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The Mechanisms of Action, Effects and Resistance Profiles of Anthelmentics Used in the Treatment of Ovine Fasciolosis

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Abstract: Fasciolosis is the common parasitic disease affecting both humans and animals all over the world. *Fasciola hepatica* and *Fasciola gigantica* are the two liver flukes commonly reported to cause fasciollosis in sheep. The disease affects the liver parenchyma and bile ducts of numerous animals. It is one of the major causes of liver condemnations at abattoirs and interferes with fertility and fecundity. The transmission of the disease is enhanced by cullucoide snail hosts. To prevent this serous disease, anthelmintics are recommended. Among these anthelmintic drugs, Albendazole and Triclabendazole are in line of curing the disease. Especially, the latter is the choice of drug against both species of *Fasciola* infection in humans and animals. However, reports showed that the disease is now developing resistance in some regions of the world. Hence, this review was focused on the mechanisms, effects and resistance profiles of anthelmintic drugs that are used against fasciollosis.

Key words: *Fasciola* · Resistance · Anthelmintic · Mechanism of Action · Prevention

INTRODUCTION

Ethiopia owns about 23.6 million sheep and 23.3 million goats [1] that are mainly kept by smallholder resource poor farmers. In Ethiopia, small ruminants are important sources of income for rural communities whose livelihood is largely based on livestock production [2, 3]. However, sheep production in the country is hindered by various factors including animal health constraints, inadequate nutrition, poor husbandry systems and lack of effective veterinary services [4].

Fascioliasis is among the important parasitic diseases in tropical and subtropical countries which limit productivity of ruminants. *Fasciola hepatica* and *Fasciola gigantica* are the two liver flukes commonly reported to cause fascioliasis in sheep [5]. *Fasciola* species infects mammals worldwide, mainly ruminants, but also humans can become infected. In ruminants and especially in sheep, the infection reduces feed conversion, growth and meat and milk production. Fascioliasis is a disease that affects the liver parenchyma and bile ducts of numerous animals, including humans, which causes economic losses and threatens public health. Moreover, it is one of the major causes of liver condemnations at abattoirs and interferes with fertility and fecundity [6]. Fascioliasis is a very serious disease that produces considerable loss in sheep and cattle worldwide [7]. In Ethiopia, ovine fascioliasis losses annually an estimated ETB of 48.4 million due to mortality, productivity (weight loss and reproductive wastage) and liver condemnation at slaughter [8].

Control and prevention measures of fascioliasis should be done on a preventative rather than curative. Three effective control strategies have been used which are: use of anthelmintic to reduce the number of liver fluke in the definitive hosts and the number of fluke eggs on the pasture, reduce the number of intermediate host and reduce exposure to infection by managing fluke prone areas. Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. Broad spectrum anthelmintics are effective against parasitic flat worms and nematodes [9].

The correct time to use anthelmintics is based on weather and climate conditions. Drugs play a crucial role in the control of fascioliasis. More frequent treatments are

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necessary if you use drugs that are only effective against advanced mature flukes aged 12-16 weeks or older. Using TCBZ based flukicides, the most effective drug against both early mature and adult liver flukes. The best control measures may be achieved if this drug use three times yearly. Namely; August/September: to prevent pasture from contamination and to eliminate adult flukes came from autumn and winter. January/February: to completely remove of flukes picked up during late spring and early summer. April/May: to remove flukes picked up during summer and early autumn [10]. Several drugs have been proposed for the treatment of fascioliasis mainly bithionol, praziquantel and benzimidazole (BZD) family of anthelmintics such as Triclabendazole (TCBZ) and Albendazole (ABZ) [11].

In Ethiopia, Fascioliasis infections in sheep are controlled by application of Albendazole and Triclabendazole. However, drug efficacy can be negatively affected by various factors such as under dosing, resistance arising from the exclusive use of drugs of the same mode of action for long periods of time, the use of substandard quality drugs and inappropriate use of the drugs. Misuse and smuggling of anthelmintics in many forms, such as illegal trading in open markets and irrational administration, are widespread in the study area and most parts of the country. This is due to an absence of strong regulatory system on the use of anthelmintics use. In addition, methods that can preserve and prolong the efficacy of anthelmintics and prevent the emergence of anthelmintics resistance, are very low all over the country [12, 13]. Hence, this review was focused on the mechanisms, effects and resistance profiles of anthelmintic drugs that are used against fasciollosis.

Overview on Ovine Fascioliasis: Fascioliasis is a worldwide zoonosis caused by *Fasciola* spp. and is often neglected despite its common occurrence in endemic areas and it is caused by two species of parasitic flatworms or trematodes that mainly affect the liver. Fasciolosis is one of the major constraint factors for ovine production development in Ethiopia by inflecting direct and indirect loss at different parts of the country. Ovine fasciolosis is an economically important parasitic disease of sheep caused by trematodes species of the genus *Fasciola*, which migrate in the hepatic parenchyma and establish and develops in the bile ducts [14].

