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Review Article

Progress of WHO-essential flukicide triclabendazole and its remarkable impact in combating fascioliasis in human and animal herbivores

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ABSTRACT

Fascioliasis is a food-borne important disease in ruminants caused by *Fasciola hepatica* and *Fasciola gigantica*. WHO classifies the disease as a neglected tropical zoonosis with an estimated millions of people infected and at risk of infection. Triclabendazole has become a medicine of choice both for animal and human fascioliasis. Owing to its high flukicidal efficacy, especially, against immature flukes, it has become established as the principal anti-fluke drug on the market. Consequently, triclabendazole resistance in livestock and humans continues to be reported necessitating a better insight of the crucial aspects of this drug, including pharmacokinetics, pharmacodynamics, and understanding of the mechanism of resistance and reversal of the drug. Despite years of research, the full mystery of triclabendazole use in medicine has not been yet fully deciphered and thus the issue well deserves to be dealt with. Keeping that in mind we have endeavored to elucidate the potential progress as well as aforementioned crucial aspects of the drug.

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1. INTRODUCTION

Fascioliasis is a food-borne parasitic disease caused by the trematode species *Fasciola hepatica*, having a cosmopolitan distribution, mainly in temperate zones, and *Fasciola gigantica*, restricted to the tropical regions of Asia including Bangladesh and Africa. In recent years there has been a dramatic increase and spread of fascioliasis caused by both *Fasciola hepatica* and *Fasciola gigantica* in the world (Cwiklinski et al., 2016). The disease occurs in cattle, sheep, buffaloes, donkeys and pigs but also horses, goats, dromedaries, camels, llamas and other herbivores. Fascioliasis is also emerging as a major zoonosis and is considered to be a serious health problem in some countries of Europe, America and Oceania where only Fasciola hepatica is transmitted and in Asia and Africa where the two species overlap (Nyindo et al., 2015). This disease in human shows an increasing importance, which relies on its recent widespread emergence related to climate and global changes and also on its pathogenicity in the invasive, biliary, and advanced chronic phases in the endemic areas, mainly of developing worlds (Mas-Coma et al., 2013).

The benzimidazole derivative triclabendazole has been considered one of the major drugs used to control fascioliasis in ruminants for over 35 years, and more recently has been used successfully to treat human cases of liver fluke infection as well as Paragonimiasis (Keiser et al., 2005; Furst et al., 2012). Triclabendazole's

established trade name for veterinary use is Fasinex, while Egaten is the brand name for human use (Wessely et al., 1988; Markwalder et al., 1988).

Triclabendazole has a high efficacy (>98%) against adult flukes and, more importantly, unique efficacy against early-immature and immature flukes (Kelley et al., 2017). Other single flukicides only target mature flukes ranging in age from 8 to 14 weeks (Fairweather, 1999). Therefore, triclabendazole rapidly became the drug of choice for treating fluke infections, especially in ruminants, because it was safe and allowed producers the relative luxury of not having to test for the stage of fluke present in their livestock (Fairweather, 2005; Brennan et al. 2007). The very success of triclabendazole has almost unavoidably led to the emergence of resistance to it and this could severely compromise its upcoming use. Triclabendazole resistance does not appear to have reached the levels seen with other anthelmintics including other benzimidazoles but, given the heavy reliance of the livestock industry on antiparasitic drugs to uphold productivity and animal health, and it is a matter for grave concern. The compound represents the first-line treatment against fascioliasis in different regions of the world (Fairweather, I. 2005; Ishii et al., 2002; Yilmaz et al., 1998; Farid et al., 1986). Triclabendazole, the only medicine recommended by WHO against all cases of fascioliasis, is active against different stages of parasites, and may therefore be employed during the acute and chronic phases. Cure rates are high, while adverse reactions following treatment are usually temporary and mild. The recommended regimen is 10 mg/kg body weight administered as a single dose in both clinical practice and preventive chemotherapy interventions. In clinical practice, where treatment failure occurs, the dosage may be increased to 20 mg/kg body weight in two divided doses 12-24 hours apart (Savioli et al., 1999; WHO, 2017a). Under this review we tried to address the latest progress of triclabendazole by revisiting its efficacy, pharmacokinetics, mechanism of actions, drug resistance and global implications.

2. Morphology, Transmission and Pathogenesis of Liver Fluke

The liver fluke Fasciola spp are leaf-shaped worms, large enough to be visible to the naked eye, adult *Fasciola hepatica* measures 20-30 mm x 13 mm and adult *Fasciola gigantica* 25-75 mm x 12 mm. The worms reside in the large biliary ducts of the mammalian host (CDC, 2015). The life-cycle of Fasciola is complex. It involves a final host where the adult worm lives, an intermediate host where the larval stages of the worm develop and a carrier entailing suitable aquatic plants. The process starts when infected animals defecate in fresh-water sources. Since the worm lives in the bile ducts of such animals, its eggs are evacuated in feces and hatch into larvae that lodge in a particular type of water snail the intermediate host. Once in the snail, the larvae reproduce and eventually release more larvae into the water. These larvae swim to nearby aquatic or semi-aquatic plants, where they attach to the leaves and stems and form small cysts called metacercariae. When the plants with the small cysts attached are ingested, they act as carriers of the infection. Grass in the marshy land, watercress and water-mint are good plants for transmitting fascioliasis, but encysted larvae may also be found on many other salad vegetables. Ingestion of free metacercariae floating on water possibly detached from carrier plants may also be a possible mode of transmission (WHO, 2017b; Phil Scott, 2016; CDC, 2015).

