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Review Article

Review on Anthelminthic Resistance in Domestic Ruminants

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Abstract

Many parasitic helminths of veterinary importance have genetic features that favor the development of anthelmintic resistance, and this becoming a major worldwide constraint in livestock production. The principal mode of control of GI nematodes is based on anthelmintics because it is simple, cheap, and offers both therapeutic and prophylactic cover against GIT helminths. Anthelmintic resistance (AR) is said to have developed when an anthelmintic drug fails to kill the exposed population of parasites using the dose that is recommended therapeutically. In Ethiopia, the problem of Anthelmintic resistance is serious and reported frequently from different parts of the country and the rural people are not aware of this anthelmintic resistance problem. Therefore this paper is aimed to review and give background information on Anthelmintic resistance. Using a combination of drugs with different modes of action will help in delaying AR development. Resistance in worms can be the result of a variety of mechanisms and can be roughly categorized as genetic changes in the drug target, changes in the drug transport, or changes in the metabolism of the drug within the parasite. There are various in vivo and in vitro methods available to assess the efficacy of anthelmintics. Different management strategies are used to prevent infestation and/or keep infestation pressure low.

INTRODUCTION

Livestock is an important source of income in most developing countries and contributes to food security. In Africa, it contributes up to 10–20% of the gross domestic product. Livestock also makes an important component of nearly all farming systems in Ethiopia and provides draught power, milk, meat, manure, hides, skins, and other products. Currently, the population of livestock found in Ethiopia is estimated to be 53.4 million cattle, 25.5 million sheep, and 22.78 million goats. Worldwide, infections with parasitic nematodes restrict the welfare and productivity of livestock. The gastrointestinal (GI) nematodes are one of the most important health problems of ruminants in all regions across the tropics and sub-tropic countries like Ethiopia, particularly where nutrition and sanitation are poor. They cause low productivity due to stunted growth, poor weight gain, feed utilization, feeding, and water intake, lower meat, wool, and milk production, cost of treatment, and mortality in young animals. The nematode infections in other parts of the world also affect the health of millions of animals, causing a huge economic loss in livestock farming [1].

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. This led to the discovery of three major drug classes available for ruminants, each with distinct modes of action: benzimidazoles (BZs), imidothiazoles and tetrahydropyrimidines (I/Ts), and macrocyclic lactones (MLS). Modern broad-spectrum anthelmintics are currently widely used in prophylaxis and treatment of helminth infections in farm animals [2]. Relatively shortly after their introduction into the market, the development of resistance against all anthelmintic drug classes has been reported [3]. The first evidence for AR in nematode populations was reported in sheep in the early 1990s [4]. Anthelmintic resistance is defined as a genetic change in the ability of parasites to survive treatments with recommended doses of anthelmintic. Anthelmintic resistance is a major problem for the control of parasitic nematodes in livestock and is of growing concern for human parasite control. However, there is little understanding of how resistance arises and spreads or of the “genetic signature” of selection for this group of important pathogens [5]. The emergence of anthelmintic resistance is leading, gradually, to direct production losses resulting from the lack of efficacy of

available anthelmintics. Resistance of gastrointestinal nematodes (Figure 1) and liver fluke has indeed become a global issue in sheep, and evidence is mounting that it is an emerging problem in cattle as well.

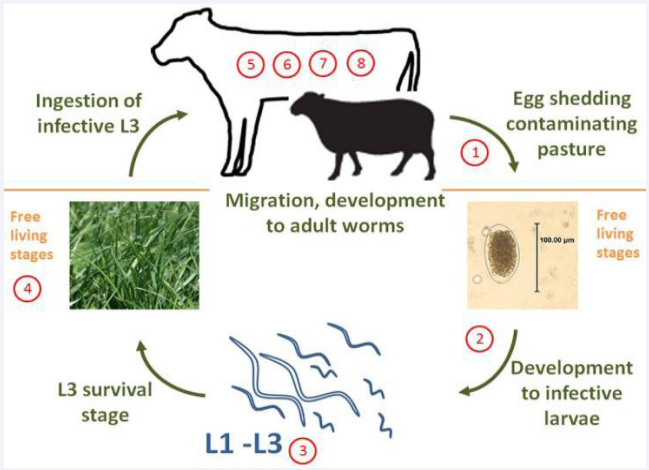

Figure 1 The Life Cycle of GI Nematodes.

There are several Conventional methods for the Detection of anthelmintic resistance (AR) in sheep and goats. In vitro tests generally involve the incubation of free-living parasite stages (eggs or larvae) of GIN in a range of drug concentrations followed by measurement of vitality in form of development, motility, or migration. Currently, five main assays are used, including (i) egg hatch test, (ii) larval development test, (iii) worm motility test, (iv) larval migration, and (v) feeding assay. In vivo tests include the (i) worm reduction test and (ii) fecal egg count reduction test (FECRT). For The worm reduction test, animals are necropsied at the end of the trial, after which the remaining worms in the digestive tract of the treated animals are compared with those from animals that did not receive any treatment. For the FECRT, the change in egg excretion after treatment is compared, depending on the study design, with either those before treatment of the same animal or with those from animals that did not receive any treatment.

