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Review on Anthelmintic Resistance

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Abstract: Helminth parasitism remains one of the most prevalent disease that affects animals and humans globally and causing a substantial threat to health, economic loss due to cost of treatment, cause loss of production and productivity. Even though Anthelmintics are the preferable method in control of helminth; dependence on therapy for prevention of helminth eventually results in gradual development of resistance against most of anthelmintics. Anthelmintic resistance is the ability of parasites to survive doses of drugs that normally kill parasites of the same species.Mechanisms of anthelmintic resistance are related to mode of action of a drug against a particular helminth species and can be specific; resulted due to mutations in intracellular targets, single nucleotide polymorphisms and non-specific resulted due to altered levels of enzymes required for drug metabolism or modified transport mechanisms that control concentration of drugs reach the receptor sites. Causes of anthelmintic resistance are related to anthelmintic drug use, hypobiosis and cystation, host parasite relationship, parasite genetics, management strategies, operational and environment factors. Special attention must be paid to appropriate selection and timing of anthelmintic use as a major force in reducing development of anthelmintic resistance.

Key words: Anthelmintic · Drug · Helminth · Mechanism · Resistance

INTRODUCTION

Livestock play a crucial role in the economies of both developed and developing countries of the world. They provide food, or more specifically animal protein in human diets, income, employment and possibly foreign exchange. For low income producers, livestock also plays as a store of wealth; provide draught power and organic fertilizer for crop production. However, the productivity of this livestock sector is lower in developing countries because of low genetic potential of the animals, poor nutrition and prevailing diseases [1].

Helminth parasitism which is one of the constraints of livestock production remains prevalent infections in grazing animals and other animals worldwide [2, 3]. Helminthes are categorized into three groups that includes nematodes (roundworms), trematodes (fluke worms) and cestodes (tapeworms) [4] and infect man and animals; causing a substantial threat to health that induce severe pathophysiological damage in infected livestock; leading to lower animal growth rate, poor body condition; dull hair coat; loss of carcass and wool quality; reduced milk yield; decreased pregnancy rate longer calving to conception interval; anorexia, diarrhea, anemia, colic and in severe cases, even death of the animal [5]. These helminthes causes economic losses in livestock, especially when raised in extensive grazing systems and negatively affects the health and welfare of infected animals resulting in production losses [6].

An anthelmintic treatment as a control approach against parasites has long been used in all parts of the world. Traditionally, the use of chemotherapeutic agents was most commonly used method to treat and control parasites of domestic animals and similarly, farmers regularly use various classes of anthelmintics to control parasites [7]. The wide spread success of anthelmintic (Benzimidazoles, Imidothiazoles and Macrocyclic lactones) for parasite control was the result of their broad therapeutic activity against different parasites species; high anti-parasitic efficacy and very low toxicity in treated animals [8].

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The intensive use of anthelmintic drugs in livestock has led to the development of progressive resistance to the available drugs. Resistance to all major classes of anthelmintics, including Benzimidazoles, Imidothiazoles/ Tetrahydropyrimidines and Macrocyclic lactones were reported worldwide [8]. The development of anthelmintic resistance poses a large threat to future production, welfare of grazing animals [9]. Climate change and global trade have also increased helminth infections in livestock by increasing the abundance of specific zoonotic parasites (or their hosts) and development of variable degrees of resistance among different species of Helminth has been observed due to frequent usage of the same group of anthelmintic; use of anthelmentic in sub-optimal doses, prophylactic mass treatment of domestic animals and frequent and continuous use of a single drug contributed to the widespread development of anthelminthic resistance [10].

