Review On Trypanocidal Drugs Resistance In Bovine And Its Management

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Abstract: Livestock are of enormous importance in Africa, economically for nutritional and agricultural purposes as well as socially. But trypanosomosis is a major constraint to livestock production in sub Saharan Africa. The distribution of the disease is influenced by the existence of tsetse and other biting flies. Tsetse transmitted trypanosomosis is encountered in many parts of Africa including Ethiopia. In those tsetse's infested African countries trypanocidal drugs (isometamidium chloride, homidium salt and diaminazene aceturate) remain the principal means of animal trypanosomosis control. However, there is a growing problem that their future effectiveness may be severely reduced by widespread drug resistance, because it is very unlikely that new trypanocidal drugs will be released in to the market in the near future. So it is essential to maintain the efficacy of currently available drugs. To make this real it is important knowing the mechanisms of drug resistance, make proper detection of drug resistance by testing in ruminants, in mice, in vitro and molecular tests, and followed by the right methods to delay the development of drug resistance like use of the correct dose, changing of drugs, sanative pair treatment, increased dosage and repeative treatment, avoid the use of quinapyramine. If once trypanocidal drug resistance appear allowing integrated control measures such as reducing vector numbers to reduce the number of drug treatment will be important.

1. Introduction

Livestock are of enormous importance in Africa, economically for nutritional and agricultural purposes as well as socially. The problem of African animal trypanosomosis, also called "Nagana", was recognized by African stockmen long before the cause of the disease was known. African bovine trypanosomosis is a collective term for a group of diseases brought about by one or more of the pathogenic trypanosome species namely: _Trypanosoma vivax, Trypanosoma congolense and Trypanosoma brucei._It is a wasting disease in which there is a slow progressive loss of condition accompanied by increasing anemia and weakness to the point of extreme emaciation, collapse and death (Uilenburg, 1998).

Trypanosomosis is widespread in African continent occupying 37 countries. It covers 9 million km^2 of sub-Saharan Africa, representing about one-third of the total land, is affected by Tsetse flies (Mattioli _et al_., 2004).With in this region, some 46–62million head of cattle and other livestock species are at risk of the disease (swallow, 2000).

Tsetse transmitted trypanosomosis is an important constraint to livestock development in sub-Saharan Africa With estimated annual losses owing to the direct and indirect effects of the disease running into billions of dollars. Approximately trypanosomosis in Africa costs livestock producers and consumers USD1340 million each year (Radosits _et al_., 2007). If lost potential in livestock and crop production are considered, then trypanosomosis is costing Africa an estimated USD 5 billion per year (ILRAD, 1994).

African animal trypanosomosis is indeed considered one of the root causes of hunger and poverty in most sub Saharan Africa countries where it represents a serious impediment to sustainable agricultural rural development about 80% of land in sub Saharan Africa is tilled byhand due to the high risk of African animal trypanosomosis threatens the survival and use of draught animals (Jemal _et al_., 2005).

As a result, in tsetse infested areas, half of the human population suffers from food insecurity and that 85% of the poor living in rural areas depend on agriculture for their livelihood (Mattioli _et al_., 2004). So by considering the serious socio-economic impacts of African animal trypanosomosis on poor rural populations, the conference of the presidents of the Organization of African Union held, in Lomé (Togo) in 2000, decided the creation of the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC). The main objective of this program is to improve food

security and therefore reduce poverty by the eradication of tsetse flies. However, eliminating tsetse flies from 9 million of km^2 of the African continent is a highly ambitious, costly and laborious project (Budd, 1999). A method such as a vaccine development is unlikely to be effective in the near future due to the unlimited antigenic variation (Kinabo, 1993; Magez and Radwanska, 2009; Magez *et al.*, 2010). However, some attempts are still being made at vaccine development using internal non-variable antigens or at immunizing agent against proteins causing pathogenic effects instead of against the parasites itself. In these tsetses infested areas trypanocidal drugs are most widely used methods of trypanosomosis controls (Clausen*et al.*, 2010).