In Ethiopia, both species co-exist at different altitudes. The snails of the genus lymnae are mainly involved as an intermediate host in the life cycle of fasciolosis. This Fasciola disease has three phases of clinical sign acute, sub-acute and chronic forms. Acute fasciolosis occurs as disease outbreak following a massive, but relatively short-term, intake of metacercariae. Death usually results from blood loss due to hemorrhage and tissue destruction caused by the migratory juvenile flukes in the liver resulting in traumatic hepatitis. Diagnosis of Fasciolosis is based on clinical sign, grazing history and seasonal occurrence, examination of feces by laboratory tests and post mortem examination [15].

Treatment of Ovine Fasciolosis: Treatment of infected animals will largely depend on the correct use of appropriate and registered anthelmintic. Several drugs have been proposed for the treatment of fascioliasis mainly, bithionol, praziquantel and benzimidazole (BZD) family of anthelmintics such as triclabendazole (TCBZ) and albendazole (ABZ). Triclabendazole is the most effective anthelmintic drug which can be destroys or kills all stage of Fasciola spp. Fasciolosis may be controlled by reducing the populations of the intermediate snail host or by appropriate anthelminthic treatment and the population of snail should be destroyed by applying Molluscicide and destroying the environment that suit for snail's reproduction [15].

Anthelmintics: Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. Broad spectrum anthelmintics are effective against parasitic flat worms and nematodes [9]. Anthelmintics expel parasites from the body by either stunning or killing them and without causing significant damage to the host. Many modern anthelmintics are effective against both adults and larval stages and an increasing number are efficacious against arrested or dormant larvae [17].

Three classes of anthelmintics are available for use in ruminants. These are Macrocyclic lactones (MLs), Benzimidazoles (BZs) and the Imidazolthiazoles Tetrahydropyrimidines (I-T). These three substance classes of anthelmintics vary in their mechanisms of action. An ideal anthelmintic should be efficient against all parasitic stages of a particular species, non-toxic to the host, rapidly cleared and excreted by the host, easily administered and the cost of an anthelmintic should be reasonable. Anthelmintics are generally used in two ways, namely, therapeutically, to treat existing infections or clinical outbreaks, or prophylactically, in which the timing of treatment is based on knowledge of the epidemiology [18]. The effect of anthelmintics treatment is manifested in many ways, such as enhanced growth rate, reproductive performance and wool production [19].

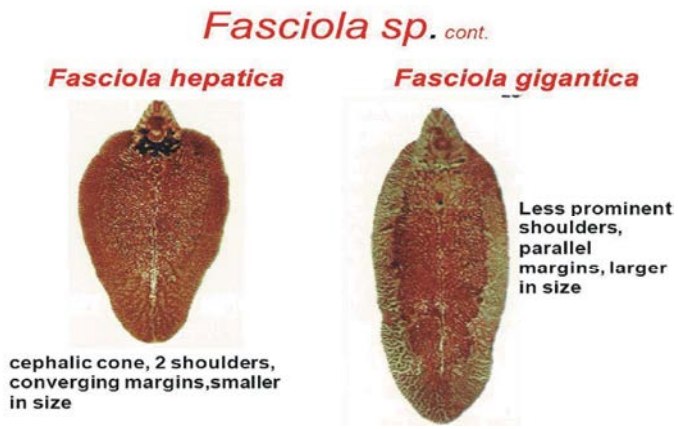

Fig. 1: Morphology of *Fasciola* species [14]

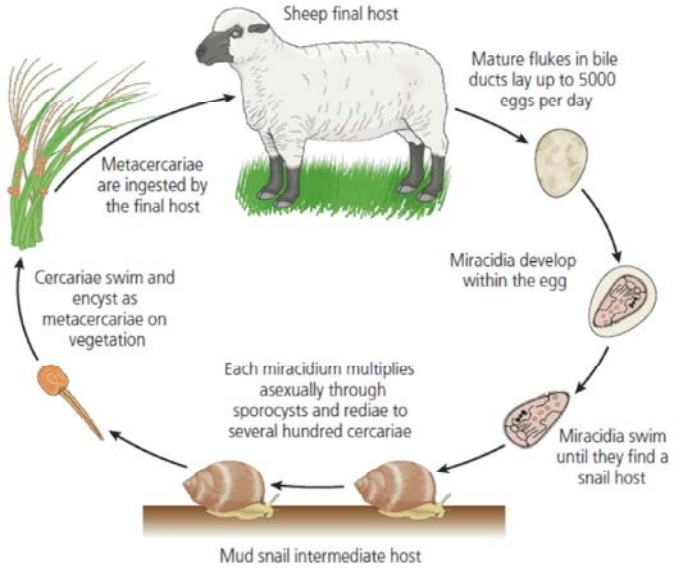

Fig. 2: The life cycle of *Fasciola* species [14]