Anthelmintic resistant parasites affecting livestock also spread to human by hybridization of parasites affecting livestock and those affecting humans as in the case of schistosomiasis and fasciolosis; leading to loss in billions of dollars and deaths of thousands of human lives every year [11]. Anthelmintic resistance is now a widespread problem for control of helminthes in almost every region of the world and is of increasing concern for control of parasites infections in cattle, sheep, goats, horses and pigs [5]. Although anthelmintic resistance is a major problem globally, the situation is aggravated in developing countries like Ethiopia by misuse of anthelmentic drugs and there is a shortage of information on anthelmentic drug resistance and due attentions were not given by service deliverers especially by veterinarians and co-workers. Therefore the objectives of this article are:

- To review on anthelmintic resistance and its mechanisms
- To review factors contributing to anthelmintic resistance and management strategies

Literature Review

Definitions: Anthelmintics are a group of anti-parasitic drugs that expel parasitic worms and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host [12]. Anthelmintic resistance is defined as capacity of a parasite population (individual parasites with in population) to tolerate doses of an anthelmintic that would kill a normal population of the same species and to transmit this resistant fitness to their progeny [13].

Mechanism of action refers to the specific biochemical interaction through which a drug substance produces its pharmacological effect or role of compound interferes with a target sites in the parasite [12].

Mechanism of Action of Anthelmintic Drugs: Currently there are broad spectrum classes of anthelmintic used to control helminth in animals and these chemicals classes are Benzimidazoles (Albendazole, Fenbendazole, Mebendazole, Oxfendazole, Oxibendazole, Ricobendazole, Thiabendazole as well as Pro-Benzimidazole (Febantel and Netobimin)), Levamisole (Morantel and Pyrantel), Macrocyclic Lactones (two sub-classes; Avermectins-Ivermectin, Abamectin, Doramectin, Eprinomectin, Selamectin and Milbemycins-Moxidectin), Amino-acetonitrile Derivatives, Piperazine, Diethylcarbamazine, Paraherquamide and Praziquantel. These drugs are either acts on parasite membrane ion-channels which have more rapid therapeutic effect and other group acts more slowly on range of biochemicaltarget sites found in parasites [12]. Membrane ion-channel actions of anthelmintic agents includes the nicotinic acetylcholine channel of nematodes, the GABA channel, the glutamate-gated Cl– channel and biochemical sites of action including β-tubulin and glycolytic enzymes [14, 15].

Benzimidazoles: These drugs bind to the ß-tubulin dimers of the growing end of microtubules of the parasite, preventing microtubules from polymerization and eventually caused the death of the parasite. The members of Benzimidazoles class of anthelmintic act by inhibiting the functions of microtubules after binding to β-tubulin, which is essential for cell structure, resulting in death of the parasite [14, 15].

Levamisole: Levamisole was released to the market in 1970 [18]. Levamisole targets to nicotinic acetylcholine receptors (nAChR) and act on the surface of somatic muscle cells in nematodes; leading to depolarization and spastic paralysis that facilitates parasite expulsion; act as an agonist's compound at the neuromuscular receptor and resulting spastic paralysis. These intestinal worms are killed by drug or pushed out by peristalsis movement of the intestine [12].

Macro Cyclic Lactones: Macrocyclic lactones were released in 1981. Macrocyclic lactones cause flaccid paralysis of somatic musculature in parasite body and disrupting food ingestion by inhibiting pharyngeal pump. These drugs interfere GABA mediated neurotransmission at glutamate-gated chloride channels (GluCl) of the helminthes, including α7 nACh receptors [19].

Table 1: Ion-channel target sites of anthelmintic

Target site and parasite group	Drugs
Nicotinic Acetylcholine receptors(in nematodes)	Levamisole, Butamisole, Pyrantel, Morantel, Bephenium, Thenium, Methyridine
GABA-receptors (in large intestinal nematodes)	Piperazine
GluCl receptor (in nematodes and insects)	Ivermectin, Abamectin, Doramectin and Moxidectin
Membrane calcium permeability in cestodes and trematodes	Praziquantel

Source: [16]