Treatment and prevention of African animal trypanosomosis nowadays relies essentially on three drugs namely: Homidium chloride (bromide), diminazene aceturate and isometamidium chloride. It is estimated that about 35 million doses per year are currently used in Africa and they remain popular with live stockowners and veterinarians because they are generally affordable and effective (Waller, 1994). However, resistance to one or more of the three-trypanocidal drugs used in cattle has been reported in many sub-Saharan Africa countries (FAO, 1998). Since most trypanocidal drugs have been in use for more than half a century, they can cause the appearance of the drug resistant strain of trypanosomes. Since there is no new products will become available in the near future, it is of utmost importance that measures are taken to avoid or delay the development of resistance and to maintain the efficacy of the currently available drugs. Professionals and livestock owners must be well aware of about drugs and drug resistance in trypanosomes (Achenef and Bekele, 2013).

Therefore, the objective of this paper is to review about drugs currently used for the treatment of trypanosomes and to give highlights on the current status and mechanisms of drug resistance, detection of drug resistance and measures to combat drug resistance.

2. Literature Review

2.1. Control of Trypanosomes

The eradication of trypanosomes from the entire African continent is an unrealistic goal. Considerable efforts have been investigated in control of this disease through the use of trypanocidal drugs, management of the vector and exploitation of the genetic resistance exhibited by indigenous breeds. There is little hope that conventional, anti-infection vaccine will be produced sometime in future. However, drug resistance developing faster than

generally thought. The control of the tsetse fly has been attempted over many decades. The decreasing efficiency of available trypanocidal drug and difficulties of sustaining tsetse control increase the imperative need to enhancetrypanotolerance through selective breeding, either within breeds or cross breeding (OIE, 2004).

2.1.1. Use of trypanotolerant cattle

Certain local breeds have developed a tolerance to trypanosome infections during the centuries spent in areas *strongly* infested by glossines. This ability, trypanotolerance, results from several biological mechanisms under multigenic control (Hanotte *et al.*, 2003). Trypanotolerant cattle can be divided into two groups: (i) the short-horned cattle represented by the breeds Baoulé, Sumba, Muturu of savannah, Lagune breed in Côte d'Ivoire, Benin, Togo, Ghana, Nigeria, Burkina Faso and Northern Cameroon and (ii) the long-horned cattle living in Southern Senegal, Mali, northern Côte d'Ivoire, Guinea, Gambia, Liberia, Sierra Leone, Bissau Guinea, Burkina Faso. Long-horned cattle are represented by the breed N'dama. Due to their small size, farmers are somewhat reluctant to breed them. This has led farmers to cross trypanotolerant livestock with Zebu to increase the size of the animals and the milk yield. The obtained crossbreds are partially trypanotolerant and represented by the breeds Borgou in Benin and Togo, Méré in Guinea, Burkina Faso, Côte d'Ivoire, Bambara in Mali and Djakoré in Senegal and Gambia (Uilenberg, 1998).

2.1.2. Vector control

Fly populations can be down-regulated by chemical control including the use of insecticides by ground application, aerial spraying, and impregnation of traps / screens or spraying / pour-on application on the back line of host animals, spraying the belly and legs of cattle. This last method is cheap and easy to implement and take advantage of the specific tropism of heamatophagous flies for the lower parts of the animals (Bouyer *et al.*, 2009). Methods such as deforestation hasbeen abandoned because of obvious environmental and economic reasons (Uilenberg, 1998). When tsetse fly population is decreased by 95% or more in isolated areas that cannot be reinvaded, eradication can be achieved by the Sterile Insect Technique (release of sterile males). This is one area wide insect pest management method where the insect pest is controlled or eradicated by affecting its reproductive capacity. It relies on the production of sterile males (target insect) in mass-rearing facilities and release in sustained numbers in the natural habitat large enough to outnumber the wild pest population (Vreysen, 2001). Males are sterilized by

radiation at the appropriate stage and then taken to the selected area and released. Eventually, so few fertile insects remain that fertile matings do not occur and the population is eliminated (Feldmann and Hendrichs, 2001).

2.1.3. Useof trypanocidal drugs

Trypanocidal drugs remain the only widely available control method for trypanosomosis. As Delespaux *et al.* (2008) showed that currently available trypanocidal drugs for use in cattle are limited to the salts of three compounds: Diminazene aceturate (Berenil, Hoechest, Veriben, Sanofi, and various other generic formulations), Homidium bromide (Ethidium, Laprovet), Homidiumchloride (Novidium, Merial) and Isometamedium chloride (Samorin/Trypamidium, Merial: Veridum. Sanofi).