Misuse and smuggling of anthelmintics in many forms, such as illegal sales in open markets and irrational administration, is widespread in Ethiopia. In addition, due to the absence of a rational policy for anthelmintic use, methods that can preserve and maintain the efficacy of anthelmintics, and delay or prevent the emergence of anthelmintic resistance are not practiced in any part of the country [6]. The challenge posed by drug resistance urgently requires the development of effective alternatives, aiming at reducing the reliance on anthelmintics and lowering the selection pressure for AR on still-effective drugs. Therefore the objective of this review is to give an insight into the current situation of anthelmintic resistance in ruminants with particular emphasis on nematode parasites.

GASTROINTESTINAL HELMINTH PARASITISM AND CONTROL

Gastrointestinal helminth parasitism

Gastrointestinal helminths are ubiquitous parasitic agents of livestock especially ruminants and are known to limit ruminant production in many areas and countries [7]. Helminth parasites of ruminants are broadly grouped into two phyla, namely nemathelminthes which are nematodes or roundworms such as Haemonchus, Trichostrongylus, Bonostomum, Oesophagostomum and Chabertia and Platyhelminthes which include cestodes (e.g. Avitellina, Moniezia, Stilesia, and Taenia) and trematodes such as Fasciola and Paramphistomum.

Helminthes adversely affect the health status of animals which may be a cause of economic losses to the livestock industry. Losses due to Gastrointestinal tract (GIT) parasitism can be categorized as direct or indirect. Direct losses are due to acute illness and death, forced premature slaughter, and rejection of parts of the carcass at meat inspection in abattoirs. What about direct losses??? Acute parasitic conditions can be recognized and affected animals are generally treated by the farmer thus some of the direct losses can be avoided or minimized [8].

The most important predisposing factors of helminth infections are grazing habits, climate, nutritional deficiency, pasture management, immunological status, vector, presence of an intermediate host, and the number of infective larvae and eggs in the environment. The effect of helminth infections is determined by a combination of factors, of which the varying susceptibility of the host species, the pathogenicity of the parasite species, the host/parasite interaction, and the infective dose are the most important [7].

Various helminth species occupy numerous niches within their mammalian hosts, ranging from the intestinal lumen to intravascular and even intracellular sites. They are responsible for substantial loss of productivity in the livestock industry. Their harmful effects on these animals range from gastroenteritis, anorexia, abdominal distention, diarrhea, emaciation, and so forth; all of which result in serious economic losses to the farmer and the nation in general. Similarly, they constitute a major impediment to efficient and profitable livestock production [9].

Nematodes: Nematodes are the most numerous animals on earth. Nematodes make up a large assemblage of worms of relatively simple structure with a widespread distribution, their cylindrical, non-segmented bodies distinguishing them easily from other helminths. The parasite has a digestive tube consisting of the mouth, esophagus, and the intestine and rectum. In most species, adult female nematodes produce eggs that are passed out of the host with feces. Under optimal conditions in the external environment, first-stage larvae (L1) can develop and hatch out of the egg within 24 hours. L1 grows and develops into the second-stage larvae (L2) which in turn grow and develop into third-stage larvae (L3), which is the infective stage. After ingestion L3 develops into fourth-stage larvae (L4), which then develop into immature adults (L5). Sexually mature adult nematodes develop within 2 to 4 weeks after ingestion of the L3 unless arrested larvae development occurs [1].

Nematode parasites are amongst the most important production-limiting diseases of ruminant livestock worldwide. Teladorsagia circumcincta, H. contortus, Trichostrongylus

vitrinus/colubriformis and Nematodirus battus are of particular relevance. These parasites cause a range of diseases in their hosts, from diarrhea to anemia, and cause significant economic losses to farmers and their keepers in terms of reduced production and treatment costs, as well as being a major welfare issue for the infected animals. They also reduce production efficiency, thereby potentially raising food prices and damaging the environment [10].

Trematodes: The trematodes of traditional veterinary and medical significance are almost all digenetic flukes that require a mollusk or snail as the first intermediate host. Prevalence studies reveal that Fasciola species are by far the most economically important trematodes of ruminants in the tropics [11]. Trematodes from the group of non-segmented flatworms, whose most relevant representatives are liver flukes (e.g. *Fasciola hepatica, Dicrocoelium dendriticum*). Liver flukes are characterized by an external life cycle involving snails and their ability to migrate outside the digestive tract of their host and establish themselves in the liver [12]. The severity of the disease depends on the number of parasites that infect the animal. Livestock becomes infected by ingesting the infective stage, the metacercaria, which contaminates grass and other vegetation. These reach the small intestine and migrate across the gut wall and directly into the liver. The juvenile flukes migrate through the liver tissue, feeding and growing until they reach the bile ducts. The migrating flukes cause liver damage, destruction of tissue, and hemorrhage. Once the fluke reaches the bile ducts, they mature into the adult egg-laying parasite. The spines on the surface of the flukes damage the mucosa as they move and the adults feed on blood [13].