Table 1: List of Currently Available Drugs for the treatment of Fascioliasis in Sheep worldwide

Flukicides: Active Compound(s)	Method of Administration Available	Age of F. hepatica Killed	Reports of Resistance On-Farm
TCBZ and TCBZ-based combinations	Oral, pour-on	From early immature	30 cases
Albendazole	Oral, intraruminal	From adult	3 cases
Clorsulon	Injectable, oral	From adult; from late immature for oral	3 cases
Closantel	Pour-on, injectable, oral	From late immature	1 case
Nitrox nil	Injectable	From adult	1 case

Source: [16]

Benzimidazole: Benzimidazoles (BZD) are broad-spectrum anthelmintic compounds widely used in human and veterinary medicine to control nematode, cestode and trematode infections. They are a group of anthelmintic drugs commonly used in ruminants for the treatment and prevention of infections caused by helminthes. Benzimidazole is the heterocyclic compound formed from benzene and imidazole ring containing nitrogen, oxygen, sulphur and its derivatives are of wide interest because of their diverse biological activity and clinical applications, they are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio. The BZs compounds currently marketed as anthelmintics can be grouped as BZD thiazolyls, BZD methylcarbamates, pro-BZD and halogenated BZD thiols. Only a few molecules within the BZD chemical family demonstrated activity against the trematode, *Fasciola hepatica* [20, 21].

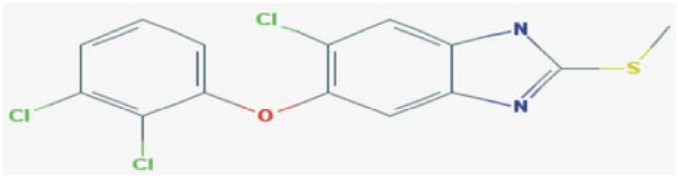

Fig. 3: Molecular structure of triclabendazole [28]

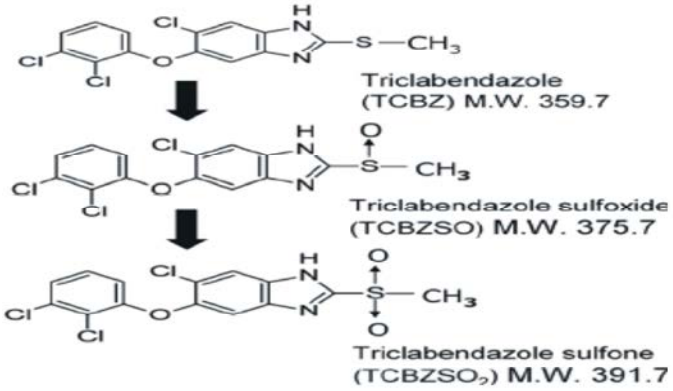

Fig. 4: Chemical structure of TCBZ and its metabolites [33]

BZD are effective against a broad range of parasites and also have wide safety margins, working at dosages of mg/kg bodyweight [22]. 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 [23]. The BZs bind to helminth â-tubulin and prevent the polymerization of the microtubules and exert its effects by interfering with cell division and the glucose uptake [24, 25].

Triclabendazole: Triclabendazole (6-chloro-5(2-3-dichlorophenoxy)-2-methyl-thio-benzimidazole), a halogenated benzimidazole-thiol derivative, is one of the major anthelminthic drugs used to control fasciolosis in sheep and cattle. TCBZ was first introduced as a flukicide during the early 1980s. It has an efficacious (> 98%) drug for both mature and immature flukes and has been used to treat and control fascioliasis [26]. Due to its efficacy for immature flukes TCBZ is the best drug of choice among other anthelminthic agents and considered as an Achilles heel in the overall control of liver fluke [27].

Triclabendazole is flukicidal BZD compounds extensively used in veterinary medicine. Effective strategies for the control of fasciolosis are mainly based on the use of drugs. TCBZ (Fasinex®, Novartis) is worldwide one of the most used drugs for the control of fasciolosis. TCBZ is usually the anthelmintic of choice against Fasciola infection in sheep, as this drug has high activity against both adult and down to 1 week old juvenile flukes [29]. TCBZ has shown no activity against nematodes. It differs from the other benzimidazole anthelmintics (e.g., albendazole, mebendazole) currently used in humans since these compounds have selective activity against nematodes and have no significant activity against flukes and other trematode helminthes [30].