There are two main strategies used when using trypanocidal drugs for controlling bovine trypanosomosis. Drugs may be used for the therapy of existing trypanosome infections, in which case they are termed as chemotherapeutic drugs. Alternatively, the drugs with a prolonged period of biological activity may be administered as suitable intervals to cattle at risk of becoming infected, in which case they are termed chemo prophylactic drugs. Some of the drugs can be used for either purpose, although dose rates and routes of administration may be adjusted for the particular circumstances (Leach and Roberts, 1981).

Chemotherapeutic drugs are mainly used where disease incidence is low and only a limited number of animals in a herd contract the disease during the course of a year. All curative drugs also possess some residual effect, but in the case of diminazene aceturate this is practically negligeable being rapidly excreted. Thus, its preventive effect is short (Uilenberg, 1998).Chemoprophylaxis implies a residual effect, as prevention depends on the persistence of the drug in the system of the animal. Often, a deposit of the drug is formed at the site of the injection where it is retained and slowly released into the circulation to maintain a concentration in the blood at a level at which no trypanosomes can exist(LemmouChi and Schacht, 1997).

Finnelle (1983) described the trypanocides used in veterinary medicine; like dimidine (diminazene aceturate-berenil) is widely used as a curative drug against *T. vivax, T. congolense* and *T. brucei* in cattle, sheep goats and equines. This drug is rapidly excreted from the circulation, phenanthridine (homidium salts) curative to *T. vivax* and *T. congolense* in cattle, sheep and goats. Isometamedium samorin (trypamidium) is widely used as curative and prophylactive drug to *T. vivax, T. congolense* and *T.brucei* in cattle, sheep, goats and equines.

2.2. Commonly Used Trypanocidal Drug

2.2.1. Diaminazene aceturate

Structurally, Diaminazene aceturate is an aromatic diamidine derived from surfen (Jensch, 1958). The compound, marketed as aceturate salts, consists of two amidinophenyl linked by atriazene bridge: p,p-diamidinodiazoamino benzenediaceturate tetrahydrate; N-1,3 diamidinophenyltriazene diaceturate tetra hydrate ($C_{22}H_{29}N_9O_6.4H_2O$, mol. wt. = 587,6)(Peregrine and Mamman, 1993).
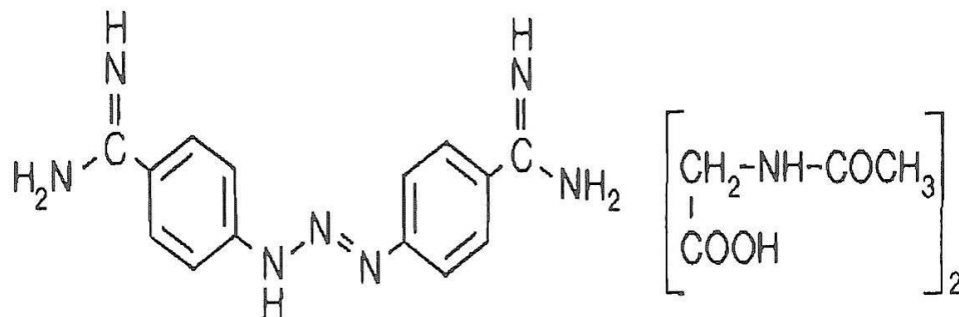

Figure 1. Molecular structure of Diaminazene aceturate(Peregrine and Mamman, 1993).

Diaminazene aceturate is marketed under the trade names Azidine, Berenil, Ganaseg, Ganasegur and Veriben as both a trypanocide and babesiocide for domestic livestock. It is recommended only for use as a therapeutic agent since it is rapidly

excreted and therefore thought to have little prophylactic activity (Fussganger and Bauer, 1958). Diminazene binds to trypanosomal kinetoplast DNA via specific interaction with sites rich in adenine-thymine (A-T) base pairs. Through this specific interaction in *trypanosomes*, Diminazene inhibits synthesis of RNA primers, resulting in accumulation of replicating intermediates, thereby inhibiting kDNA replication (Brack and Delain, 1975). Shapiro and Englung (1990) have shown that Diminazene specifically inhibits mitochondrial type II topoisomerase in viable trypanosomes.