Cestodes: Cestodes are segmented flatworms that dwell as adult worms in the digestive tract of their definitive hosts but also infect intermediate hosts during their pre-adult life stages. Although cattle and sheep are definitive hosts to some cestode species such as Moniezia spp. many cestodes use cattle and sheep as intermediate hosts where ingested larvae migrate outside the intestine and form cysts in different places within the host body. The cycle of the parasite is then completed when the intermediate host and the cysts are consumed by a potential definitive host. Particularly relevant in the livestock industry are cestodes for which human is a definitive or accidental hosts because of their zoonotic potential (e.g. Taenia spp., Echinococcus spp.) [12]. The life cycle of *Taenia saginata* starts when eggs contained in tapeworm segments (proglottids) are passed into the feces of an infected human. They can survive a few months out in the environment. If a cow (the intermediate host) feeds on contaminated vegetation, it ingests the matured eggs or gravid proglottids. In the small intestine the larvae known as oncospheres hatch, penetrate the intestinal wall, enter the bloodstream and migrate to the muscle tissue (rarely to the liver or other organs), where they encyst into cysticerci.

Treatment and control of GIT helminth parasitism

Non-chemical control methods: The best forms of control of helminths will require a basic understanding of certain principles bordering on the parasite's developmental cycle, mode of transmission, and predisposing factors to infection. Apart from the conventional chemical agents or anthelmintics, several alternatives are being practiced or on trial with three main principles of action. The first one is to limit the contact between the hosts and the infective larvae in the field through grazing management methods. The latter were described since the 1970s and, at present, they benefit from innovations based on computer models [14]. Several biological control agents have also been studied in the last three decades as potential tools to reduce the infective larvae in the field. The entire philosophy of using biological control agents against GIN nematodes in animals is to reduce the number of infective stages that are available to be picked up by grazing susceptible individuals of the different species of livestock. The second principle aims at improving the host response against GIN infections relying on the genetic selection between or within breeds of sheep or goats, crossbreeding of resistant and susceptible breeds, and/or the manipulation of nutrition. These approaches may benefit from a better understanding of the potential underlying mechanisms, in particular regarding the host's immune response against the worms. The third principle is the control of GIN based on non-conventional AH materials (plant extracts or mineral compounds). Worldwide studies show that non-conventional AH materials can eliminate worms and/or negatively affect the parasite's biology [14]. Copper wire particles have also been tried with varying success [15].

Chemical control: chemotherapy: The current methods of gastrointestinal nematode (GIN) control are based on the repeated use of synthetic anthelmintic drugs. Anthelmintic Drugs are chemotherapeutic agents commonly used either for prophylactic purposes, in which the timing of treatment is based on a knowledge of epidemiology or for therapeutic purposes to treat existing infections or clinical outbreaks. However, most anthelmintics leave residues in meat, milk, and their products. On the other hand, ivermectin is excreted in feces in sufficient quantity to have a detrimental effect on invertebrates that usually degrade dung heaps, and hence on organisms higher up the food chai. Anthelmintics are drugs that reduce parasite burdens in the animals to a tolerable level; they kill the parasites (vermicide), inhibit their growth, or paralyze them (vermifuge). They also reduce the build-up of infective worm larvae on the pasture, or eggs in the environment. Anthelmintic drugs are widely and routinely administered to grazing livestock to control gastrointestinal nematodes and other parasites [16]. Veterinary anthelmintics available to treat veterinary helminthiasis belong to the classes of probenzimidazoles and benzimidazoles, macrocyclic lactones, imidazothiazoles, salicylanilides, and substituted phenols, tetrahydropyrimidines, spiroindoles, amino-acetonitrile derivatives, and Cycloocta depsipeptides.

Also, based on their mode of action, anthelmintics can be classified as follow: Nicotin agonist, an acetylcholinesterase inhibitor, GABA agonist, GluCl potentiator, calcium permeability increase, B-tubulin binding, proton ionophores, an inhibitor of malate metabolism, an inhibitor of phosphoglycerate kinase and

mutase, and inhibitor of arachidonic acid. Based on the spectrum of action, anthelmintics can be classified as a broad spectrum (killing a wide variety of worms) or a narrow spectrum [17].

Benzimidazoles

The antihelmintic drugs derived from benzimidazole are the largest chemical family used to treat endoparasitic diseases in domestic animals and humans. They are the first chemical class of modern anthelmintics developed. They include drugs such as albendazole, albendazole, fenbendazole, flubendazole, mebendazole, oxfendazole, oxibendazole, albendazole, and triclabendazole some of which are used against both nematodes and flukes as broad- spectrum anthelmintics [18]. The chief flukicides among this group is triclabendazole which holds excellent efficacy against the adult and juvenile stages of Fasciola hepatica even down to one-week-old flukes. Their anthelmintic efficacy is due to their ability to compromise the cytoskeleton through selective interaction with b-tubulin.