Pharmacokinetics and Pharmacodynamics of Triclabendazole

Pharmacokinetics of Triclabendazole: Following oral administration, triclabendazole is absorbed from the gastrointestinal tract; absorption is increased twofold to threefold when triclabendazole is taken after a fatty meal. Triclabendazole and its metabolites attain high concentrations in the biliary tract, through which they are excreted back into the intestine over a period of several days; less than 1% of orally administered triclabendazole is distributed into breast milk [31]. Triclabendazole is oxidized to sulfoxide (the primary metabolite) and sulfone (present in lesser amounts) over the first 24 hours following oral administration.

Briefly, TCBZ is completely removed from the portal blood by the liver and cannot be detected in the plasma. It is oxidized to the sulphoxide (TCBZ.SO) and sulphone (TCBZ.SO2) metabolites, which are the main metabolites present in the plasma. Hydroxylation of TCBZ and its two

metabolites takes place in the liver, too, giving rise to the corresponding hydroxy metabolites, which are excreted in the bile [32].

The flavinmonooxygenase (FMO) pathway is the main pathway involved in the conversion of TCBZ to TCBZ.SO, while it contributes equally with the cytochrome P450 (P450) enzyme system to the sulphonation of TCBZ.SO to TCBZ.SO_2 [34, 35]. It has been shown that the rumen microflora are capable of carrying out the sulpho reduction of TCBZ.SO and OH-TCBZ.SO to TCBZ and OH-TCBZ, respectively, suggesting that the rumen can act as a reservoir of TCBZ compounds. This could serve as a slow-release system for the further availability of TCBZ in the digestive tract, from where it could be absorbed and passed to the liver [35]. TCBZ can also be oxidized to TCBZ.SO by digestive microflora prior to its absorption or by the intestinal wall during absorption. It is evident, that the mechanisms of TCBZ metabolism are complex, but serve (together with the strong binding to plasma proteins) to maintain active concentrations of TCBZ compounds in the host for considerable periods of time and this undoubtedly enhances drug efficacy. Elimination of the drug is done through Fecal-Approximately 95% of orally administered triclabendazole (unchanged or as the primary metabolite) is excreted in the feces and renal approximately 2% is excreted in the urine [36].

Triclabendazole is marketed in combination with other anthelmintics and there may be interactions between the drugs that affect its pharmacokinetics. One such combination is TCBZ plus ivermectin. Ivermectin itself has no activity against trematodes such as Fasciola [37], but a recent study has shown how it can affect the disposition of TCBZ and its metabolites [38].

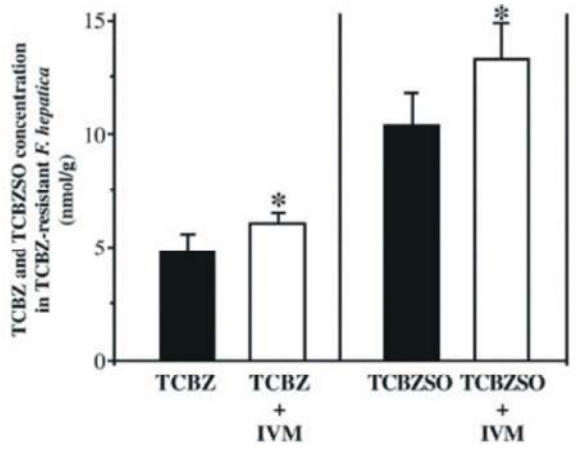

Fig. 5: Decreased efflux of TCBZ and TCBZ.SO in TCBZ-R flukes following co-incubation with Ivermectin [26]

Pharmacodynamics of Triclabendazole: The extensive metabolism of TCBZ by the host means that (potentially) the adult fluke is exposed to a number of different forms of TCBZ. Moreover, each of the compounds is capable of entering the fluke via diffusion, entry being closely related to their lipophilicity. TCBZ, TCBZ.SO and TCBZ.SO2 demonstrate a similar ability to diffuse into the fluke and their level of diffusion is higher than that for the corresponding hydroxyl compounds [34].

Entry of TCBZ compounds into the fluke has been shown to take place principally by means of diffusion across the tegument, rather than by oral ingestion, a result that is surprising, perhaps, given the strong binding of the metabolites to plasma proteins. Two approaches have been used to confirm this idea, one pharmacological and the other one is morphological. Both made use of flukes that had been ligatured to prevent oral entry of drug. Following incubation in TCBZ.SO, its concentration in the fluke was similar, irrespective of whether the fluke had been ligatured or not. When an excess of bovine serum albumin (BSA) was added to the incubation medium, in order to allow most of the drug to bind to it, the concentration of TCBZ.SO was reduced (by 85%) in both ligatured and non-ligatured flukes [34].