2.2.2..Isometamidium chloride

Chemically, isometamidium chloride is known as 8-[(m-amidinophenyl-azo) amino]-3-amino-5-ethyl-6-phenylphenanthridinium chloride hypo chloride ($C_{28}H_{25}ClN_7HCl$; MW: 531.5) and differs from homidium by an additional moiety of m-amidinophenyl-azo-amine (Wragg *et al*., 1958) (Figure 2). In other words, ISM is synthesized by coupling homidium with a part of the diminazene molecule (Delespaux and De Koning, 2007).
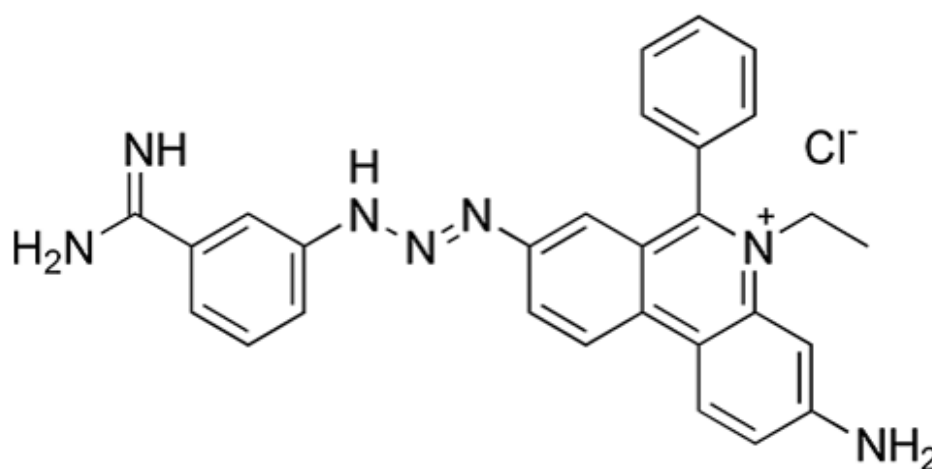

Figure 2. Molecular structure of *Isometamidium chloride* (Delespaux *et al*., 2010)

Isometamidium chloride is an amphiphilic cationic drug, which is commercialized as adark reddish-brown powder. It is less soluble in pure organic solvents and labile under low and high pH conditions and at a high temperature. Its solubility in water is about 6% at 20°C (Kinabo and Bogan, 1988). As marketed (Trypamidium, Samorin), the product contains 70% of ISMand 30% of a mixture of its two isomers and a small proportion of a bis-compound (bis designates the number of each type of ligand in the complex ion) and homidium (Novidium and Ethidium). ISM is used in aqueous solution (1 or 2%) mainly by deep intra muscular route at doses between 0.25 and 1 mg/kg b.w., depending on the risk of trypanocidal drug resistance. To clear infections with *T. vivax* and *T. congolense* in bovines and small ruminants, the drug is recommended at doses between 0.25 and 0.5 mg/kg b.w.. Moreover, it protects animals that received doses of 0.5 to 1 mg/kg b.w. fora period between 2 to 4 months (Chartier *et al*., 2000).

2.2.3. Homidium salts

Chemically, homidium is a 3, 8-diamino-5-ethyl-6-phenylphenanthridinium. Homidium is better known by its chloride salt or Novidium ($C_{21}H_{20}ClN_3$; MW: 349.86) and its bromide salt or ethidium bromide (Ethidium; $C_{21}H_{20}BrN_3$; MW: 394.31) (Figure 3).
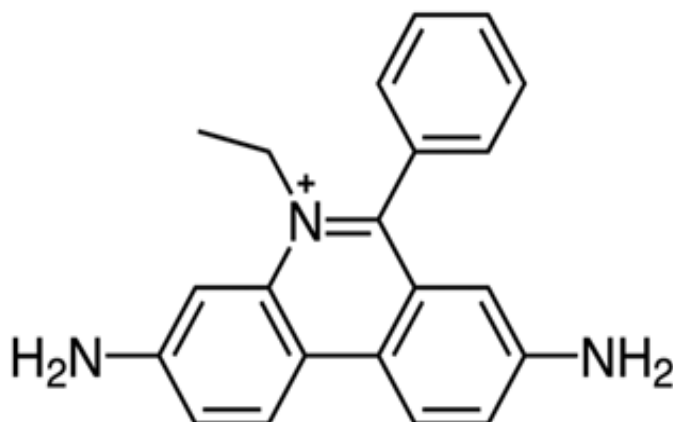
Figure 3. Molecular structure of homidium (Delespaux *et al*., 2010)