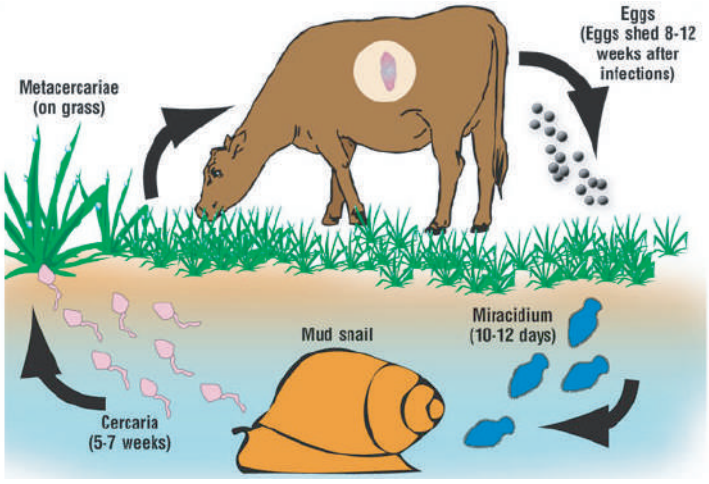

Fig 1: Fascioliasis in Herbivores (Phil Scott, 2016)

After the larvae are ingested with contaminated food or water a symptomless incubation period starts, lasting for a few days to a few months. This is followed by an acute and a chronic clinical phase. The acute phase, lasting 2-4 months, begins when the immature worms penetrate the intestinal wall and the peritoneum, the protective membrane surrounding the internal organs. From here, they puncture the liver's surface and eat their way through its tissues until they reach the bile ducts. This invasion kills the liver cells and causes intense internal bleeding. Typical symptoms include fever, nausea, a swollen liver, skin rashes and extreme abdominal pain. The chronic phase begins when the worms reach the bile ducts, where they mature and start producing eggs. These eggs are released into the bile and reach the intestine, where they are evacuated in feces, thereby completing the transmission cycle. Symptoms include intermittent pain, jaundice and anemia. Pancreatitis, gallstones and bacterial super-infections may also occur.

Animal or humans with chronic infections experience fibrosis of liver as a result of the long-term inflammation (WHO, 2017b; Phil Scott, 2016; CDC, 2015).

The severity of liver fluke infection depends on the condition of the herbivore and the number and species of fluke it ingests. Cattle with a light infection of the common liver fluke may show no symptoms. However, when infections accumulate, cattle show a gradual loss of condition or reduced milk production in dairy animals. Liver damage caused by migrating or encysted flukes results in economic loss when the animal's liver is condemned and discarded at slaughter. Sheep, goats and llamas are very susceptible to the common liver fluke and may experience high mortality when infected. The giant liver fluke in cattle, bison and moose rarely matures to produce eggs. Establishing and maintaining an infection in cattle and sheep depends on their cohabitation with infected cervids like elk or deer that are suitable hosts of *Fascioloides magna*. Under high stocking densities and limited pastures, fluke numbers may build up, resulting in heavy infections that may affect herd health and productivity and cause increased liver condemnations (WHO, 2017b; Phil Scott, 2016; CDC, 2015).

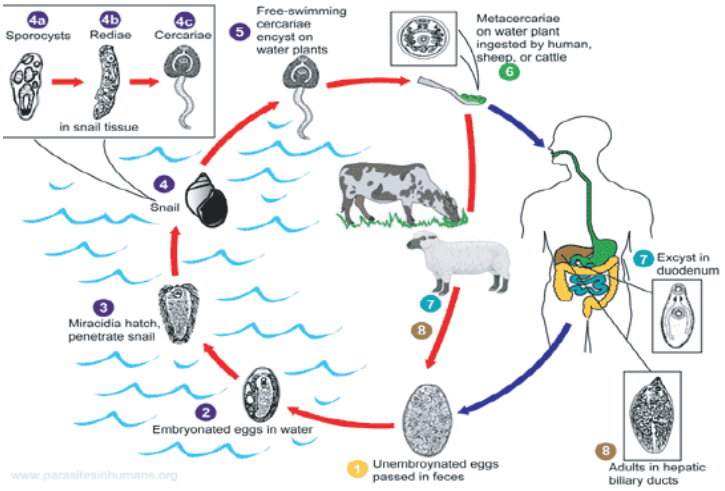

Fig 2: The flow chart depicts human fascioliasis, a trematode zoonosis of interest in public health (CDC, 2015)

3. Pharmacokinetics of Triclabendazole:

Orally administered triclabendazole (TCBZ) is well absorbed into the bloodstream. Then in the liver the drug is metabolized into a number of compounds and quickly oxidized to its effective flukicidal sulfoxide (TCBZ.SO) and sulphone (TCBZ.SO_2) derivatives. The drug TCBZ is rapidly removed by the liver. Peak plasma levels of the TCBZ.SO are reached about one day after administration. The sulfoxide itself is further metabolized to the TCBZ.SO_2 derivative, which is ineffective (Alvinerie & Galtier, 1986; Mohammed Ali et al., 1986; Hennessy et al., 1987). Peak plasma levels of the sulfone are reached 3 days after treatment. These two metabolites are usually detected in tissues and milk, whereas the parent molecule remains almost undetectable. Both metabolites bind strongly (>99%) to plasma proteins, specifically to albumin. The TCBZ.SO binds reversibly to albumins, and is partly released back in the liver tissues, which increases its bioavailability in the host. Hydroxylation of TCBZ and its two main metabolites also occurs in the liver, but

the products are secreted into the bile, mainly in conjugated form. Maximum levels of the hydoxylated compounds are reached after 8 h (OHTCBZ), 21 h (OH-TCBZ.SO) and 36 h (OH-TCBZ.SO_2), respectively, after administration (Hennessy et al., 1987).