A parallel morphological study has been carried out, to compare drug-induced changes to the tegument and gut following incubation with TCBZ.SO. Disruption to the tegument, as assessed by scanning electron microscopy (SEM), was similar in ligatured and non-ligatured flukes, indicating that restricting the oral uptake of drug does not affect the ability of TCBZ.SO to enter the fluke and exert its effect. Incubation with TCBZ.SO in the presence of an excess of BSA led to a reduction in the level of tegumental disruption. In all experiments, the gut remained unaffected by TCBZ.SO action, suggesting that the oral uptake of drug plays only a (very) minor role in drug entry [39].

In terms of drug action, the fluke is known to play a more active role than simply being subject to the passive uptake of drug and its diffusion to the site of action, as it has been shown to be capable of metabolizing TCBZ to TCBZ.SO and TCBZ.SO to TCBZ.SO2 [34, 40]. The precise mechanism remains to be fully elucidated, but there is more evidence for an action against microtubules and microtubule-based processes than for other possibilities, such as against energy metabolism or neuromuscular co-ordination. Since TCBZ.SO is the main metabolite present in both blood plasma and bile, it has been assumed to be the active form of TCBZ. Moreover, TCBZ.SO2 has been shown to have some activity in its own right in vivo: it caused a 41% reduction in worm burden against a juvenile fluke infection in sheep [29].

The level of surface disruption induced by the three compounds varied from region to region and overall was similar, but that caused by TCBZ was slightly greater than that of produced by the two metabolites. Internal changes observed were greatest following treatment with TCBZ.SO2 and, while TCBZ.SO was also disruptive, TCBZ was far less disruptive. Combining the results for surface and internal changes, the order of severity of disruption was TCBZ.SO2, .TCBZ.SO and TCBZ. So, TCBZ.SO2 may well contribute to drug action in vivo and is not the inactive metabolite that it was previously thought to be. It may further disrupt flukes already affected by TCBZ.SO. So drug action may be the combined effect of several metabolites, rather than being due to a single compound [41].

Mechanism of Action of Triclabendazole: TCBZ is a BZD derivative and all available evidence from gastrointestinal round-worms indicates that BZD anthelmintics bind to α- and β-tubulins within the cells of the parasite, causing disruption of vital processes, such as feeding and digestion. Several morphological studies of the effects of TCBZ and its active metabolites on Fasciola species, have examined on the tegument, vitellaria and testis of the fluke; all three tissues showed significant signs of Ultra structural disruption, consistent with inhibition of microtubule-based processes which, in turn, would prevent the movement of secretory bodies from the cell bodies to the tegmental surface [26]. There is inhibition of mitosis in the vitelline and spermatogenic cells; disruption of transport processes in the tegument (the outer layer of a trematode), which leads to progressively severe damage of the tegmental surface [29]. There is also a concurrent loss of tubulin immuno sustaining in the tegmental syncytium, further implicating an interaction with tubulin as the primary mode of action of TCBZ [42].

Fasciolicidal not only against the adult worms present in the biliary ducts, but also against the immature larval stages of *Fasciola* migrating through the hepatic parenchyma; the mechanism of action is not thoroughly understood; however, triclabendazole is shown to penetrate into liver flukes by transtegumentary absorption followed by inhibition of the parasite's motility, probably related to the destruction of the microtubular structure, resulting in the death of the parasite; the immobilizing effect is paralleled by changes in the parasite's resting tegumental membrane potential, strongly inhibiting the release of proteolytic enzymes, a process that appears critical to the survival of the parasite [43]. Recently, TCBZ was reported to inhibit adenylatecyclase activity in yeast and/or inhibit the association of GTP-Ras with adenylatecyclase [44]. Most of the studies on the mechanism of action of TCBZ have been carried out with TCBZ.SO. The precise mechanism remains to be fully elucidated, but there is more evidence for an action against microtubules and microtubule-based processes than for other possibilities, such as against energy metabolism or neuromuscular co-ordination, [29].

Efficacy of Fascilocide Drugs: Not all compounds are equally effective against all stages of development of Fasciola species in the body. Early effective treatment is the cornerstone of helminthes control. Effective drugs will exhibit a selective toxicity for the pathogen as compared to the host [45]. For the treatment of acute Fasciolosis, it is essential to choose a product highly effective against the juveniles that damage the liver parenchyma. For chronic disease, a compound active against adult fluke is required. Triclabendazole (Fasinex) is considered as the most common drug due to its high efficacy against adult as well juvenile flukes. It is ovicidal and well kills any Fasciola species eggs present in the bile duct or the alimentary tract at the time of treatment [7]. The efficacy of the main compounds used to treat fasciolosis and their spectrum of activity against flukes of different ages is summarized in Figure 6.