Homidium used at the dose of 1 mg/kg b.w. intra muscularly. It is active against *T. congolense* and *T.vivax* infections in cattle. The compound is essentially used as a curative drug in the field, even if some studies reported a prophylactic effect varying from 2 to 19 weeks (Stevenson *et al*., 1995). Homidium was widely used during the 1960s but due to the spread of resistance and its mutagenic activity, its use has greatly decreased. The guideline is actually to forbid it for treating animals. This is highly understandable when considering the precautions taken by laboratory technicians when using this compound (Ethidium bromide) for DNA staining (Greets *et al*., 2010).

2.3. Trypanocidal drug resistances

Trypanocidal drug resistance is defined as the decreased or absence of sensitivity of trypanosome strains to standard quality trypanocidal drugs at the dose recommended by the manufacturer and administered according to the good veterinary practice (Peregrine *et al*., 1991). It is caused by the exposure of trypanosomes to sub-therapeutic drug concentrations, resulting from under-dosing and the irrational use of drugs and the lack of proper diagnosis (Whiteside, 1962). The prolonged and frequent use of trypanocides in high tsetse challenge areas, even when used at the right doses, is also likely to cause resistance (Clausen *et al*., 1992; Greets and Holmes, 1998).

2.4. Current Situation of Trypanocidal Drug Resistance in Africa and Ethiopia

Table 1: Summary on drug resistant trypanosomes in Africa (Source; Biniam *et al*., 2015).

Country	Trypanosome species	Resist to(*)	References
Burkina Faso	Tc	I	Pinder and Authie, 1984
		I,D,H	Clausen *et al*.,1992
	Tv	I,D	McDermott *et al*., 2003; Sow*et al*., 2012
Mali	Tc	I,D	Mungube *et al*.,2012
	Tv	I	Mungube *et al*.,2012
Mozambique	Tc	I,D	Jamal *et al*.,2005
Kenya	Tc	I	Gray *et al*.,1993
	Tc	I,D,Q	Peregrine *et al*.,1997
Zambia	Tc	I,D	Chitanga *et al*.,2011
Zimbabwe	Tc	I,D	Joshua *et al*,1995
Kenya/Somalia	Tv	I	Schonefeld *et al*,1987
		H	Ainanshe *et al*,1992

Nigeria	Tv	D,H,I	Ilemobade,1979
	Tb	D,I	Kalu,1995
Sudan	Tc,Tv,Tb	H	Abdel *et al.*,1981
Uganda	Tb	D,I	Matovu *et al.*,1997
Ethiopia	Tc	D,H,I	Mulugeta *et al.*,1997
	Tc	I,D	Afewerk *et al.*,2000
	Tc,Tv,Tb	I	Tewelde *et al.*,2004
	Tv	I,D	Desalegn *et al.*,2010
	Tc	D,I	Moti *et al.*,2012

(*) D = Diminazene Aceturate; H = Homidium Bromide (Ethidium); I = Isometamidium Chloride; Q = Quinapyramine; Tc = *T. congolense*; Tv = *T. vivax*; Tb = *T. brucei*

The first case of drug resistance in trypanosomes was reported in 1967 in northern Nigeria (Naʾisa, 1967). At present, there are twenty-one African countries (some of them include Burkina Faso, Chad, Côte d'Ivoire, Ethiopia, Kenya, Nigeria, Somalia, Sudan, the United Republic of Tanzania, Uganda, Zimbabwe, the Central African Republic, Zambia, Cameroun, Mozambique, Benin, Ghana and Togo) in which trypanocidal drug resistance has been reported (Delespaux *et al.*, 2008; Chitang *et al.*,2011).