TCBZ.SO and TCBZ.SO_2 are the two main (unconjugated) metabolites in both plasma and bile and their excretion pattern is more than 90% through bile and feces, around 2% and 1% via urine and milk, respectively (Hennessy et al., 1987). The same experiment by Hennessy (1987) also reveals that the excretory half-life of the administered drug was 6 days. Whether the fluke ingests or absorbs, an elevated excretion of triclabendazole metabolites using bile obviously exposes the parasites to the higher concentration of drug and greatly justifies its therapeutic potentiality. In ruminants, the slow passage through the complex stomach prolongs the time of triclabendazole absorption. Direct admi-nistration into the abomasums, due to the esophageal groove reflex, strongly diminishes the absorption and consequently its efficacy. In ruminants, reducing the amount of feed slows down the exit flow of the rumen and prolongs the time, and therefore the flukicidal drug remains there which increases its bioavailability and the length of its efficacy (Parasitipedia, 2017a; Fairweather, 2005).

Two routes of drug entry into the fluke are available: oral ingestion and transtegumental diffusion. The binding of TCBZ.SO and TCBZ.SO_2 to plasma proteins suggests that they enter the fluke predominantly via the mouth. However, TCBZ and all of its metabolic products can enter the fluke by diffusion, although the diffusion of the hydroxy derivatives is lower than that for TCBZ, TCBZ.SO and TCBZ.SO_2, which showed a similar ability to enter the fluke (Mottier et al., 2004). The uptake of the compounds is closely correlated with their lipophilicity (Mottier et al., 2004). Diffusion of TCBZ into the fluke is less than more lipophilic compounds such as albendazole and fenbendazole; moreover, the uptake is reduced in the presence of bile (Alvarez et al., 2004). *In vitro* study revealed that uptake of TCBZ occurs even when the *Fasciola* mouth has been closed by ligation (Bennett and Kohler, 1987), which suggests that diffusion does play a role in drug uptake in vivo. The presence of a variety of metabolites in the bile provides the opportunity for uptake by both diffusion and oral ingestion. The fluke has been shown to be capable of metabolizing parent TCBZ to its sulphoxide and sulphon metabolites (Mottier et al., 2004).

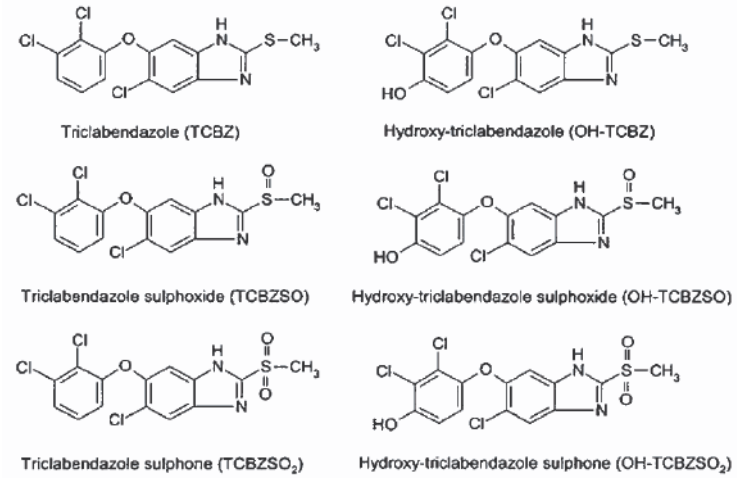

Fig 3. Chemical structures of triclabendazole and its main metabolites (Fairweather, 2005)

3.1. Mechanism of Action

The molecular mode of action of all benzimidazoles, including triclabendazole, consists in binding to tubulin, a structural protein of microtubules. These microtubules are important organelles involved in the motility, the division and the secretion processes of cells in all living organisms. In *Fasciola* and *Paragonimus*, triclabendazole are generally accepted to bind to the β-tubulins and prevent the polymerization of the microtubules of which they are part causing microtubule blockage that perturbs their vital processes, such as uptake of glucose and digestion, which eventually empties the glycogen reserves. This blockage severely compromises the whole energy management mechanism of the worms leading to

paralysis and death or explusion from the host (Parasitipedia, 2017b). Conversely, triclabendazole binds to a specific "tubulozole" receptor in the microtubules of flukes, which interferes mainly with the intracellular transport in the cells and not with cell division. For these reason it has no teratogenic effect as other benzimidazoles. Triclabendazole also inhibits protein synthesis in liver flukes, which affects enzyme production and therefore digestion, tegument maintenance, as well as egg and sperm production (Parasitipedia, 2017b; Fairweather, 2005).