Table 2: Biochemical target sites of Anthelmintic

Target site and parasite group	Drugs
β-tubulin (in nematodes)	Thiabendazole, Cambendazole, Oxibendazole, Albendazole
β-tubulin (in nematodes, cestodes and trematodes)	Fenbendazole, Oxfendazole, Mebendazole, Flubendazole, Febantel, Netobimin, Thiophanate, Triclabendazole
Proton Ionophores (blood suckers: flukes, Haemonchuscontortus, Oestrusovis)	Closantel, Rafoxanide, Oxyclozanide, Brotianide, Nitroxynil, Niclopholan, Hexachlorophene, Dibromosalan, Niclosamide
Malate metabolism (in immature Fasciola)	Diamphenethide
Phosphoglycerate kinase and mutase (in Fasciola)	Clorsulon
Arachidonic acid metabolism and innate immunity of host (effective against filaria)	Diethylcarbamazine

Source: [17]

Amino-Acetonitrile Derivatives: Amino-acetonitrile derivatives are novel synthetic anthelmintic drugs recently discovered and tested experimentally for veterinary use only, at the moment. A newer version of the drug, AAD-1566 (Monepantel) was to be developed for better pharmacological actions [20]. The mode of action of Amino-acetonitrile derivatives involves the ability of the drug to interfere with a unique clade of acr-23 nicotinic acetylcholine receptor sub-unit which causes paralysis of the worms. At the moment, AADs overcomes existing resistances of worms to the current available anthelmintic drugs and are well tolerated with low toxicity to mammals [14, 20, 21].

Piperazine: It was commercialized in 1950 and suitable for the treatment of filariasis and thread worm. Its mode of action is acts as an antagonist against GABA receptors and causes flaccid, reversible paralysis of helminthes body wall muscle as studied in Ascarissuum [22].

Paraherquamide: Marcfortine A and Paraherquamide A is isolated from PenicilliumRoqueforti and Penicilliumparaherquei respectively. Both drugs belong to members of the Oxindole alkaloid family. In studies, Marcfortine A was confirmed to be active against C. elegans at a high dose. On the other hand, Paraherquamide and its derivatives were found to induce flaccid paralysis in many parasitic nematodes during in vitro studies [23].

Pharmacological analysis of the effects of these drugs on acetylcholine-stimulated body wall muscle contractions in A. suum muscle strips in vitro has shown that they act as typical competitive antagonists, shifting concentration-response curves to the right in a parallel fashion. These drugs have no apparent direct effect on A. sum body wall muscle tension or membrane potential [13].

Paraherquamide also blocks actions of nicotinic agonists, but not equipotent. Interestingly, this antagonist seems to distinguish nicotinic receptor subtypes on muscle and has greater affinity for the receptors mediating the response to Levamisole than receptors that mediate response to nicotine. One might therefore expect that Paraherquamide would be an effective antagonist of the Levamisole-selective receptor on *C. elegans* body wall muscle. Importantly, the mode of action of this class of anthelmintics differs from the more established drugs that interfere with cholinergic transmission [23].

Praziquantel: Praziquantel, a drug used in the treatment of schistosomiasis and thought to act by disrupting calcium homeostasis has no activity against nematodes. Praziquantel works by causing severe spasms and paralysis of the worms' muscles. This paralysis is accompanied by a rapid Ca 2+ influx inside schistosomes. Morphological alterations are another early effect of Praziquantel. These morphological alterations are accompanied by an increased exposure of schistosomes' antigens at the parasite surface [17].

The worms are then either completely destroyed in the intestine or passed in the stool. An interesting quirk of Praziquantel is that it is relatively ineffective against juvenile schistosomes. While initially effective, effectiveness against schistosomes decreases until it

reaches a minimum at 3-4 weeks. Effectiveness then increases again until it is once again fully effective at 6-7 weeks. Glutathione S-transferase, an essential detoxification enzyme in parasitic helminths, is a major vaccine target and a drug target against schistosomiasis. Schistosomes' calcium ion channels are currently the only known target of Praziquantel [24].