In addition, the occurrence of multiple drug resistance to DA, ISM and homidium has been reported in ten African countries (Delespaux*et al*, 2008), some of them include Nigeria, Kenya, Burkina Faso, Sudan and Ethiopia.

In Ethiopia Resistance to trypanocidal drugs has been reported from different parts of the country including Ghibe valley. Tewelde *et al* (2004), reported ISM resistant *T.congolense*, *T. vivax* and *T. brucei* in cattle in the upper Didessa valley of Western Ethiopia. Mulugeta *et al.* (1997) documented multiple drug resistance in *T. congolense* in the Ghibe river basin. Chaka and Abebe (2003) reported the existence of resistant *T. congolense* originally isolatedfrom cattle in the Southwest of Ethiopia, namely, Ghibe, Bedelle, Sodo and Arbaminch. Afewerk *et al.* (2000) also showed that clones of *T. congolense*, which were derived from primary isolates collected from relapsed cattle in the field after treatment with 1 mg/ kg b.w. of isometamidium, were resistant to both DA and ISM when tested in mice; this indicates the appearance of a multiple drug resistant *T. congolense* population in northwestern Ethiopia. The occurrence of *T. vivax* resistant population due to indiscriminate and frequent use of DA and ISM was also reported in the Tselemti Woreda (Desalegn *et al.*, 2010).

Table 2. Multiple and single trypanocidal drug resistance reported in Ethiopia (Source: Shiferaw *et al.*, 2015).

Site of study area		Drug tested	Species of parasites	References
	Ghibe valley	ISM,DA	TC	Moti *et al.*,2012
	Upper didesa	ISM	TC,TV,TB	Tewelde *et al.*,2004
Oromia Regional state	Ghibe valley	ISM,DA	TC	Chaka and Abebe,2003
	Bedele	ISM,DA	TC	Chaka and Abebe,2003
	Ghibe valley	ISM,DA,H	TC	Mulugeta *et al.*,1997
Benshangul gumz	Metekel	ISM,DA	TC	Afewerk *et al.*,2000
	Metekel	ISM,DA	TC	Afewerk,1998
SNNPRS	Sodo	ISM,DA	TC	Chaka and Abebe,2003
	Arbaminch	ISM,DA	TC	Chaka and Abebe,2003
	Omo valley	ISM,DA	TC	Ademe,1998
Tigray	Tselemet	DA,ISM	TV	Desalegn *et al.*,2010

ISM (Isometamidiumchloride), DA (Diaminazeneaceturate), H (Homidiumsalt), TC (Trypanosomacongolense), TV (Trypanosomavivax), TB (Trypanosomabrucei), SNNPRS (Southern Nation and Nationality People Regionality State)

Wide spread use, the irregular use of prophylactics drugs, their discontinuation while livestock remain at risk, the high incidence of trypanosomosis and mis use of drugs has contributed to the development of drug resistance in the population of *trypanosoma congolense* parasites(Afewerk *et al.*,2000).The magnitude of drug resistant trypanosomes across Ethiopia is not well documented. However, some study on a few isolates of *trypanosoma congolense* indicated the potential risk for the future on the greater part of tsetse infested areas, where the proportional infection rate of cattle by *trypanosoma congolense* is increasing and where dependence on regular drug treatment for trypanosomosis control, which is a common practice in Ethiopia, may lead to the risk of major drug resistance development (Abebe and Jobre, 1996).

2.5. Mechanisms of Trypanocidal Drug Resistance

2.5.1. Isometamidium chloride

Isometamidium chloride is firstly seen in the cytoplasmic compartment of the trypanosome from where the drug is driven to its primary site of accumulation i.e. the kinetoplastic compartment (Wilkes *et al*., 1995). From the outside environment, ISM is driven down the concentration gradient and enters the cell via a facilitated diffusion, which therefore does not require an expenditure of metabolic energy. Then, ISM is actively transported into the kinetoplastic compartment probably due to the mitochondrial electrical potential (Wilkes *et al*., 1997) or through an as yet unidentified energy consuming transmembrane transporter (Delespaux *et al*., 2005). When placed in an ISM-free medium, no difference in ISM diffusion out of the cell was observed between sensitive and resistant strains. Under the same conditions, a large proportion of ISM is retained sequestered within the mitochondrion of sensitive strains(Wilkes *et al.,* 1997).