In order to elucidate whether triclabendazole exerts its effect by binding with parasite β-tubulins, a number of morphological studies on the *Fasciola* tegument, vitellaria and testes with the active triclabendazole metabolites showed significant signs of ultra structural disruption connected with β-tubulins (Brennan et al. 2007). In a number of other cases it was also observed that triclabendazole treated parasite showed compromised tubulin immunostaining in the tegument syncytium, further implicating an interaction with tubulin as the primary mode of action of the drug (Robinson et al. 2002, McConville et al. 2006). Recently, Triclabendazole was reported to inhibit adenylate cyclase activity in yeast and/or inhibit the association of GTP-Ras with adenylate cyclase (Lee et al. 2013). Interestingly, *F. hepatica* has one of the most active adenylate cyclase activities in biology and this activity is associated with the membrane fraction of the parasite (Mansour, 1979). One of the first signs of triclabendazole induced damage in *F. hepatica* is tegumental blebbing and disruption of the tegumental ultrastructure (Fairweather, 2011c; Stitt et al., 1993; Stitt et al., 1994). If triclabendazole was shown to inhibit adenylate cyclase activity, the effects of triclabendazole on the metabolism of the fluke would likely be pleiotropic due to the second messenger function of cyclic adenosine monophosphate and its effects on protein kinases, carbohydrate metabolism, and motility (Mansour, 1979). These results suggest that an evaluation of the sensitivity of fluke adenylate cyclase to inhibition by triclabendazole would be informative. In addition, an analysis of sequence polymorphisms in adenylate cyclase gene(s) and/or GTP-Ras gene(s) in resistant and susceptible fluke populations is warranted, to determine whether there is any selection on those sequences in resistant flukes (Kelley et al., 2017). The mode of action of triclabendazole and/or the effects on fluke metabolism are complicated, but the advent of new technologies such as affinity purification, metabolomics, mass spectrometry or nuclear magnetic resonance could allow the target of triclabendazole to be discerned in the foreseeable future. Hence, a blend of approaches may be necessary to entirely characterize on-target and off-target effects of triclabendazole and to clearly define the mechanism(s) of action.

3.2. Dosing and Efficacy

Triclabendazole is highly effective against adults of the common liver flukes *F. hepatica, F. gigantica* and *Fascioloides magna* as well as all immature stages of the parasites, including early immature larvae of 1 to 9 weeks old. Triclabendazole is on the WHO's list of essential medicines and is the only flukicide effective against all early immature liver fluke larvae. Efficacy against these early immature larvae is important, because these larvae significantly damage the liver tissue when migrating towards the bile ducts. In contrast with most of the other benzimidazoles and their derivatives, triclabendazole has no efficacy against nematodes or cestodes. This flukicide has only a limited residual effect. This means that a single administration will kill the parasites present in the host at the time of treatment and protect against re-infestations for a few days, but not for weeks. However, since triclabendazole kills not only the adult flukes but also the immature stages, the treated animals recover more completely and for a longer period of time. It is used abundantly in small and large ruminants mainly as a drench, or in the form of boluses, tablets often mixed with other broad-spectrum nematicides such as ivermectin, levamisole, tetramisole, albendazole, fenbendazole and abamectin. It is not used on pig, poultry, dogs or cats. For administration of triclabendazole it is advisable to access to feed especially to fresh pasture, not to water 24 hours before administration. For increase bioavailability of the drug, it is better to keep the animals away from food for about 6 hours after drenching. However sick, weak, or pregnant animals should not be kept away from food and fasting animals should have access to water. The drug is sold under the brand name Egaten® for the medication of human fascioliasis and Fasinex® is a widely used brand name for veterinary use. The drug is safe during pregnancy at certain dose, however biliary colic may occur due to dying worm (Trifina et al., 2011; Stitt et al., 1993; Wessely et al., 1988).

Table 1. Dosing recommendations for Triclabendazole (Parasitipedia, 2017b; WHO, 2017a; Sanyal, 1996; Slavica et al., 2006; Foreyt et al., 2010

Species	Parasites	Dose	Delivery
Cattle	*Fasciola hepatica, Fasciola gigantica*	12 mg/kg	Oral
Bison	*Fasciola hepatica, Fascioloides magna*	40 mg/kg	Oral
Sheep	*Fasciola hepatica, Fasciola gigantica*	10 mg/kg	Oral
Goats	*Fasciola hepatica, Fasciola gigantica*	10 mg/kg	Oral
Horses	*Fasciola hepatica*	10-12 mg/kg	Oral
Deer, Elk, Impala, Moose	*Fascioloides magna*	10 mg/kg in feed for 7 days (higher doses up to 60 mg/kg)	Oral
Buffalo	*Fasciola gigantica*	12 mg/Kg (24-36 mg/Kg proven to be more effective)	Oral
Camels, Ilamas, Alpacas	*Fasciola hepatica, Fasciola gigantica*	10-15 mg/kg	Oral
Human	*Fasciola gigantica*	10 mg/Kg	Oral
	Fasciola hepatica	10 mg/kg	Oral

3.3. Triclabendazole for the medicament of human fascioliasis

In recent years, fascioliasis has come out as a major zoonotic disease, with an increase in the number of human cases, and it is revealed as a serious health problem in a number of countries like Bolivia, Chile, Equador, Peru, Egypt, Iran, Portugal, Spain, France and South-East Asia (Mas-Coma et al., 2005 and WHO, 2007). Following successful use of triclabendazole as a medicament of fascioliasis in livestock, a number of studies also proved drugs equal efficacy against *Fasciola hepatica* infections in humans (Laird & Boray, 1992 and Apt et al., 1995). This led to it being placed on the WHO Model List of Essential Medicines in 1998 (Savioli et al., 1999). It has now become the drug of choice for human fascioliasis and the human formulation is marketed as 'Egaten®'. Triclabendazole is administered as a single dose, though in severe cases may require more than one dose (Richter et al., 2002; Talaie et al., 2004). Triclabendazole is also widely used for the treatment of human paragonimiasis (Calvopina et al., 1998, 2003). Triclabendazole proved to be highly effective against selective treatment program targeted to the school children in Egypt (Cutale et al., 2005).