Mechanisms of Anthelmentic Resistance: The mechanisms of anthelmintic resistance are intrinsically related with mode of action of a drug against particular helminth species and ability of parasite to overcome the drug activity [25]. The accumulation of resistant genes in helminth population is evolutionary process depends on genetic diversity of parasite populations under selection for anthelmintic resistance, selection pressure (i.e. anthelminthic treatment) and time. Resistance is inherited and selected for during treatment, as resistant helminthes escape the effect of treatment and pass resistance to the next generation. The resistance genes that occur through mutation are rare in the population but, as selection continues, their relative proportion in the population increases and proportion of resistant parasites increases too [26].

Specific Mechanism of Anthelmintic Resistance: Specific mechanisms of anthelmintic resistance also known as targeted resistance, usually due to modification of the receptors that are target sites for the drugs and therefore the mode of action of the drug is affected [22]. Targeted resistance can be mainly due to single nucleotide polymorphisms or any other genetic modifications which alter amino acid sequence of the drug receptors and affinity of receptors to bind drugs; altered ancillary proteins or other substances which affect receptor functionality and changes in regulatory components that modify the expression level of receptors or ancillary proteins [27].

Benzimidazole: Were introduced into commercial market in 1961 and resistance was reported in 1964. Changes and occurrence of resistance varies between helminthes species. Resistance in nematodes can be due to a mutation in the gene coding for the target site, same mutation does not cause resistance to Triclabendazole in the trematode *Fasciola hepatica* [28]. In addition, within a single worm species, different mutations can lead to resistance to the same anthelmintic. For instance, Benzimidazole resistance in *Haemonchuscontortus* is caused by the phenylalanine to tyrosine mutation at amino acid position 200 of the isotope 1β-tubulin gene [29].

Levamisole: The laboratory produced Levamisole resistant *Caenorhabditiselegans*worms showed that a large number of genes are involved in the observed resistance. The mechanism of clinical Levamisole resistance in parasitic nematodes is less well understood and may incorporate different mechanisms and genes. The mechanism of resistance to Levamisole is most likely due to a change in the ability of target sites to bind the drug. Studies conducted on the pig nematode; *Oesophagostomumdentatum,* involving different members of this class of Levamisole and Pyrantel showed that there were a lower percentage of opened ion channels in resistant worms compared to the counterparts, suggesting an increased desensitization of drug receptors in resistant isolates [30, 31].

Macrocyclic Lactones: The mechanism of Ivermectin resistance is still unclear, as genetic analysis of a resistant isolate of *H. contortus*indicated that only one major gene is associated with resistance to Ivermectin, while other studies suggest that this mechanism seems to be influenced by more than one gene (Ardelli and Prichard, 2004). In the nematode *C. elegans*, high-level resistance to Ivermectin was reported to be associated with simultaneous mutation of three genes (*avr-14, avr-15* and *glc-1*) that encode GluCl channel alpha type subunits. Ivermectin resistance is more likely due to changes in target sites, the receptors on pharyngeal muscles. There is still little evidence that resistance to *H. contortus* is associated with genetic changes to GluCl channels [32]. Another major mechanism implicated for the development Macrocyclic Lactones resistance in nematodes is increased drug transport through trans-membrane protein because Macrocyclic lactones are good substrates of these transporter proteins [27].

Non-Specific Mechanisms of Anthelmintic Resistance: Generally, drug resistance is associated with more than one genetic change and quite often non-receptor based mechanisms also contribute to the development of resistance [33]. The major risk associated with this type of resistance is that several classes of drugs having different modes/targets of action may be equally impacted and their efficacies can be compromised by the modified pharmacokinetics [27].

Resistance to one anthelmintic due to non-receptor based mechanisms may lead to cross resistance to other anthelmintic to which the parasite has not been exposed, as indicated by reduced sensitivity of *C. elegans* to Levamisole due to selection for Ivermectin resistance [34]. This type of resistance is associated either with altered levels of enzymes required for drug metabolism, defensive molecules required for survival of the cells, or modified transport mechanisms that control concentration of drugs that reach the receptor sites. The continuous intrusions of environmental toxic substances direct organisms to develop special cellular mechanisms to combat effects of such substances. These mechanisms include altered drug transport and modified drug metabolism [12]. Modified drug metabolism includes increased drug metabolism and inactivation and reduced activation in case of pro-drugs [27].