Benzimidazole has been used as a lead structure and part of the central scaffold in some metals and serine protease inhibitors as well, because of its potential in H bonding and π-π stacking interactions with the imidazole ring of HIS residue which is required for the activity of these enzymes. Once the benzimidazole molecule has been absorbed from the gastrointestinal tract, it is rapidly distributed by the circulatory system throughout the entire body. During this process, the metabolic process necessary to facilitate its elimination commences. The drugs transfer into the target parasites through transcuticular diffusion. This is usually the predominant pathway through which the drugs reach the nematodes. It is however worthy of note that the external surface of the nematode (cuticle) and that of the cestodes and trematodes (tegument) influence the mechanism of entry of this drug. The mechanism of drug entry to types of helminths seems to be dependent on lipophilicity as a major phytochemical determinant of drug capability to reach therapeutic concentrations within the target parasites.

Imidazothiazoles

Tetramisole is the first imidazothiazole anthelmintic that was introduced into the veterinary market in 1967. However, the current and the most available imidazothiazole anthelmintic worldwide is levamisole. The other compound available is butamisole which is a derivative of levamisole. Levamisole acts selectively as a cholinergic agonist at the synaptic and extra-synaptic nicotinic acetylcholine receptors on nematode muscle cells. Tetramisole is a mixture of two optically active isomers, of which the laevorotatory (L) isomer, levamisole, was responsible for its efficacy against nematodes. The absorption rate and bioavailability of the drugs in this group depend on the route of administration. The drug is most rapidly absorbed following intramuscular or subcutaneous injection in cattle. The drug is widely distributed in the organism being more recovered in the tissues such as muscle, fat, kidney, and particularly the liver at two-hour post-oral and subacute administration. The drug is rapidly and extensively metabolized in the liver through oxidation, hydrolysis, and hydroxylation. It is rapidly eliminated from the body in urine and feces within 24 hours and consequently has a short withdrawal period of 3 days for meat and 1 day for milk. (Reference).

Macrocyclic lactones

Macrocyclic lactones (MLS) are now considered the most widely used broad-spectrum antiparasitic drugs in veterinary medicine. They possess unique features such as exceptional potency, high lipophilicity, and prolonged persistence of their potent broad-spectrum activity. The avermectins and milbemycins are the macrolides produced through fermentation by soil-dwelling actinomycetes called streptomyces. It is a unique combination that kills both endo-and ectoparasites thus, giving it the name “edectocides” by which macrocyclic lactones are now recognized. The avermectin family includes a series of natural and semisynthetic molecules, such as abamectin, ivermectin, doramectin, eprinomectin, and selamectin. Abamectin is the naturally occurring avermectin approved for animal use and the starting material for the production of ivermectin (reference). The macrolides act through selective toxic effects on insects, acarines, and nematodes. However, they do not possess efficacy against cestode and trematode parasites. The macrocyclic lactones induce a reduction in motor activity and paralysis in both arthropods and nematodes. The parasitic effects are mediated through GABA and/or glutamate-gated chloride channels (GluCl), collectively known as ligand-gated chloride channels. The newly proposed mechanism of action of the MLS is hyperpolarization and flaccid paralysis of the invertebrate somatic muscles. The endectocides cause paralysis and death of both arthropod and nematode parasites due to their paralytic effects on the pharyngeal pump which affects nutrient ingestion, and on the parasite somatic musculature limiting its ability to remain at the site of predilection in the host. In addition, MLS causes inhibitory effects on the female reproductive system and causes reductions in parasite egg production.

Tetrahydropyramidines (Pyrantel, Morantel)

Pyrantel is the first compound within the tetrahydro pyrimidines family introduced in 1966 as a broad-spectrum anthelmintic to treat gastrointestinal nematodes in sheep and thereafter, it was developed for use in cattle, swine, horses, dogs, and cats. Later, pyrantel methyl esters called morantel; as well as the methoxyphenyl analog called oxantel were introduced as nematocidal compounds. Pyrantel is prepared for use as pyrantel tartrate or pyrantel pamoate (embonate) and citrate salts. Pyrantel pamoate is insoluble in water and alcohol while tartrate salt is more water-soluble. Morantel is mainly formulated as tartrate salt. Aqueous solutions are subject to isomerization on exposure to light, with a resultant loss in potency; therefore, suspensions should be kept out of direct sunlight. These drugs act selectively as an agonist at synaptic and postsynaptic nicotinic acetylcholine receptors on nematode muscle cells and produce contraction and spastic paralysis. Pyrantel and morantel are 100 times more potent than acetylcholine, although slower in initiating contraction. Metabolism is rapid, and the metabolites are excreted rapidly in the urine (40% of the dose in dogs);

some unchanged drug is excreted in the feces (principally in ruminants). Blood levels usually peak 4-6 hr after administration of PO. (reference)

