positions 165, 167, 198 and 200 of the β-tubulin sequences. Molecular modeling studies corroborated the binding mode of the BZs in the β-tubulin binding site and were also in agreement with β-tubulin susceptibility reports based on the treatment with BZs. The mutated and unsusceptible β-tubulin models suggest that the possible cause of resistance to BZs is mainly due to amino acid modification at position 198 because of the loss of hydrogen bonding interactions. On the other hand, the substitution of phenylalanine for Tyr at positions 167 and 200 suggests that the inhibitory mechanism may take place during the opening of the binding site or during the internalization of the ligand. The mechanism by which the principal single point mutations Phenylalanine167Tyr, Glutamate198Alanine and Phenylalanine200Tyr could lead to resistance to BZs. The binding site pocket of the four structures consists of several highly conserved hydrophobic amino acids with a few hydrophilic residues in which the main differences are observed at positions165, 167, 198 and 200. The different amino acids at these positions could be of great importance to determine the possible cause of susceptibility and resistance to BZs between organisms [36].

Resistance to Imidazothiazoles: The molecular mechanisms of LEV activity and expression of resistance remain largely unknown in parasitic. In contrast, genetic screens for mutants that survive exposure to LEV in the free-living nematode *Caenorhabditis elegans* (*C. elegans*) have led to the identification of five genes (*unc-38, unc-63, unc-29, lev-1 and lev-8*) that encode a LEV sensitive acetylcholine receptor (L-AChR). Loss of these genes leads to LEV resistance [23].

The natural ligand typically binds at the interface between an alpha-type and its adjacent subunit, causing a change in physical structure of the channel, opening a gate that allows ion flow into, or out of, the cell [37]. Numerous genes encode cation-channels in *C. elegans*; they can be grouped into 5 clusters named after specific subunits: *acr-16, acr-8, unc-38, unc-29 and deg-3* and in addition, many subunits whose function is not clearly defined [38].

The candidate gene strategy developed revealed an unexpectedly high diversity of L-AChR subunits specific to the trichostrongylide parasites that are a principal target for the drug LEV. [39] reviewed the pharmacology of LEV resistance, where it is caused either by a reduction of the number of nicotinic acetyl cholinesterase receptors or by a decreased affinity of these receptors for the drug. Although polymorphism at the amino acid level could be demonstrated, there is no evidence that alleles at this locus were involved in selection for resistance to LEV [40]

The cholinergic anthelmintics act on nematode nicotinic acetylcholine receptors located on somatic muscle cells. The receptors of several genes are subject to modulation of several other proteins. Mutations altering these proteins could alter sensitivity to the cholinergic anthelmintics and thus lead to resistance. The possibility of resistance to the cholinergic anthelmintics is not necessarily the result of a single mutation but may well be polygenic in nature. Additionally, the mutations resulting in resistance may vary between different species or between resistant isolates of the same species [41].

Resistance mechanisms to the macrocyclic lactones

IVM and other MLs affect gastro-intestinal by causing paralysis via opening chloride channels, which are thought to be associated with α-subunits of glutamate-gated channels located on muscles of the pharynx and possibly the somatic musculature [42] also compared IVM-resistant helminthes, susceptible *H. contortus* populations and found that resistance is due to an alteration in the binding of IVM to glutamate gated chloride channel receptors. Phospho-glycoprotein (Pgp) is also involved in resistance to IVM in helminth species [43].

ABC (ATP binding cassette) transporters of the subfamily B, the so-called Pgps have been frequently implicated in IVM resistance and are a major cause of multi-drug resistance in protozoa and helminthes. The Pgp inhibitor verapamil dramatically enhanced susceptibility of the cattle parasitic nematode *C. oncophora* to ivermectin. Moreover, verapamil completely restored susceptibility to IVM in a resistant isolate resulting in virtually identical dose-response curves of susceptible and resistant isolates in the presence of verapamil. Further characterization of the molecular mechanisms resulting in Pgp-mediated IVM resistance is still hampered by the lack of molecular and biochemical information for Pgps of parasitic nematodes. The Pgp sequences contribute important data required to systematically screen resistant C. oncophora isolates for up- or down-regulation of Pgps and for the detection of single nucleotide polymorphisms in Pgps to detect selection of specific Pgp alleles by anthelmintics as early as possible [43].