Certain defensive molecules are produced inside cellular machinery of parasitic nematodes; alter drug metabolism and make compounds less toxic. Glutathione and thioredoxin systems work in parallel to oxidize or reduce the toxins, scavenge the free radicals and protect the cells from oxidative damage [12]. Modulation of drug transport includes increased efflux of the drugs from target cells and decreased uptake of the drugs. P-gps are members of a large superfamily of trans-membrane transport proteins (ABC transport proteins) have been associated with anthelmentic resistance in nematodes [35]. These proteins are important players in the development of resistance to Ivermectin. There have been reports describing an over-expression of multiple P-gps in different nematodes resistant to this class of anthelminthic [36]. The up-regulation of helminth P-gps gene expression enhances the parasites ability to survive Ivermectin exposure and also supports its role in the development of resistance to this drug [35].

Causes of Anthelmintic Resistance

Hypobiosis and Cystation: Hypobiosis and cystation is behaviors of the parasites that exhibited by undergoing avoidance mechanism from host immune attack while they are not metabolically fit with the host in case anthelminthic drugs fail to inhibit the parasite activities and there after these parasites become re-activated later and exposed to residual drugs either in the host or in the environment that increases their resistance capacity from time to time [37].

Parasite Biology: Some Parasites have short generation time and highly prolific. So there is high increase in generation with spread of resistance alleles in the population and their life cycle contributes resistance. For example, in indirect life cycle parasite population tend to be mobile as hosts are moved and leaving low levels of untreated parasites in refugia [38].

Parasite Genetics: Many parasites have genetic features that favor the development of anthelminthic resistance. Among the most important of these are rapid rates of nucleotide sequence evolution and extremely large effective population sizes that give these worms an exceptionally high level of genetic diversity [37, 38].

Treatment Factors

Anthelmintics under Dosing: Under dosing is an important factor in the development of AR because sub therapeutic doses might allow the survival of heterozygous resistant worm. Laboratory experiments show under dosing contribute to the selection of resistant or tolerant strains [38]. Treatment factors such as under dosing are likely to favor the survival of heterozygous individuals, possibly enhancing the selection pressure for resistance and frequency of dosing, frequent and repeated use of same class of anthelmintic is determined to be a considerable risk factor for development of resistance. The consequence of inappropriate anthelmintic treatment procedures causes development of resistance to classes of broad-spectrum anthelmintic drugs like Benzimidazoles, Imidothiazoles and Macrocyclic lactones [37, 39].

Mass Treatment: Prophylactic mass treatments of domestic animals contributed to the widespread development of AR in helminthes. This approach ensures 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 [37, 38].

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. Long-term use of Levamisole in cattle also led to the development of resistance, although the annual treatment frequency was low and cattle helminthes develop resistance less easily than do worms in small ruminants [38].

Host-Parasite Relationship: The epidemiology of nematode parasites of ruminants is also strongly influenced by aspects of host-parasite biology after infection occurs and larvae of important nematodes are undergo a period of arrested development in host abomasal or intestinal mucosae. Following infection, larvae become metabolically inactive for several months. Although the immune status of host also has an influence on rates of hypobiosis; the greatest proportion of larvae usually becomes arrested when conditions in the external environment are least favorable for development and survival of eggs and larvae that reduces the refugia to drug of arrested larvae inaccessible [37, 39].

Environment Factors: Relaying on anthelmintics to control parasites; rather than changing management practices using only drugs instead of incorporating methods to preserve refugia and better management of pasture can spread up anthelmintic resistance. In certain livestock management practices, when a producer administers a parasite-control product and fails to see result; efficacy of that product is in question. The intensive use of anthelmintic drugs also increases the risk of drug residues in animal products [38].