Monitoring the therapeutic efficacy of anthelmintics is therefore a fundamental component of treatment strategies. As the parasite evolves continuously to develop resistance to drugs, continuous global monitoring and reporting of drug efficacy and parasite resistance are needed [4]. Only a program of surveillance of therapeutic efficacy and anthelmintic drug resistance will allow detection of changing patterns of parasite susceptibility and timely revision of national and global parasite treatment policies [30].

Efficacy of Triclabendazole: TCBZ was first introduced as a flukicide during the early 1980s. It has an efficacious (> 98%) drug for both mature and immature flukes and has been used to treat and control fasciollosis [26]. Due to its efficacy for immature flukes TCBZ is the best drug of choice among other anthelminthic agents and considered as an Achilles heel in the overall control of liver fluke [27]. This over-reliance on TCBZ to treat sheep and, to a lesser extent, cattle, has resulted in selection for flukes resistant to TCBZ. Studies on the efficacy of TCBZ have centered mainly on field trials involving pre- and post-treatment fluke counts, faecal egg count reduction (FECRT) and clinical chemistry [46].

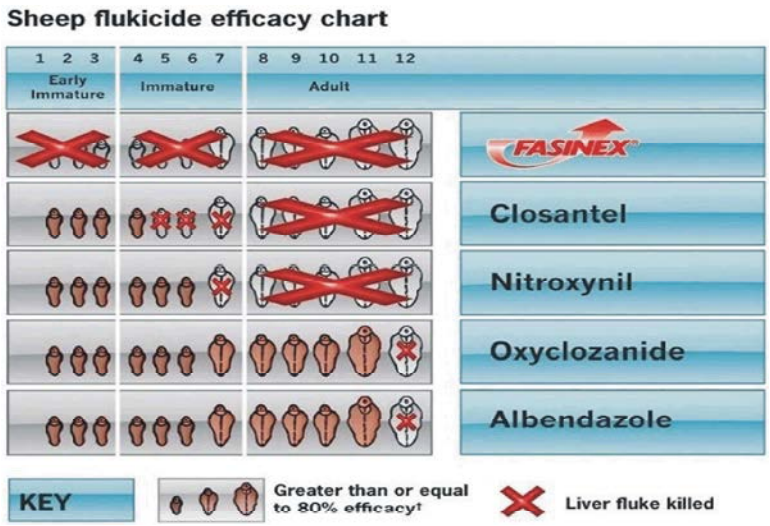

Fig. 6: Flukicides and their efficacy [7]

Unlike other BZD compounds, the halogenated derivative TCBZ has been shown to have an excellent efficacy against the mature and immature stages of Fasciola species. However, TCBZ activity appears to be restricted to the liver fluke and the lung fluke, *Paragonimus* species. Since the drug is inactive against nematodes, cestodes and other trematode parasites. The intensive use of TCBZ in endemic areas of fascioliasis has resulted in the development of liver flukes resistant to this compound, which is considered a major problem for veterinary therapeutics [46, 47].

Anthelmintics Resistance: Anthelmintic resistance (AR) is defined by Køhler as genetically transmitted loss of sensitivity of a drug in worm populations that were previously sensitive to the same drug and the heritable ability of the parasite tolerate a normally effective dose of the anthelmintic" [48]. Since resistance is inherited, the surviving worms will pass their resistance alleles to their progeny. In a worm population, alleles coding for resistance will be present as a result of mutations, also in unexposed populations. Resistance will develop if there are survival advantages for parasites carrying these alleles. Treating worms with drugs corresponding to the "resistance" alleles will give these worms an advantage and the frequency of resistant worms in the population will increase. The frequency of alleles coding for resistance at the time of exposure to a drug will be important for the rate of the development of a resistant population [48].

Frequent dosing (extensive use), adoption of common management and therapeutic strategies, long-term utilization, inappropriate handling, rapid reinfection, under dosage and inefficiency against arrested or dormant larva may be some of the reasons for the reduced efficacy and for the increasing development of AR [17]. The availability of fake and adulterated anthelmintics in the drug market in many of the under developed countries is another important contributing factor to the development of resistant strains. Generally, the presence of AR depends upon factors associated with the host, the parasite, type of anthelmintic, animal management and climatic characteristics, thus increasing the difficulties for the establishment of preventive measures, which should vary according to the animal production systems [49].

Triclabendazole Resistance: Resistance to triclabendazole was first described in the United Kingdom (UK) in the late 1990's and has now been reported on numerous occasions in fluke populations affecting sheep and cattle. Mechanisms involved in the development of resistance to the TCBZ can result from changes in the target molecule, in drug uptake/efflux mechanisms and in drug metabolism. The failure of TCBZ to kill liver fluke could be due to several factors ranging from problematic drug delivery, reduced host liver metabolism of TCBZ to active pro-drug, or management practices that select for TCBZ resistant parasites [50].