3.4. Triclabendazole Resistance

Resistance of *F. hepatica* to triclabendazole is first discovered in mid 1990's in a group of sheep in Australia. Contemporarily, another benzimidazole drug, albendazole was also observed to be resistant to liver flukes in small ruminants. Likewise, tricla-bendazole resistance (TCBZ-R) has also become a challenging issue in other countries such as Argentina, Ireland, New Zealand, Spain and UK and in cattle too (Overend et al., 1995). But the triclabendazole resistance issue is not as grave as for nematode resistance to benzimidazoles and other anti-nematodal drugs. Nonetheless, in some parts of the world triclabendazole is not efficiently protecting livestock populations against liver fluke infections (Overend et al., 1995). TCBZ-R has compromised fluke control in livestock in 11 countries. Resistance has likely appeared due to generally poor understanding of liver fluke biology by farmers and confounding factors, such as incorrect dosing, inappropriate product choice, and lack of testing for efficacy (Fairweather, 2011a; Fairweather, 2011b; Anon 2015). The high frequency of TCBZ use, effectively TCBZ monotherapy with no anthelmintic rotation, was a major contributing factor towards the development of TCBZ-R (Overend et al., 1995; Moll et al., 2000).

The main method used to identify TCBZ-R in the field is the fecal egg count reduction test (FECRT), with the recommended post-treatments sample collection time point at 21 days (Wood et al., 1995; Brockwell et al., 2014). 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 (Flanagan et al, 2011a; Flanagan et al., 2011b). The use of the FECRT and the new coproantigen ELISA (cELISA) (Mezo et al., 2004), in the form of a coproantigen reduction test (CRT), is now becoming common research practice (Brockwell et al., 2014; Gordon et al., 2012), but has yet to be routinely used in the field. Given that TCBZ kills most stages of a fluke infection in the host animal, a significant

reduction (>95%) in egg count or coproantigen should occur if using an oral TCBZ formulation in a susceptible fluke population (Brockwell et al., 2014). However, when adult flukicides are tested, the egg counts and coproantigen levels may not be reduced to zero at the time of retesting (21days post-treatment), even in a susceptible population, since young parasites not targeted by the adult flukicide will subsequently mature and release eggs or coproantigens. Several studies have shown that the signal in the cELISA is related to fluke numbers and could be used to indicate the relative level of fluke infection (low, moderate, or high), allowing the targeted treatment of animals, but further work is required to validate the cELISA under field conditions (Charlier et al., 2014; Fairweather, 2011c; Mezo et al., 2004; Charlier et al., 2008; Brockwell, et al., 2013).

In Europe, most reports of TCBZ-R have come from the lower-lying northwestern countries, such as The Netherlands (Moll et al., 2000). There is a growing gradient in the prevalence of *F. hepatica* west-to-east and south-to-north in Europe, with prevailing climatic and/or underlying geological conditions probably pivotal. The implication of this spread of liver fluke is of serious concern in relation to TCBZ-R, since farmers in traditionally fluke-free regions

will need to treat animals that may have been exposed to TCBZ- resistant flukes. Recent data from Great Britain revealed TCBZ-R on seven out 25 farms tested using a composite FECRT method (Daniel et al., 2012). Six of these farms were in South Wales and one in Scotland. In some cases, resistance is absolute i.e., no reduction in FEC observed. On certain farms, egg counts increased post-TCBZ treatment, which is of major concern. In Northern Ireland, a recent FECRT and CRT survey of sheep flocks, supported by fluke histology, found demonstrable TCBZ-R on five out of 13 farms tested, with absolute resistance on two farms (Hanna et al., 2015). Interestingly, a subsequent study revealed a change in farmer behavior over recent years presumably as a result of perceived TCBZ-R (McMahon et al., 2015). For example, there was a significant shift away from the use of TCBZ over time in favor of closantel, with farmers also tending to treat earlier in the year (Hanna et al., 2015).

The first incidence of TCBZ treatment failure in humans was reported in a livestock farmer in the Netherlands, with further recent reports of four cases from Chile, one case from Turkey, and seven cases from Peru (Winkelhagen et al., 2012; Gil et al., 2014; Gulhan et al., 2015; Cabada et al., 2016). Clearly, TCBZ-resistant zoonotic infections are a serious emerging issue. Given the unlikelihood of any new drugs against fascioliasis being developed in the foreseeable future, the emergence of animal resistance to triclabendazole represents an important threat (Alvarez-Sanchez et al, 2006). The only chemical options for the control of TCBZ-resistant fluke are, depending on the host species, treatment with clorsulon, nitroxynil, closantel, albendazole, or oxyclozanide (Fairweather, 1999; Fairweather, 2011c). The fact that these chemicals are available to control TCBZ-resistant flukes is of benefit, but none of these chemicals is administered as a single active dose that is able to kill the early immature stage of the parasite (<5weeks of age). This raises the issue of what to do, practically, in the face of an acute fluke outbreak, especially in sheep, when the flukes are likely, or confirmed, to be TCBZ resistant (Fairweather, 2011c). Closantel is a viable alternative for TCBZ in sheep, but it is only effective against more than 8-week-old flukes (Boray et al., 2007). The use of dual-active flukicides has been recommended to control *Fasciola* spp infection. Synergy has been seen with several dual-active flukicides (e.g., TCBZ+clorsulon or TCBZ+luxabendazole) against TCBZ-resistant fluke in sheep (Fairweather, 1999; Fairweather, 2011c). Over-dosing of some anthelmintics is toxic to animals and suboptimum dosing often leads to drug resistance (Fair-weather, 2011c). To avoid resitance, 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, where possible, regularly rotating effective products (Crilly et al., 2015).