Salicylanilides (Rafoxanide, clozantel, oxyclozanide): Nitroxyl is effective against adult stages of Fasciola hepatica i.e. 8 weeks post-infection and F. gigantica. It is not effective for the treatment of flukes younger than 6 weeks. It is also used to control Hemonchus contortus in sheep, Oesophagostomum species, Parafilaria bovicola, and Bunostomum species in both sheep and cattle. Nitroxyl acts by producing a rapid spastic paralysis of the flukes causing severe disruption of the tegument of Fasciola hepatica. Nitroxyl is available in oral, intraluminal, subcutaneous, and intramuscular preparations. The subcutaneous route has become the method of choice in practice. Closantel acts as an uncoupler of oxidative phosphorylation in the liver flukes. These result in metabolic changes such as an increase in glucose uptake decrease in glycogen content, changes in respiratory intermediates, and a decrease in ATP synthesis. Rafoxanide was developed in 1969 and has been used extensively against fasciolosis and haemonchosis, bunostomosis in sheep and cattle as well as a nasal bot in sheep. The mode of action of rafoxanide is similar to that of the closantel discussed above. It is effective against flukes, and Moniezia species (tapeworms) in sheep and cattle. It is available as an oral drench (aqueous suspension) containing oxycloxanide only or in combination with levamisole hydrochloride or oxfendazole. Reference

ANTHELMINTIC DRUG RESISTANCE

Definition and distribution of anthelmintic drug resistance

The control of GI nematode infections in livestock, over the past decades and still today, is primarily based on the preventive or curative use of chemotherapeutics. However, by way of their inherent genetic diversity, GI nematodes have consistently found ways to circumvent existing control measures. As a consequence, we are currently faced with an escalating spread of anthelmintic resistance (AR) and infection patterns that may be altered by a changing climate, altered land use, and associated farm husbandry changes.

Anthelmintic drug resistance is the heritable reduction in the sensitivity of a parasite population to the action of a drug. The reduction is expressed as the decrease in the frequency of individual parasites affected by exposure to the drug, compared to the frequency observed in the same population upon initial or prior exposure. Although not unequivocal but generally considered the most adequate, this dentition encompasses two biologically distinct but not always distinguishable processes: (i) existing drug-tolerant parasite lines may become more frequent, particularly under drug pressure, and (ii) previously susceptible parasites may undergo genetic mutations, possibly induced by drug exposure, and be selected under drug pressure [19].

Different types of resistance are side resistance, cross-resistance, and multiple resistances. The side and cross resistances are the condition in which a drug-selected population has a gene coding for a mechanism that defeats the toxicity of the drugs within a mode of action families and from a different mode of action families, respectively whereas multiple drug resistance (MDR) is a state in which a population has been selected independently by drug from different mode to produce different but concurrent mechanism of evasion [20].

Resistance to anthelmintics has particularly become a major problem in small ruminants infected with gastrointestinal nematodes of the family Trichostrongylidae. The nematode Haemonchus contortus, which parasitizes the abomasum of small ruminants, was the first parasite ever to develop resistance. Resistance to phenothiazine was reported in the USA in 1957 within two decades of the drug's introduction onto the market. Resistance has developed mainly in H. contortus, Teladorsagia circumcincta, Trichostrongylus colubriformis, Ostertagia spp., and Cooperia spp., affecting Australia, New Zealand, South Africa, many European countries, several Asian countries, and both American continents.

Resistance is probably an inevitable consequence of the use of anthelmintic and the history of resistance to anthelmintic starts with the first report on phenothiazine resistance approved in 1957. In most regions of Africa, the development of anthelmintic resistance could be expected to be slow, because of high refugia and low frequency of treatment. The exception is South Africa, where in large-scale commercial sheep farms the intensive use of anthelmintics for several decades has led to very high levels of multiple anthelmintic resistances. However, the overall prevalence of anthelmintic resistance has not been extensively investigated throughout the African continent, and anthelmintic resistance in sheep and goat parasites has been reported in at least 14 countries [20].

The extensive use of anthelmintics for the control of helminth infections on grazing livestock has resulted in the development of resistance that has become a major practical problem in many countries of Africa [21,22], Europe [23-25], Asia [26], South America [27,28] and Australia.

A similar situation has been reported in eastern Ethiopia by [39] where nematodes have shown resistance to albendazole, tertramisole, and ivermectin at prescribed dosages in small ruminants. On the other hand, an experimental study on Heamonchus contortus infection in sheep has shown 100% efficacy of ivermectin [30]. Highly prolific species such as *H. contortus* with a relatively short life expectancy of adult worms have a higher risk of developing diverse resistance alleles due to spontaneous mutations than the less prolific *T. colubriformis* [31].