Changes in the sequence and expression of these Ligand-gated chloride channels can cause resistance to the ML, such as *C. elegans*. Mutations in multiple GluCl subunit genes are required for high-level ML resistance in *C. elegans*, and this can be influenced by additional mutations in gap junction and genes. Parasitic nematodes have a different complement of channel subunit genes from *C. elegans*, but a few genes, including *avr-14*, are widely present. A polymorphism in an *avr-14* orthologue, which makes the subunit less sensitive to IVM and glutamate, has been identified in *C. oncophora* and polymorphisms in several subunits have been reported from resistant isolates of *H. contortus*. This has led to suggestions that ML resistance may be polygenic. There is no specific resistance associated sequence changes have yet been identified. The *avr-14* of *C. oncophora* showed

a Leu256Phenylalanine polymorphism change between a resistant and a susceptible isolate, which causes a reduction in sensitivity to IVM. But this polymorphism has not been reported so far in other isolates of *C. oncophora* or other parasite species [44].

Cross resistance, active transport and its role in anthelmintic resistance

Cross-resistance occurs through drug receptor-independent mechanisms and arises because the mechanism of resistance to several drugs is the same, through identical genetic mutations [46]. In any case, cross-resistance involving two mechanisms.

Specific mechanism: Specific resistance mechanisms as those that confer resistance to only one class of drugs, with little or no effect on other classes. These forms of resistance might be expected to be caused by changes in the drug target site or in specific activating enzymes, rather than by an increase in the expression of detoxifying enzymes, like P450, or efflux pumps, like the ABC pumps in the plasma membrane. The generation of drug-resistant strains of *C. elegans* and the genetic and molecular characterization of the mutations responsible for this resistance was an extremely productive and informative strategy for studying the mechanisms of action of all the current anthelmintic classes [46].

Nonspecific mechanisms: Mechanisms that alter drug concentration are sometimes referred to as nonspecific mechanisms because they may affect pharmaceuticals from different chemical and mode of action classes. Nonspecific resistance mechanisms can include drug transport mechanisms with relatively low specificity, usually involving ABC transport proteins, or drug metabolism such as oxidation by cytochrome P450. There is little evidence that oxidative drug metabolism is very active in parasitic nematodes and there is no evidence this drug metabolism plays a significant role in resistance to existing anthelmintic drugs [47]. Mechanism acquired through direct exposure to one drug and may generate resistance to one or more other drugs to which the pathogen has not been exposed. Non-receptor mechanisms of resistance include altered levels of enzymes involved in drug metabolism or transport mechanisms which modulate subsequently the concentration of the drug that reaches the effect or site on a receptor, such as:

(i) increased efflux of the drug from cells containing the receptors

(ii) reduced uptake

(iii) increased drug metabolism and inactivation or

(iv) reduced activation of drugs [49].

However, drug efflux mechanisms may play a significant role in resistance to some anthelmintics. Initial evidence that IVM is an excellent substrate for efflux mechanisms by mammalian P-glycoprotein. Some of the most common mechanisms of drug resistance involve altered levels or altered drug specificities of ABC transporters, such as P-glycoprotein [48].

ABC transporters belong to an evolutionarily well-conserved family of membrane proteins whose main function is the ATP-dependent transport of a number of structurally unrelated endogenous and exogenous compounds including a large range of drugs. Among these transporters, the Multidrug Resistance (MDR) BC transport protein called P-glycoprotein was the first active pump described for over expression in tumor cells, leading to MDR [49].

Source: [49],

(A) Constitutive expression of MDR transporters on cell membranes of target organism: basal efflux of drugs (example with macrocyclic lactones).