4. Concluding remarks and future perspectives

It is obvious that various aspects of triclabendazole, for example, mechanisms of action, true extent of resistance and the pharmacodynamic interactions between the fluke and its metabolites remain to be elucidated. Reliable information on the current scenario of this drug is missing owing to a lack of dependable tests, a lack of standardized protocols and a dearth of precision in how data is assessed and reported. Given the dominant position of triclabendazole in the market-place, the spread of resistance to it is a matter of great concern. The problem needs to be addressed by updating the current knowledge before the situation becomes as serious. Human therapy should be accompanied by advocacy and educational messages to avoid uncooked

vegetables. Drug's efficacy should be maintained by enlightening the farmer to implement improved flock or herd health management program. Affinity purification of target proteins using triclabendazole as ligand, or metabolomic studies of the parasitic response to triclabendazole could be a good prospect for detection of the protein target(s) or sensitive molecular marker(s), which in turn helps for the eventual control of fascioliasis in humans and animals.

Conflict of Interest

The authors declare that there are no commercial, personal or financial relationships with this manuscript that could be construed as a potential conflict of interest.

5. REFERENCE

Alvarez, L. I., Mottier, M. L. and Lanusse, C. E. 2004. Comparative assessment of the access of albendazole, fenbendazole and triclabendazole to *Fasciola hepatica*: effect of bile in the incubation medium. Parasitology, 128:73-81.

Alvarez-Sanchez, M. A., Mainar-Jaime, R.C., Perez-Garcia, J., Rojo-Vazquez, F.A. 2006. Resistance of *Fasciola hepatica* to triclabendazole and albendazole in sheep in Spain. Veterinary Research, 159(13):424-5.

Alvinerie, M. and Galtier, P. 1986. Assay of triclabendazole and its main metabolites in plasma by high performance liquid chromatography. Journal of Chromatography, 374: 409-414.

Anon, 2015. Raising awareness of sustainable management of liver fluke in sheep. Veterinary Research, 177: 246.

Apt, W., Aguilera, X., Vega, F., Miranda, C., Zulantay, I., Perez, C., Gabor, M. and Apt, P.1995. Treatment of human chronic fascioliasis with triclabendazole: drug efficacy and serologic response. American Journal of Tropical Medicine and Hygiene, 52: 532-535.

Barduagni, P., Hassanein, Y., Mohamed, M., El Wakeel, A., El Sayed, M., Hallaj, Z. and Curtale, F. 2008. Use of triclabendazole for treatment of patients co-infected by Fasciola spp. and S. mansoni in Behera Governate, Egyptian Parasitology Research, 102: 631-633.

Bennett, J. L. and Kohler, P. 1987. *Fasciola hepatica*: action in vitro of triclabendazole on immature and adult stages. Experimental Parasitology, 63: 49-57.

Boray, J. C. et al. 2007. Liver fluke disease in sheep and cattle. Department of Primary Industries NSW, Australia. Prime facts, 446:1-10.

Brennan, G. P. et al., 2007. Understanding triclabendazole resistance. Experiment and Molecular Pathology, 82: 104-109.

Brockwell, Y. M. et al., 2013. Comparative kinetics of serological and coproantigen ELISA and faecal egg count in cattle experimentally infected with *Fasciola hepatica* and following treatment with triclabendazole. Veterinary Parasitology, 196(3-4):417-26.

Brockwell, Y. M. et al., 2014. Confirmation of *Fasciola hepatica* resistant to triclabendazole in naturally infected Australian beef and dairy cattle. International Journal of Parasitology: Drugs and Drug Resistance, 4: 48-54.

Cabada, M. M. et al., 2016. Treatment failure after multiple courses of triclabendazole among patients with fascioliasis in Cusco, Peru: a caseseries. PLoS Negl.Trop.Dis. 10: e0004361.

Calvopina, M., Guderian, R.H., Paredes, W. and Cooper, P.J. 2003. Comparison of two single-day regimens of triclabendazole for the treatment of human pulmonary paragonimiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 97: 451-454.

Calvopina, M., Guderian, R. H., Paredes, W., Chici, M. and Cooper, P.J. 1998. Treatment of human pulmonary paragonimiasis with triclabendazole: clinical tolerance and drug efficacy. Transactions of the Royal Society of Tropical Medicine and Hygiene 92, 566-569.

CDC, 2015. DPDx - Fascioliasis. www.cdc.gov. Retrieved 2015-07-16.

Charlier, J. et al., 2008. Qualitative and quantitative evaluation of coprological and serological techniques for the diagnosis of fascioliasis in cattle. Veterinary Parasitology, 153:44-51.

Crilly, J. P. et al., 2015. Triclabendazole resistant liver fluke: issues and strategies. Livestock, 20: 6-14.

Curtale, F. 2006. Treatment of fascioliasis in human infections: a reply. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100: 187-188.

Curtale, F. 2008. Treatment of human fascioliasis with triclabendazole: good news. Transactions of the Royal Society of Tropical Medicine and Hygiene, 102: 508.

Cwiklinski, K., O'Neill, S.M., S. Donnelly, S., Dalton, J.P. 2016. A prospective view of animal and human Fasciolosis. Parasite Immunology, 38(9): 558-568.

Daniel, R. et al., 2012. Composite faecal egg count reduction test to detect resistance to triclabendazole in *Fasciola hepatica*. Veterinary Research, 171: 153-157.