Operational Factors: The chemical nature of the drug (mechanism of action and rotation of chemicals) no rotation and frequent rotation contributes spread of resistance. Frequent routine deworming without performing diagnostic tests or determining if treatment is necessary, herd variation, this stands to reason because no two cattle operations managed equally; deworming when environmental refugia is low; treating animals when few eggs are on the pasture after a harsh winter or hot, dry summer; increases the proportion of resistant eggs in the environment. Using anthelmentic for unapproved uses like to increase weight gain in the short-term; increases opportunities to eliminate susceptible parasites; leaving resistant parasites [38].

Detection of Anthelmintic Resistance: Apart from developing new anthelmintic and adapting alternate strategies to slow down the development of resistance; detection of resistance at an early stage is also very important. Advances in molecular technology increased understanding mechanisms of resistance in worms [35].

***In vivo* Tests for the Detection of Anthelmintic Resistance**

Faecal Egg Count Reduction Test: The faecal egg count reduction test (FECRT) is most widely used method for detection of AR in the field and the only readily-available technique to diagnose drug resistance on farm and provides estimation of anthelmenthic efficacy based on percentage of reduction in faecal egg counts after administration of anthelmintics. Anthelmenthic drugs may cause temporary suppression of egg output by resistant female nematodes that survived treatment; hence, the FECRT can give a false negative result if FECRT are analyzed during this period. In general, temporary worm egg suppression lasts for 3 days after Levamisole treatment, 8 days after Benzimendazole treatment and 10-14 days after Macrocyclic Lactones treatment [40].

Animals treated with Levamisole which has no effect on immature nematode stages should be sampled 7-10 days post-treatment to avoid the detection of eggs from females not affected by the drug during their larval development [23]. In addition, the fecundity of some nematode species like Ostertagiaostertagi in cattle can influenced by density-dependent mechanisms; resulting in reduced egg excretion following an increase parasite burden, while an increased and/or highly variable fecundity could occur when low worm burdens are present [41]. In addition, eggs from Trichostrongyle gastrointestinal nematodes are similar in size and shape except eggs from Nematodirus species. Hence, FECRT should combine with identification of the resistant parasites surviving treatment. The most common procedure is culturing of faeces to isolate infective third-stage (L3) larvae for morphological differentiation at genus level. FECRT also detects AR in *Fasciola hepatica* [42].

Controlled Efficacy Test (CET): The CET is gold standard and the most reliable method to assess the efficacy of anti-parasitic drugs and to diagnose AR. This method involves anthelmentic treatment of naturally or experimentally infected animals and following post-mortem worm recovery and identification of surviving or resistant parasites. Anthelmenthic efficacy is then calculated by comparing the number of parasites between treated and untreated control animals. However, the high costs of CET in terms of labor, equipment and slaughter of animals, makes this technique unpractical for routine use in commercial farms and is almost completely restricted for research purposes [40].

***In vitro* Tests for the Detection of Anthelmintic Resistance**

Egg Hatch Assay: EHA is used to measure the effects of anthelmintics on hatching of the nematode eggs. The ability of anthelmintics to prevent nematode egg hatching

is measured, therefore, the assay is not suitable for anthelmintics lacking ovicidal effects, for example Ivermectin. EHA is most frequently used to detect Benzimendazole resistance in ruminant nematodes. At the moment, only the EHA can be reliably used in field samples due to the consistent EC50 threshold value related with Benzimendazole resistance observed in different species of nematodes of small ruminants, cattle and horses > 0.1mg Thiabendazole/Kg40, 43]. The EHA has also good correlation with molecular methods and the FECRT to detect Benzimendazole resistant and susceptible nematode strains and has a high reproducibility between laboratories. In addition EHA detects Triclabendazole-resistant in *F. hepatica* eggs [44].

Larval Development Assay: The effect of anthelmintics on development of parasites provides a chance to develop techniques useful for detection of anthelmentic resistance. In the LDA, the eggs or L1 larvae are exposed to different concentrations of anthelmintics incorporated into agar wells in 96-well plates or in a small test tube containing growth medium [25]. Methods based upon development inhibition are more laborious and time consuming than for the EHA but are useful to detect resistance to all the major groups of anthelmintics including MLs. The larval development assay (LDA) is more sensitive than the FECRT as it identifies resistance when it is present in a worm population at levels down to 10% [45].