Their long-term utilization, inappropriate handling, and under dosage may be some of the reasons for their reduced efficacy and the increasing development of drug resistance. A study done on the blood-feeding parasite, *H. contortus* has demonstrated the existence of multiple resistances to repeated applications of benzimidazoles, levamisole, and ivermectin [21]. In this study, all three drugs were almost 100% effective against ivermectin susceptible isolates while only closantel proved efficacious on the ivermectin-resistant strain.

Anthelmintic drug resistance situation in Ethiopia

Since anthelmintics within each drug class act similarly, resistance to one anthelmintic in a given drug class is likely to be accompanied by resistance to other anthelmintics of that same class (side resistance). There is also the likelihood for the development of cross-resistance from anthelmintics of one drug class to those of another if the two drug classes share similar targets. Hence, the widespread occurrence of resistance across the majority of anthelmintic drug classes.

Anthelmintic resistance has increased to become an important economic problem in several animal industries. Modern broad-spectrum anthelmintics are currently widely used in the prophylaxis and treatment of helminth infections in farm animals. The problem of resistance to chemotherapeutic drugs has gradually grown from its rather sporadic occurrence in the early 1960s to the current status where anthelmintic resistance threatens the sustainability of many intensive systems of production [23].

The history of parasite resistance to anthelmintics starts with the first report on phenothiazine resistance in 1957. H. contortus was the first nematode to develop resistance against the different anthelmintics. Benzimidazoles are the oldest class of authorized anthelmintics; thiabendazole was introduced in the 1960s. The first report of decreased efficacy of thiabendazole against H. contortus strains dates from 1964, just 3 years after its introduction to the market. The problem of anthelmintic resistance in GI nematode of Ruminant is worldwide and well-documented reports of anthelmintic resistance have been made from South Africa, Australia, New Zealand, Malaysia, Spain, France, Denmark, the UK, Brazil, and the United States.

Mechanisms of Anthelminthic Resistance

Anthelmintic Resistance mechanisms include mutation or deletion of one or more amino acids in the target genes, reduction in the number of receptors, decreased affinity of receptors for drugs, and absence of bioactivation enzymes. Due to modern molecular technology, mechanisms of resistance in worms are becoming further understood. Resistance in worms can be the result of a variety of mechanisms and can be categorized as genetic changes in the drug target, in the drug transport, or in the drug metabolism. The cause of resistance in worms is often complex. Whereas nematode resistance to benzimidazoles can be due to a mutation in the gene coding for the target site, the same mutation [32].

There are several phases in the process of resistance development. Firstly, there is an initial phase of susceptibility where the number of resistant individuals within the parasite population is low with continued exposure to the same drug group. An intermediate phase then follows in which the frequency of heterozygous resistant individuals within the population increases. Finally, sustained selection results in a resistant phase where homozygous resistant individuals predominate within the population. The speed of this process will depend on how severe the selection pressure is on the parasite population. It is known that this is linked to the frequency of treatment and the fact that widespread and excessive use (8 to 12 times per year) of the drugs without considering the ecology of the parasites, has led to the development of resistance of the parasites to drugs**.**

Analysis of resistance mechanisms in several organisms is warranted as their general biochemical framework of resistance is often similar. Cells may evade drug action by hiding in sanctuaries; drug uptake may be thwarted by loss of uptake systems or alteration of membrane composition; once inside, drugs may be inactivated, excreted, modified and excreted, or routed into vacuoles; drug activation mechanisms may be suppressed or lost; the interaction of drug with the target may be made less effective by increasing the level of competing substrates or by altering the target to make it less sensitive to the drug; the cell may learn to live with a blocked target by passing the block [33].

The consensus is that anthelmintic resistance appears to be a pre-adaptive heritable phenomenon with the gene or genes conferring resistance being present within the parasite population even before the drug is used for the first time. Under these circumstances, resistance arises as a result of selection through the exposure of the worm population to an anthelmintic. When an animal has optimally exposed to an anthelmintic the only worms that should survive are those that carry the genes that confer resistance. For a short period (until the animal becomes re-infected with drug-susceptible worms from pasture) the resistant survivors are the only worms laying eggs and in this way, the gene pool for resistance is increased. The rate of development of resistance is influenced by many factors, of them, significant ones are described here**.**

Detection of Anthelminthic Drug Resistance

Different methods have been described to detect the presence of resistance to anthelmintic. These methods can be divided into in vivo and In vitro techniques. The in vivo methods are suitable for all types of anthelmintic, including those that undergo metabolism in the host to chemically active compounds. In vitro techniques offer rapid, sensitive, and considerably more economic methods of screening but suffer from certain limitations [32].

In vivo Tests: Invivo tests include the (I) Worm Reduction Test (WRT) and (II) The Faecal Egg Count Reduction Test (FECRT). For The worm reduction test, animals are necropsied at the end of the trial, after which the remaining worms in the intestinal tract of the treated animals are compared with those from animals that did not receive any treatment. For the FECRT, the change in egg excretion after treatment is compared, depending on the Study design, with either those before treatment of the same animal or with those from animals that did not receive any treatment.