Fairweather, I. 2005. Triclabendazole: newskills to unravel an old (ish) enigma. Journal of Helminthology., 79: 227-234.

Fairweather, I. 2011a. Liver fluke isolates: a question of provenance. Veterinary Parasitology., 176: 1-8.

Fairweather, I. 2011b. Raising the bar on reporting 'triclabendazole resistance'. Veterinary Record., 168: 514-515.

Fairweather, I. 2011c. Reducing the future threat from liver fluke: realistic prospect or quixotic fantasy? Veterinary Parasitology., 180: 133-143.

Fairweather, I. and Boray, J. C. 1999. Fasciolicides: efficacy, actions, resistance and its management. Veterinary Journal, 158: 81-112.

Farid, Z., Trabolsi, B., Boctor, F., Hafez, A. 1986. Unsuccessful use of praziquantel to treat acute fasioliasis in children. Journal of infectious disease, 154: 920-921.

Flanagan, A. M. et al., 2011a. Comparison of two assays, a fecal egg count reduction test (FECRT) and a coproantigen reduction test (CRT), for the diagnosis of resistance to triclabendazole in Fasciola hepatica in sheep. Vetrinary Parasitology, 176: 170-176.

Flanagan, A. M. et al., 2011b. Standardization of a coproantigen reduction test (CRT) protocol for the diagnosis of resistance to triclabendazole in *Fasciola hepatica*. Veterinary Parasitology, 176: 34-42.

Foreyt, W. J. and Drew, M. L. 2010. Experimental infection of liver flukes, *Fasciola hepatica* and *Fascioloides magna* in Bison. Journal of Wild Animal Disease, 46 (1): 283-6.

Furst, T., Duthaler, U., Sripa, B., Utzinger, J. and Keiser, J. 2012. Trematode infections: liver and lung flukes. Infectious Disease Clinics of North America, 26: 399-419.

Gil, L. C. et al., 2014. Resistant human fascioliasis: report off our patients. Revista Medica De Chile, 142: 1330-1333.

Gordon, D. K. et al., 2012. On farm evaluation of the coproantigen ELISA and coproantigen reduction test in Scottish sheep naturally infected with *Fasciola hepatica*. Veterinary Parasitology, 187: 436-444.

Gulhan, B. et al., 2015. Partial hepatectomy for the resistant Fasciola hepatica infection in a child. APSP Journal of Case Reports, 6, 27.

Hanna, R. E. B. et al., 2015. Fasciola hepatica: a comparative survey of adult fluke resistance to triclabendazole, nitroxynil and closantel on selected up land and low land sheep farms in Northern Ireland using faecal egg counting, coproantigen ELISA testing and fluke histology. Veterinary Parasitology, 207: 34-43.

Hennessy, D. R., Lacey, E., Steel, J. W. and Prichard, R.K. 1987. The kinetics of triclabendazole disposition in sheep. Journal of Veterinary Pharmacology and Therapeutics, 10: 64-72.

Ishii, Y., Nakamura-Uchiyama, F., and Nawa, Y. 2002. A praziquantel-ineffective fascioliasis case successfully treated with triclabendazole. Parasitology International, 51:205-209.

Keiser, J., Engels, D., Buscher, G. and Utzinger, J. 2005. Triclabendazole for the treatment of fascioliasis and paragonimiasis. Expert Opinion in Investigational Drugs, 14: 1513-1526.

Kelley, J. M., Elliott, T. P., Beddoe, T., Anderson, G., Skuce, P., and Spithill, T.W. 2017. Current Threat of Triclabendazole Resistance in *Fasciola hepatica*. Trends in Parasitology, 1490: 12.

Laird, P. P. and Boray, J. C. 1992. Human fascioliasis successfully treated with triclabendazole. Australian and New Zealand Journal of Medicine, 22: 45-47.

Le Jambre, L. F. et al., 1999. Selection for anthelmintic resistance by macrocyclic lactones in Haemonchus contortus. International Journal of Parasitology, 29: 1101-1111.

Lee, Y. J. et al., 2013. The small molecule triclabendazole decreases the intracellular level of cyclic AMP and increases resistance to stress in Saccharomyces cerevisiae. PLoS ONE 8, e64337.

Mansour, T. E. 1979. Chemotherapy of parasitic worms: new biochemical strategies. Science, 205: 462-469.

Markwalder, K., Koller, M., Goebel, N., Wolff K. 1988. *Fasciola hepatica* infection. Successful therapy using triclabendazole. Schweiz Med Wochenschr, 118:1048-1052.

Mas-Coma, S. et al., 2005. Fascioliasis and other plant-borne trematode zoonoses. International Journal of Parasitology, 35:1255-1278.

Mas-Coma, S., Agramunt, V. H., Valero, M.A. 2013. Direct and indirect affection of the central nervous system by *Fasciola infection*. Handbook of Clinical Neurology, 114: 297-310.

McConville, M. et al., 2006. Adult triclabendazole-resistant *Fasciola hepatica*: surface and subsurface tegumental responses to in vitro treatment with the sulphoxide metabolite of the experimental fasciolicide compound alpha. Parasitology, 133: 195-208.

McMahon, C. et al., 2015. Liver fluke control on sheep farms in Northern Ireland: a survey of changing management practices in relation to disease prevalence and perceived triclabendazole resistance. Veterinary Parasitology, 216: 72-83.