(B) Over expression of MDR transporters in response to drug pressure: increased efflux of drug and development of resistance.

(C) Inhibition of MDR-mediated efflux with MDR reversal agents: enhancement of drug concentration and toxicity into the target cells.

Table 1: Summary of genes and molecular changes that are associated with resistance. Source [45]

Anthelmintic	Site of action	Gene Carrying	Molecular change
Benzimidazole	β-tubulin	ben-1 (isotype-I)	F200y E198A F167Y
Levamisole	Body muscle nAChR	Unc-38 Unc-29	Decreased expression
Macrocyclic **Lactones**	GluCl channel Transporter	Avr-14 Pgp	L256F Increased expression

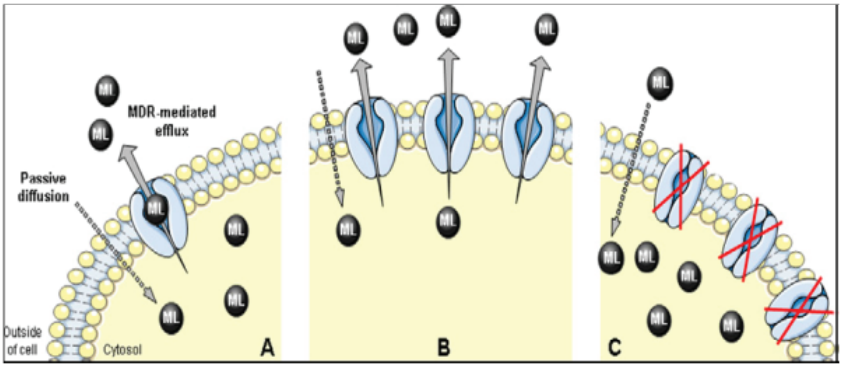

Figure 1: Multidrug transporter mediated efflux of drug, its contribution to the development of resistance and mechanism of reversion

Factors leading to anthelmintic resistance

The development of anthelmintic resistance has occurred relatively rapid. It is facilitated by the large population size and inherent high genetic variability that is typical among nematode parasites and compounded by the movement of their host species [50].

Modern anthelmintics are used at an efficiency of around 99% against susceptible strains of helminthes [26]. However, a small number of worms still survive due to variety of reasons. The rate of resistance is influenced by many reasons like genetic, biological or operational, the most important of which are operational factors, which can be manipulated by farmers and form the basis of resistance in management programs [51].

Treatment frequency: One of the major factors that predispose to anthelmintic resistance is frequent use of the same group of anthelmintic regularly [51]. The frequency of treatment determines how rapidly resistance will develop. It has been observed that frequent usage of the same group of anthelmintic may result in the development of AR [52]. AR in *H. contortus* has been reported in some humid tropical areas where 10 to 15 treatments per year were used to control this parasite in small ruminants [53].

Anthelmintics under dosing: Under dosing is generally considered as a major cause for anthelmintic resistance, because sub-therapeutic doses allow survival of heterozygous resistance worms [54]. Variation in bioavailability of many drugs in different host species also promotes anthelmintic resistance. The presence of generic products of substandard quality, repacked and reformulated or expired drug products are most widely distributed in pharmacies and veterinary clinics, where use of such drugs promotes development of AR [51].

Under dosing is contributes to the selection of resistant or tolerant strains [55]. Moreover, variation in bioavailability in different host species also is crucial for making a decision about correct dose [56]. Most of the currently applied anthelmintics are in fact sub curative in at least part of the population [57].

Mass treatment: Prophylactic mass treatments of domestic animals have contributed to the widespread development of AR in helminthes. This approach would ensure that the progeny of the worms surviving treatment will not consist only of resistant worms. Leaving a part of the group untreated, especially the members carrying the lowest worm burdens should not necessarily reduce the overall impact of the treatment. In worm control in livestock, regular moving of the flocks to clean pastures after mass treatment and planning to administer treatment in the dry seasons is a common practice to reduce rapid reinfection. However, these actions result in the next helminth generation that consists almost completely of worms that survived therapy and, therefore, might contribute to the development of AR [54]. A parasite resistant to one anthelmintic in a drug class will usually be resistant to all anthelmintics within the class, this is known as side resistance [58].