Faecal Egg Count Reduction Test (FECRT):-This is the most common test to study anthelmintic resistance. The ability of the anthelmintic in question to reduce the concentration of eggs per gram of feces (EPG) by more than 95 percent, measured 10-14

days after treatment, in comparison with the EPG measured at the time of treatment. Failure to do so is indicative of resistance. This test was originally designed for sheep, but can be used also for cattle, swine, and horses. A cut-off value for drug efficacy in FECRT is 95% and 90%, macrolides and benzimidazoles/ pyrantel, respectively.

The Controlled Test: The controlled test is considered the gold standard in measuring the efficacy of anthelmintics, which is the most reliable method of assessing anthelmintic efficacy against mixed nematode infections. This test the efficacy of an anthelmintic by comparing parasite populations in groups of treated and recommended untreated animals. 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 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 and is not extensively used except in cases of special interest or when confirmation of resistance is required at the species level and for evaluation of the effect on larval stages. 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.

In vitro Test: Several different in vitro tests are available but the majority are almost exclusively used for research purposes. These tests can be used to quantify the level of resistance but they require considerable technical expertise and in some cases, expensive laboratory equipment. Ideally, these tests require mono-specific infections. The maintenance of standard laboratory strains, both drug-susceptible and resistant is necessary for comparative purposes. The main in vitro bioassays are listed in Table 1,2 [20].

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 are used to calculate the 50% of the 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.

The principle is based on the determination of the proportion of the eggs that fail to hatch in the solution of increasing drug concentration about the control wells enabling the user of the test to develop a dose-response line plotted against the drug concentration [20]. The long-term stability of thiabendazole in solutions of DMSO is not known but a reduction in anticipated concentrations may occur when stock solutions are diluted in water [34].

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 parasites, as embryonated proceeds. The test has only been shown to work

Table 1: Some drugs are used in the treatment of helminths in livestock.

Nematodes	Trematodes	Cestodes
Benzimidazoles	Praziquante	Benzimidazoles
Ivermectin	Closantel	Niclosamide
Levamisole	Triclabendazole	
Pyrantel	Nitroxynil	
Piperazine	Oxyclozanide plus	
Closantel		
Emodepside		

Table 2: Bioassays for the diagnosis of anthelmintic resistance.

List of Assays	Application
Egg hatch Assay	Benzimidazoles/levamisole/morantel
Larval paralysis	Levamisole/morantel
Tubulin binding	Benzimidazoles
Larval development	All drugs
Adult development	Benzimidazoles

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.

Larval Paralysis and Motility Assay:- The principle is that it estimates the proportion of the third-stage larvae in tonic paralysis after incubation with a range of levamisole and drug concentration to differentiate between resistance and susceptible strain of parasites. It is relatively easy to carry out and has fairly good reproducibility of the test [20]

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. LDA is the only one that allows the detection of resistance against all drugs irrespective of their mode of action. In this test, nematode eggs isolated from fecal samples are applied to the wells of a microtiter 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.

Tubulin Binding Assay:- The test is based on the differential binding of benzimidazoles to tubulin, an intracellular structural protein from susceptible and resistant nematodes. Tubulin binding assay involves the incubation of a crude tubulin extract from adult parasites, infective larvae, or eggs, with a titrated benzimidazole until equilibrium is reached. The mechanism of benzimidazole resistance appears to be associated with a reduced affinity of tubulin for the anthelmintics. The free, unbound drug in test suspension after incubation is removed using charcoal and the tubulin-bound label is sampled and counted by liquid scintillation spectrophotometry. Tubulin extracts from resistant parasites bind substantially less strongly than those from susceptible parasites. The test is considered to be rapid, highly reproducible, and sensitive to minor changes in the resistance

status of parasite populations, but it is unsuitable for routine field assays [20].

Adult Development Assay:- The adult development assay is used for detecting benzimidazole resistance in trichostrongylid nematodes has advanced significantly [20], and H. contortus has been cultured through to the adult egg-laying stages, although this test is mainly for research purposes.

Molecular based tests: The most common molecular mechanism that confers benzimidazole resistance in trichostrongyles in small ruminants involves a phenylalanine to tyrosine mutation at residue 200 of the isotype 1 β-tubulin gene. However, in addition, a similar mutation at codon 167 may be involved in benzimidazole resistance in nematodes. An allele-specific polymerase chain reaction (AS-PCR) has been used to detect this mutation in H. Contortus and Teladorsagia circumcincta adult and larval stage. The key issue is that only when a diagnosis based on using pooled larval DNA samples can be obtained will it be possible to bring molecular-resistant testing to routine use. Testing representative numbers of single stages are prohibitively expensive. Also, the available molecular tests mainly address resistance in species where the problem is widespread and in some cases may be too common to justify testing. The most common molecular mechanism that confers BZ resistance in trichostrongyles in small ruminants involves a phenylalanine to tyrosine mutation at residue 200 of the isotype 1 b-tubulin gene [34].