Mezo, M. et al., 2004. An ultrasensitive capture ELISA for detection of *Fasciola hepatica* coproantigens in sheep and cattle using a new monoclonal antibody (MM3). Journal of Parasitology, 90: 845-852.

Mohammed Ali, N. A. K., Bogan, J. A., Marriner, S.E. and Richards, R. J. 1986. Pharmacokinetics of triclabendazole alone or in combination with fenbendazole in sheep. Journal of Veterinary Pharmacology and Therapeutics, 9: 442-445.

Moll, L. et al., 2000. Resistance of *Fasciola hepatica* against triclabendazole in cattle and sheep in The Netherlands. Veterinary Parasitology., 91: 153-158.

Mottier, L., Virkel, G., Solana, H., Alvarez, L., Salles, J. and Lanusse, C. E. 2004. Triclabendazole biotransformation and comparative diffusion of the parent drug and its oxidized metabolites into *Fasciola hepatica*. Xenobiotica, 34: 1043-1057.

Nyindo, M. and Lukambagire, A. H. 2015. Fascioliasis: An Ongoing Zoonotic Trematode Infection. BioMed Research International, doi.org/10.1155/2015/786195.

Overend, D. J. and Bowen, F. L. 1995. Resistance of *Fasciola hepatica* to triclabendazole. Australian Veterinary Journal, 72: 275-276.

Parasitipedia. Triclabendazole: safety summary for veterinary use in sheep, goats and cattle (http://parasitipedia.net/index.php?option=com_content&view=article&id=2704&Itemid=2957), 2017a. Accessed 19 September 2017.

Parasitipedia. Triclabendazole, anthelmintic for veteri-nary use in cattle, sheep and goats against liver flukes (http://parasitipedia.net/index.php?option=com_content&view=article&id=2519&Itemid=2792), 2017b. Accessed 20 September 2017.

Phil Scott, 2016. Liver Fluke Control in Cattle. National Animal Disease Information service (NADIS), online edition.

Richter, J., Knipper, M., Gobels, K. and Hausinger, D. 2002. Fascioliasis. Current Treatment Options in Infectious Diseases, 4: 313-317.

Robinson, M. W. et al., 2002. Triclabendazole-resistant *Fasciola hepatica*: β-tubulin and response to in vitro treatment with triclabendazole. Parasitology, 124: 325-338.

Sanyal, P. K. and Gupta, S. C. 1996. Efficacy and pharmacokinetics of triclabendazole in buffalo with induced fascioliasis. Veterinary parasitology, 63 (1-2):75-82.

Savioli, L., Chitsulo, L. and Montresor, A. 1999. New opportunities for the control of fascioliasis. Bulletin of the World Health Organization, 77; 300.

Slavica, A. et al., 2006. Treatment of fascioloidosis *(Fascioloides magna)* in free ranging and captive red deer at eastern Croatia. Veterinary Archive, 76 (suppl.): S9-S18.

Stitt, A. W. and Fairweather, I. 1993. *Fasciola hepatica*: tegumental surface changes in adult and juvenile flukes following treatment in vitro with the sulphoxide metabolite of triclabendazole(Fasinex). Parasitology Research, 79: 529-536.

Stitt, A. W. and Fairweather, I. 1994. The effect of the sulphoxide metabolite of triclabendazole on the tegument of mature and immature stages of the liver fluke, *Fasciola hepatica*. Parasitology, 108: 555-567.

Talaie, H., Emami, H., Yadegarinia, D., Nava-Ocampo, A. A., Massoud, J., Azmoudeh, M. and Mas-Coma, S. 2004. Randomized trial of a single, double and triple dose of 10 mg per kg of a human formulation of triclabendazole in patients with fascioliasis. Clinical and Experimental Pharmacology and Physiology 31, 777-782.

Trifina, E., Spenger, J., Zandieh, S., Haller, J., Auer, H., Osterreicher, C., Klaushofer, K., Mikosch, P. 2011. Multiple liver lesions accompanied by eosinophilia - a case report of fascioliosis. Wien Med Wochenschr, 161(17-18):448-54.

Wessely, K., Reischig, H. L., Heinerman, M., Stempka, R. 1988. Human fascioliasis treated with triclabendazole (Fasinex) for the first time. Transactions of the Royal Society of Tropical Medicine and Hygiene, 82:743-744.

WHO, 2007. Report of the WHO Informal Meeting on use of triclabendazole in fascioliasis control, held at WHO Headquarters, Geneva, Switzerland, October 2006. 39 pp.

Winkelhagen, A.J.S. et al., 2012. Apparent triclabendazole resistant human *Fasciola hepatica* infection, the Netherlands. Emerging Infectious Diseases, 18: 1028-1029.

Wood, I.B. et al., 1995. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Veterinary Parasitology, 58: 181-213.

World Health Organization (WHO) of the United Nations. Report of the WHO expert on Foodborne trematode infections: Fascioliasis diagnosis, treatment and control strategy (http://www.who.int/foodborne_trematode_infections/fascioliasis/fascioliasis_diagnosis/en/), Geneva, Switzerland, 2017a. Accessed 20 September 2017.

World Health Organization (WHO) of the United Nations. Report of the WHO expert on foodborne trematode infections: Fascioliasis (http://www.who.int/foodborne_trematode_infections/fascioliasis/en/), Geneva, Switzerland, 2017b. Accessed 19 September 2017.

Yilmaz, H., Oner, A. F., Akdeniz, H., Arslan S. 1998. The effect of triclabendazole (Fasinex) in children with fasciolosis. Journal of Egyptian Society of Parasitology, 28:497-502.