Single drug regimens: Frequent and continuous use of a single drug leads to the development of resistance. For example, a single drug, which is usually very effective in the first years, is continuously used until it no longer works [59]. Frequent use of single drugs without alternation with other drugs has also been reported as the reason for the fast development of resistance [60].

Transmission of resistance: Studies examining changes in the prevalence of AR have suggested that initially "on farm" selection is the crucial process. However, as resistant parasite populations become more common, animal movement is one of the key factors that account for the rapid changes that occur during the last stages of the developmental process of the parasite [61].

Possible management of Anthelmenthics resistance

Refugia: Refugia describe the proportion of a parasite population that is not exposed to a particular drug, thereby escaping selection for resistance. Most parasitologists now consider levels of refugia as the single most important factor contributing to selection for anthelmintic resistant parasites [62].

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. For many years, parasitologists and veterinarians have recommended that all animals should be treated with an anthelmintic at the same time. However, this strategy has turned out to be unsustainable, and parasitologists now favor a selective approach where only animals in need of treatment actually receive medication [63].

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. Climatic conditions have fundamental effects on the numbers in refugia. Few free-living stages survive in arid climates, so the pasture refugium is small [64].

Adoption of strict quarantine measures: Effective management strategies to prevent development of anthelmintic resistance are worthless if producers purchase resistant worms residing in breeding stock. Therefore, strict quarantine procedures should be instituted for all new additions. This practice is more important, as in recent years several farms with high-quality breeding stock dispersed herds [65].

Combination drug strategy: Treating simultaneously with two drugs from different anthelmintic classes is one method of preventing the development of AR. This strategy is used when the drugs are first introduced, before there is any selection for resistance to either drug, appreciable resistance will not develop for over 20 years. However, once resistance alleles accumulate in worm populations, this strategy will probably not be successful. 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. Once high-level resistance to both drugs is present, the synergistic effect is unlikely to produce acceptable levels of efficacy [66].

Genetic improvement: There is considerable evidence that part of the variation in resistance to helminths infection is under genetic control. Resistance is most likely based on inheritance of genes that play a principal role in expression of host immunity. Several breeds around the globe are known to be relatively resistant to infection [67].

Although such a strategy may be acceptable to some, selection for resistant animals within a breed also is a viable option. Within a breed, animals become more resistant to infection with age as their immune system becomes more competent to combat infection. Some animals within such a population do not respond well and remain susceptible to disease; therefore, the majority of the worm population resides in a minority of the animal population [68].

Nutrition: The strongest link between nutrition and parasitism has been illustrated between protein intake and resistance to gastrointestinal nematode infection [69]. Immunity is closely related to protein repletion. Supplementation with phosphorus has been shown to prevent worm establishment. Cobalt deficiency also has been associated with reduced immunity [70].

Pasture management: Reducing exposure of susceptible hosts in control programs is paramount. The goal of pasture management is to provide safe pastures for grazing. Stocking rate is an important consideration in parasite control as it affects exposure to infective larvae and contamination of the pasture [71].

CONCLUSION AND RECOMMENDATION

Ability to detect molecular mechanism of anthelmintic resistance (AR) is quite limited. The reason behind this is complexity of molecular mechanism of the resistance. Currently molecular mechanism of AR has not been investigated widely in most developing countries due to absence and/or lack of technologies. Mechanism of resistance is difficult to study, but nowadays some people used *C. elegan* nematode for further investigation of mechanism AR in some anthelmintics.

Changes in gene expression are associated with resistance to several different classes of anthelmintic. It is therefore one of the key issues to identify the mechanisms of anthelmintic resistance, to develop improved tools, especially molecular tools, to detect and to monitor for AR and to seek means to overcome the resistance mechanisms in order to maintain the current status of helminthes control.

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