The suitability of the LDA for detection of resistance to Pyrantel in livestock nematodes has also been established. On the other hand, some report indicates that the LDA is not suitable fordetecting Macrocyclic Lactones resistance in T. circumcincta while the assay was able to detect resistance to Benzimendazole and Levamisoleanthelmintics[25]. This test is considered as reliable, inexpensive and suitable for use in the field investigations of AR. The test can also utilize L1; therefore, there is no prerequisite for undeveloped eggs or fresh faecal samples [45].

Larval Paralysis Test: LDT is established to detect resistance against Levamisole and Morantel. After exposure of larvae to different drug for 24hr; proportion of paralyzed larvae for each concentration is calculated and dose dependent response is referenced to control replicate [45].

Larval Motility Assay: The larval motility assay is used to identify resistance to Benzimendazole, Macrocyclic Lactones and Levamisole or Morantel. However in Morantel, definitive discrimination among resistant and susceptible strains is not always possible. A micro motility meter was developed for measuring motility of larval and adult nematodes after exposure to anthelmintics [46]. The instrument uses microprocessor technology to measure light refraction at the meniscal interface. Movement of larvae in solution is claimed to change the angle of light refraction entering the photodiode. This light deviation is measured and information passed to a computer to give a motility index. An *in vitro* assay using dog hookworms (*A. caninum*and *A. ceylanicum*), human hookworm (*N. americanus*)and *Strongyloides* species demonstrating the effects of Benzimendazole and Ivermectin has been established [47].

Larval Arrested Morphology Assay: The larval arrested morphology assay is performed by exposing hookworm larvae to different concentrations of anthelmintics, the presence of which alters the posture of the larvae into a dormant state. This assay is validated for dog hookworm using isolates of intermediate susceptibility to Pyrantel. The sensitivity of this assay is demonstrated significantly higher than larval a motility and migration assay; which makes it more appropriate tool for detecting resistance to Pyrantel in dog hookworms [46].

Larval Migration Assay: The larval migration assay was developed as a modification of the motility assay to detect the sheep nematodes resistant to Ivermectin. The test is useful for investigating the action of a range of paralyzing agents like Ivermectin. Infective stage larvae (L3) are exposed to various dilutions of Ivermectin for 48hr and then allowed to migrate through an agar/ filter mesh system fitted over a receiver plate, for the next 24hr [48]. The assay is able to detect a 10% Ivermectin resistant fraction in a population of *H. contortus* but it proved to be ineffective for two other nematodes, including *Tr. colubriformis* and *O. circumcincta*. For instance, detection of anthelmintic effects of enzyme systems generating oxygen radicals and in detection of inhibitory effects of double stranded RNA (DsRNA) with L3 *H. contortus* [49].

Molecular Techniques: Different molecular techniques have been developed for the detection of specific mutations that are associated with AR, which include restriction enzyme digestion, direct sequencing, pyro sequencing and diagnostic PCR. These techniques used to reveal a pattern of substitution associated with Benzimendazole resistance. DNA polymorphisms investigated in genome of resistant and susceptible larvae

and adult *H. contortus*using restriction fragment length polymorphisms preceded by southern blotting. It was suggested that Benzimendazole resistant worms possess a transformed, perhaps decreased, pair of β-tubulin genes in contrast to susceptible worms [32].

Later on, an allele specific PCR was introduced capable of identifying transformation of a single amino acid (Phenylalanine to Tyrosine) at codon 200 in β-tubulin isotope I gene in *H. contortus,* which was implicated for the development of Benzimidazole resistance. A similar substitution at P167 has also been reported in *H. contortus*. There is currently little evidence to support a definite molecular mechanism involved in resistance to Ivermectin and Levamisole[32]. In the case of Levamisole, previous studies suggest that the development of genetic mutations alter target sites, resulting in an inability of drugs to bind to the receptors [50]. Polymerase chain reaction (PCR) is capable of detecting resistance even when 1% of the worms are resistant in a population, which makes it more sensitive than any other available techniques. Although more high-throughput and rapid techniques are now available as direct real-time PCR, sequencing and pyro-sequencing are being used to detect the proportion of susceptible to resistant genes in worm populations [50, 51].