Triclabendazole resistance (TCBZ-R) has likely appeared due to poor understanding of liver fluke biology by farmers and con-founding factors, such as incorrect dosing, inappropriate product choice and lack of testing for efficacy. The high frequency of TCBZ use, effectively TCBZ mono therapy with no anthelmintic rotation, was a major contributing factor towards the development of TCBZ-R [47]. Resistance of TCBZ described in different

parts of the world mostly in European countries such as Netherland, Britain, Russia, Scotland and main land of Europe [29].

Methods for Detection of Triclabendazole Resistance: Different methods, both in vivo and in vitro methods, have been used to detect and monitor AR. Faecal egg count reduction test (FECRT) is the most used in vivo method and gives an estimation of the efficacy of the drug by comparing the egg counts pre and post treatment. The accuracy of the method depends on a correlation between egg counts and worm burdens which is not always present. From in vitro methods the egg hatch assay (EHA) was the most commonly used which is first described by Le Jambre for the detection of BZD- resistance. Modification of the original method is developed by [51] and the method is mostly used for the detection of possible BZD resistance in sheep and horses [52].

***In vitro* Method:** The EHA is based on the ovicidal properties of some BZs and on the capacity of eggs from resistant isolates to embrionate and hatch at higher concentrations than those ones from a susceptible isolate [53]. Although the EHA was originally designed to detect AR in gastrointestinal nematodes some studies have been carried out with *F. species* eggs from gall bladder and/or faeces using TCBZ and their sulphoxide metabolites [54].

A commercial formulation of TCBZ (Fasinex®) diluted in dimethyl sulfoxide (DMSO) was used to carry out the EHAs. The concentration of TCBZ in this commercial formulation was 50 mg/ml. Dilutions of 10, 40, 200, 1000 and 5000 µg/ml were prepared to obtain a final concentration in the wells of 0.05, 0.2, 1, 5 and 25 µg/ ml after adding 10 µl of each dilution to a total volume of 2 ml. In all EHAs, control wells with 10 µl of DMSO were included. Eggs from faeces were obtained by sedimentation, *F. species* eggs were directly recovered from the gall bladder and washed several times with tap water [53].

***In vivo* Method:** The main method used to identify TCBZ-R in the field has been the faecal egg count reduction test (FECRT), with the recommended post-treatment sample collection time point at 21 days [55]. Other studies using experimental infections have used 14 days for post-treatment sample collection, which may not allow sufficient time for all eggs from dead parasites to pass out of the gall bladder and be excreted. The FECRT is probably most often used, with drug treatment being regarded as successful if there is a 95% reduction in fluke egg counts by 14 days post-treatment. However, it is known that eggs can be stored in the gall bladder for several weeks, so they may still be present, even though the flukes have been successfully removed; this can lead to false positive results. Moreover, egg production by flukes ceases within 2 days of successful TCBZ treatment [56, 57].

Other disadvantages of the test include the fact that there is no standard method (i.e. sedimentation, floatation, individual or composite samples) and faecal egg counts are not related to fluke numbers; also, for diagnosis of infection, it only detects patent infections and egg shedding is irregular. Fluke counts may be more accurate but are not always carried out and this data runs into problems of trial design and how the flukes are recovered. The FECRT is often used for field cases, though it suffers from the problems outlined above and is not always linked to fluke count data [57].

Management Strategies to Prevent Development of Triclabendazole Resistance

Use of Other Drugs and Their Combinations: The chemotherapy options for the control of TCBZ-R flukes are, depending on the host species, treatment with clorsulon, nitroxynil, closantel, Albendazole, or oxyclozanide [58]. The use of dual-active flukicides has been recommended to control Fasciola infection that was resistant to TCBZ. Dual formulations have a synergistic effect (i.e., have greater efficacy than the sum of the actives), this may increase the lifespan of the respective actives. Synergy has been seen with several dual-active flukicides (e.g., TCBZ+ clorsulon or TCBZ+ luxabendazole) against TCBZ-R fluke in sheep [59].

Vaccination: An alternative approach to control TCBZ-R would be the development of a livestock vaccine for Fasciola species infection, which would reduce fluke burdens irrespective of the drug resistance. However, no commercial liver fluke vaccine exists, although several experimental vaccines for livestock are under development. No vaccine has shown reproducibly high enough efficacies (> 60%) in sheep to warrant commercial production, although the leucine amino-peptidase (LAP) vaccine has shown high efficacy (up to 89%) [60]. Thus, until a new anthelmintic is developed that kills all developmental stages, including the early immature fluke, a vaccine is the only alternative treatment that could provide ongoing control of fluke infections in livestock in regions where TCBZ-R is endemic [61].