Development of resistance could therefore be due to (i) a decrease in diffusion through the mitochondrial membranes (lowered mitochondrial electrical potential); (ii) modification of a possible transporter located in the inner mitochondrial membrane; (iii) increased extrusion of the drug by a transporter located in the cytoplasmic membrane or (iv) a combination of these processes (Figure 4). The direct or indirect ATP consumptions of an extrusion system(primary or secondary transporter) would simultaneously decrease the mitochondrial electrical potential and consequently the accumulation of ISM within the kinetoplast (Delespaux *et al.,* 2008).
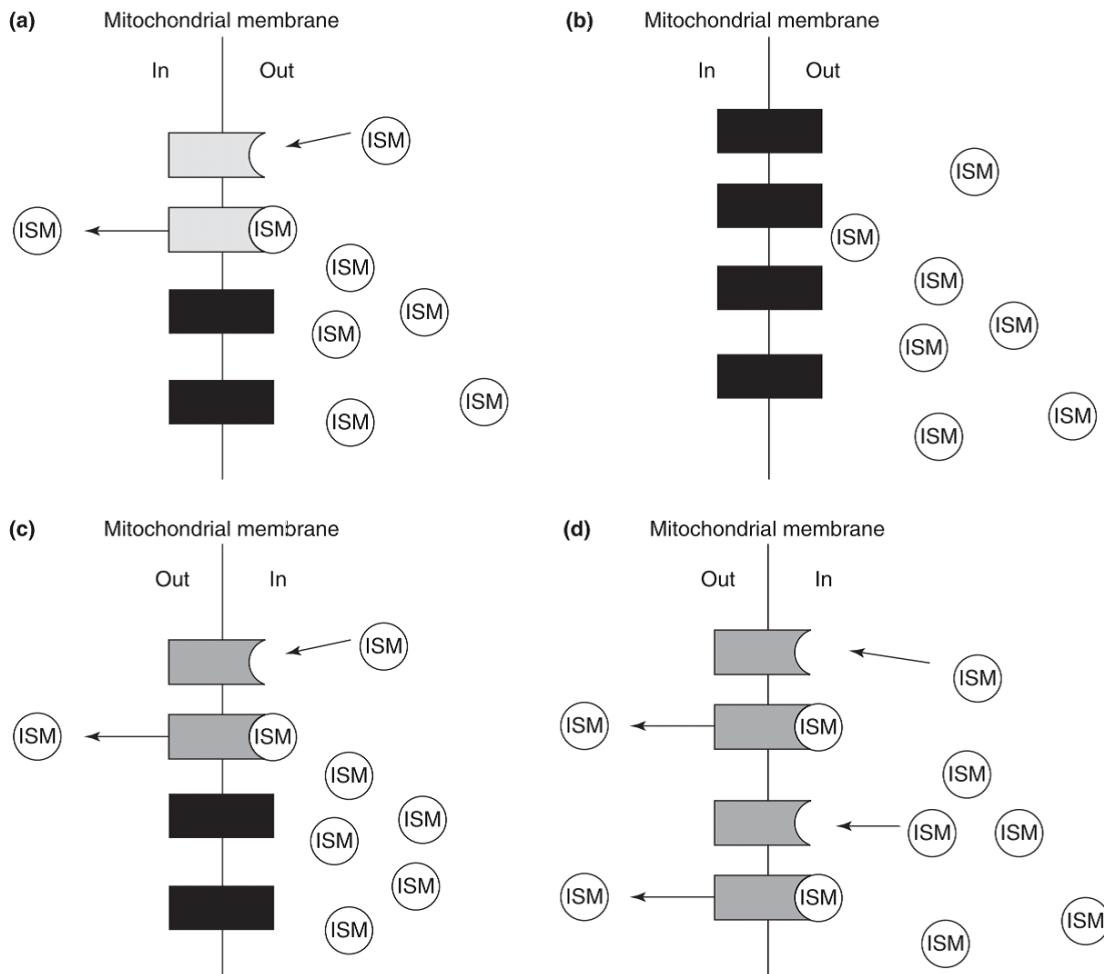

Figure 4: Model of the uptake of ISM by *T. congolense* mitochondria (Delespaux *et al*., 2008).
(a, b) Drug importer models: (a) Heterozygous wild-type (light grey) and mutated (black) importers with decreased activity. (b) Homozygous mutated importers with decreased affinity. Only the homozygous mutated importers will be resistant to ISM. (c, d) Drug exporter models: (c) Heterozygous wild-type (black) and mutated exporters with increased affinity (dark grey). (d) Homozygous mutated exporters leading to a resistant phenotype. Heterozygous and homozygous mutated exporters would both be resistant to ISM to different degrees (Delespaux et al., 2008).