Management Strategies to Delay the Development of Resistance

Correct Use of Anthelmintics: The prudent use recommendations currently established have overall aim to target treatment in the best possible way so as to reduce unnecessary exposure and thus limit the risk for resistance. Recommendations for prudent use of anthelmintics are generally based on an in-depth understanding of the helminth epidemiology [52]. It is stressed that deworming is based on confirmation of worm burden and treatment with a relevant product is applied at the right time in relation to the life cycle of the parasite so as to obtain sufficient effect without unnecessary exposure [52, 53]. To avoid unnecessary exposure, it is prudent to limit use of broad-spectrum products (with nematocidal and flukicidal activity) only when all substances included in product are necessary to effectively treat animal [54].

Refugia: Resistance is promoted if parasites carrying mutations that bring about reduced susceptibility to anthelmintics are provided with a survival advantage in the population. Refugia concept aims to keep the proportion of resistant worms within the population at a low level and it is thus advocated as a tool to slow the progress of anthelmentic resistance. The success refugia (dilution) strategies rely on maintaining a sufficiently large susceptible population of worms [53].

Parasites in refugiaare those that have not been exposed to an anthelmenthic, including those present as free-living stages in environment and those in untreated individuals [55]. The selective deworming of those animals that are predicted to be most infested by nematodes and/or to contribute towards pasture contamination is implemented to slow the development of anthelmintic resistance but maintain parasite population in refugia[52, 53]. The value of maintaining a population of parasites in refugia to slow down development of anthelmintic resistance has been demonstrated in a bioeconomic model. In this model, besides the number of flock treatments, proportion of worm population in refugia has significant influence on development of anthelmintic resistance [56, 57].

Use of Multiactive Anthelmintic Products: It is currently under discussion whether combination products that contain two or more active substances targeting the same helminthes but through different mode of actions (multiactive anthelmintic products) is advantageous with respect to delaying the emergence of resistance. Modeling studies and some field data indicated that such products delay the development of resistance to new active substances or delay development of anthelmintic resistance to existing anthelmintic classes [58].

Other Options: Other measures to control helminth infestation in animals are different pasture management routines like removal of faeces from pasture to reduce the level of infective larvae, reducing stocking densities, preventing high degree of infestation or improving drainage of pastures to decrease the risk of liver fluke infestations [52]. To be effective, such measures would have to be tailored according to the specific epidemiology situation on the individual farm recommend that the farm epidemiological picture is determined by means of a detailed diagnosis of the affected pasture and the group of animals before implementing appropriate measures against *Fasciola hepatica* in dairy cattle [59]. Appropriate quarantine protocols are also recommended as a useful measure to prevent introduction of resistant helminthes, other biological control methods like vaccines and the selection for livestock that is genetically less susceptible to helminth infestation [60].

CONCLUSION AND RECOMMENDATIONS

The health and productivity of domestic animals are threatened by parasitic diseases caused by helminths both in developed and developing countries throughout the world. Infection of livestock with parasitic worms poses a great burden on health that eventually leads to great losses in productivity of animals and economic losses to the owners. Control of parasitic diseases has historically focused on the use of chemotherapy and chemoprophylaxis all over the world. However, dependence on anthelmintic as sole means for prevention of helminths only to end up with the problem of anthelmintic resistance just because some of the parasites were smart enough to handle the drugs on their own by means of mutation in its genome.

Based on the above conclusion the following recommendations were forwarded:

- There should be rational uses and appropriate management strategies of Anthelmintics
- There should be awareness creation on Anthelmentic resistance
- Appropriate institution should test the efficacy of anthelmintics before use

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