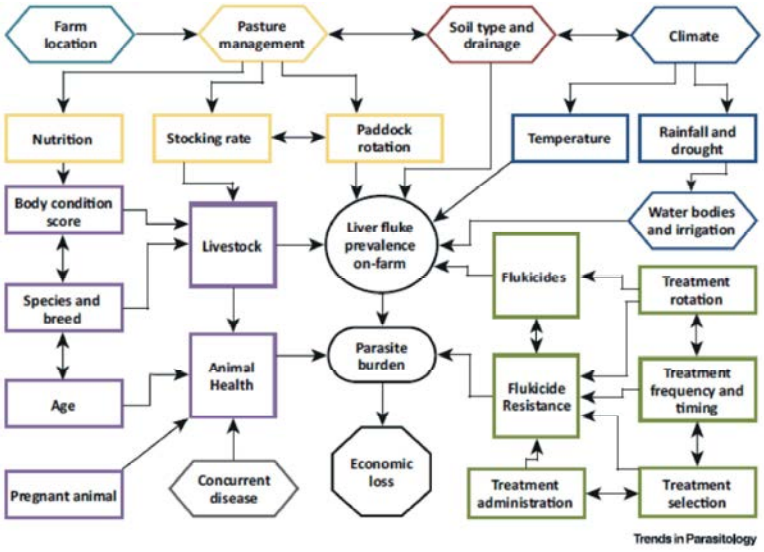

Fig. 7: Integrated Parasite Management [16]

Integrated Parasite Management for Farms: The management practices on farms generally rely solely upon anthelmintics and appear to have contributed to the development of resistance [62]. Management practices must change to preserve the longevity of existing flukicides, because the likelihood of any new flukicides coming to market in the near future is low [60]. Throughout the year, there are periods in which the risk of fluke infection is higher and these periods fluctuate depending upon location and prevailing climatic conditions [63].

Well-executed strategic treatments will minimize the need for further treatments throughout the year and, therefore, help to preserve the efficacy of existing flukicides. Regular drug efficacy testing, using FECRT to preserve the efficacy of existing flukicides is essential to allow producers to avoid using products with reduced efficacy and prevent economic losses resulting from unidentified resistance [60]. Flukicides should always be administered according to the product specifications and best-practice methods, which include: weighing individual animals or the heaviest in the herd to determine dose, calibrating drench equipment before use and during treatments, selecting the most potent formulations of product and regularly rotating effective products. In addition, we must also look at pastures, drinking water and irrigation can be better managed to decrease the likelihood of Fasciola infection. Pasture management can allocate low-risk pastures (such as newly sown paddocks, hay, or silage paddocks) to young animals during the high-risk periods, to limit the chances of parasite transmission [60].

Ethno-Veterinary Medicine: Ethno-veterinary medicine covers people's knowledge, skills, methods, practices and beliefs about the care of their animals. It provides valuable alternatives to and complements for veterinary medicine. It can play an important role in grassroots development which seeks to empower people by enhancing the use of their own knowledge and resources. Many indigenous veterinary beliefs and practices persist in a wide majority of stock raisers and farmers, particularly in the developing countries.

A number of medicinal plants have been used to treat parasitic infections in man and animals. Among the most common medicinal plants which have anthelmintics effect for Fasciola species are*, Artemisia species* plant is the most commonly one used in different parts of the world. This plant can be a promising future for the control of liver flukes which had previously shown resistance to synthetic drugs [60].

CONCLUSIONS

Treatment of infected animals with *Fasciola* is largely dependent on the correct use of appropriate and registered anthelmintic. Several drugs have been proposed for the treatment of fascioliasis mainly, bithionol, praziquantel and benzimidazole (BZD) family of anthelmintics such as triclabendazole (TCBZ) and albendazole (ABZ). Triclabendazole is the most effective anthelmintic drug which can be destroys or kills all stage of Fasciola spp. Fasciolosis may be controlled by reducing the populations of the intermediate snail host or by appropriate anthelminthic treatment and the population

of snail should be destroyed by applying Molluscicide and destroying the environment that suit for snail's reproduction. Thus, so as to maintain and prolong the lifespan of the efficacy of triclabendazole currently in use the following recommendations are forwarded. Strict supervision on the usage of Triclabendazole drugs should be implemented. Professionals and livestock owners should be well aware of about Triclabendazole drugs and its effectiveness. Regular monitoring for anthelmintic resistance in livestock production communities is essential to keep a track of their efficacy. Research should be done with aim of Triclabendazole efficacy and its resistance in Ethiopia.

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