MANAGEMENT OF ANTHELMINTIC DRUG RESISTANCE

The key areas of concern in the management of anthelmintic resistance throughout the world are A) Drug-related factors (pharmacokinetics, formulation, and mode of application of anthelmintics). B) Management-related factors (incorrect dosing of anthelmintics, frequency of anthelmintic treatment, use of the same anthelmintic class for several years, pasture management of livestock). C) Parasite-related factors (number of nematodes in refugia, frequency of genes for resistance in an unselected parasite population, genetic factors as the mode of inheritance, fitness, and fecundity of resistant nematodes, generation time.

Considering the increasing concern regarding the development of drug resistance, the use of pharmacology-based information is critical to design successful strategies for future helminth parasite control in livestock. Integrated pharmacokinetic/pharmacodynamic and clinical pharmacology knowledge is required to preserve both well-established and modern anthelmintics. Assessment of drug disposition in the host and comprehension of the mechanisms of drug influx/efflux/detoxification in different target helminths have signified relevant progress in anthelmintic therapy in ruminants. Moreover, different pharmacokinetic-based approaches to enhance parasite exposure (pharmacokinetic optimization) and the use of a mixture of molecules from different chemical families (drug combinations) have been assessed as valid strategies to control resistant parasites and to slow the selection for further resistance [35].

Alternatives to the use of chemical compounds such as grazing management, improving the resistance of the parasites through selective breeding, vaccination, and provision of good nutrition are also of paramount importance. Control of pasture can reduce the impact of worm infection in livestock. Another approach is through the use of the pasture for different animals at different times such as bringing equine or cattle to the pasture for one season and using the pasture for sheep grazing in the next season. The reason is that sheep and cattle or equines do not share many of the important helminth parasites such as *Haemonchus contortus*. However, implementation of this method needs good knowledge about the epidemiology of the helminth parasites that are endemic to that area, such as the knowledge about the time at which the helminth eggs are hatched and the larval populations reach the infective stage.

A safe pasture has not had sheep or goats grazed on it for 6 months during cool/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 of susceptible animals to large numbers of infective larvae (L3). There is considerable evidence that part of the variation in resistance to nematode infection is under genetic control. Resistance is most likely based on the inheritance of genes that play a principal role in the expression of host immunity. Based on survival of the fittest management conditions, several breeds of sheep around the globe are known to be relatively resistant to infection. Such breeds include Scottish Blackface, Red Maasai, Romanov, St. Croix, Barbados Blackbelly, and the Gulf Coast Native [29].

The most promising vaccine for small ruminant worms is based on a ‘‘hidden gut’’ antigen and specifically targets H contortus. This antigen is derived from the gut of the worm and, when administered to the animal, antibodies are produced. When the worm ingests blood during feeding, it also ingests these antibodies. The antibodies then attack the target gut cells of the worm and disrupt the worm’s ability to process the nutrients necessary to maintain proper growth and maintenance, thus killing the worms. This vaccine has been tested successfully only in sheep under experimental conditions and has had limited success under field conditions.

On the other hand, reducing the host’s exposure to infection through biological control on pasture such as by using nematophagous or nematode-trapping fungi has also shown great promise. Research with nematode-trapping fungi has documented the potential as a biological control agent against the free-living stages under experimental and natural conditions. These fungi occur in the soil/ rhizosphere throughout the world where they feed on a variety of free-living soil nematodes. These fungi capture nematodes by producing sticky, sophisticated traps on their growing hyphae. Of the various fungi tested, Duddingtonia flagrans have the greatest potential for survival in the gastrointestinal tract of ruminants. After passing through the gastrointestinal tract, spores germinate and looped hyphae trap the developing larval stages in the fecal environment.

This technology has been applied successfully under field conditions and is an environmentally safe biological approach for the control of worms under sustainable, forage-based feeding systems. Biological control of parasitic nematodes in livestock, therefore, aims to establish a situation where the grazing animals are exposed to a low level of infective larvae, at which naturally acquired immunity will develop in the animals.

CONCLUSION AND RECOMMENDATION

At present, the problem of anthelmintic resistance occurs in several genera and classes of helminths with all three groups of commercially available anthelmintics as:-Benzimidazoles, Imidazothiazoles, and Macrocyclic Lactones. The main form of parasite control for most farmers 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 helminths 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 diagnosed through in vivo and in vitro techniques. But the fecal egg count reduction test is the best at the farm level in the field even though the 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:-.

- Use of correct dose supply, rotation anthelmintics, and the right dose in the right way at the right time.
- Reducing dependence on anthelmintic treatment rather, than using alternative worm control
- Biological control of parasites is a good management strategy to overcome resistance development.
- Avoid frequent and unnecessary treatment with anthelmintics.
- Veterinarians and all concerned stakeholders should establish the correct strategies to control and monitor drug quality.
- Frequent training of the local veterinarian and farmers, on how to use and handle newly introduced drugs available on markets.

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