ISM resistance mechanism could also include the alteration or modification of the targeted site of the drug since it has been suggested that the main mode of action of ISM was the cleavage of kDNA-topoisomerase complexes (Shapiro and Englund, 1990). The silencing of the mitochondrial topoisomerase gene by RNA interference or by the use of specific to poisomerase II inhibitors induces the progressive shrinking and disappearance of the kinetoplast DNA network (Wang and Englund, 2001; Cavalcanti *et al.,* 2003).

2.5.2. Homidium salts

Although their mutagenic activity has been known for a long time (Macgregor and Johnson, 1977), homidium chloride and especially homidium bromide or ethidium are still widely used as trypanocidal drugs. The mechanism of their antitrypanosomal action is not well understood. However, it has been shown that the drugs interfere with glycosomal functions, the function of an unusual adenosine monophosphate-(AMP) binding protein, trypanothione metabolism and the replication of kinetoplast minicircles (Wang, 1995). The mechanism of resistance by trypanosomes to these drugs is unknown. There are indications, however, that it is similar to that described for ISM (Peregrine *et al*., 1997).

2.5.3. Diaminazene aceturate

The molecular basis of resistance to diminazene in trypanosomes is not clear. It has been shown that the accumulation of diminazene was markedly reduced in arsenical-resistant *T. brucei* owing to alterations in the nucleoside transporter system (P2) (Carter and Fairlamb, 1993; Carter *et al.,* 1995). Similarly to ISM, contradictory reports have also been published on the stability of resistance to diminazene. Mulugeta *et al*. (1997), however, showed that the phenotype of multiple drug-resistant (including diminazene) *T. congolense* remained stable over a period of four years.

2.6. Detection of Drug Resistance

Several methods have been described to identify drug resistance in trypanosomes (Peregrine, 1994). At present, four types of technique are commonly used to identify drug resistance: tests inruminants; tests in mice; *in vitro* assays; and molecular detection (Shiferaw *et al*., 2015).

2.6.1. Tests in ruminants

Tests in ruminants provide direct information by using recommended doses of trypanocide. It is done by infecting a group of cattle or small ruminants with the isolate under investigation and later, when the animals are parasitaemic, treating them with various levels of trypanocide. The animals are then regularly monitored over a prolonged period (up to 100 days) to determine the effective dose (ED) and curative dose (CD) (Sones *et al.,* 1988). For these studies, the cattle or small ruminants must be kept in fly-proof accommodation or in a non-tsetse area in order to eliminate the risk of reinfection during the study (Sones *et al.,* 1989).

A useful indication of the level of resistance can be obtained from studies in ruminants (and mice) by recording the length of time between treatment and the detection of breakthrough populations of trypanosomes. The shorter the period, the greater the level of resistance (Ainanshe *et al.,* 1992). The advantages of studies in ruminants are that most trypanosome isolates of cattle are able to grow in these hosts and that the data obtained are directly applicable to the field. The disadvantages are the long duration (a follow-up of 100 days) is necessary to allow the detection of relapses) and the cost (purchase and maintenance of the animals are expensive). Furthermore, if only one isolate per animal is tested, it is usually impractical and too expensive to examine a large number of isolates (FAO, 1998).

2.6.2. Test in mice's

Either single-dose or multi-dose tests are conducted in mice to provide information on resistant trypanosome isolates from a given area. After expansion of an isolate in a donor mouse, experimental mice are inoculated with the test trypanosome isolate and treated with a trypanocidal drug. Tail blood wet smears are checked 2-3 times per week for parasites for a period of up to 60 days. The ED50 and ED95 (effective dose that gives temporary clearance of the parasite in 50% or 95% of the animals, respectively) can be calculated as can the CD50 and CD95 (curative dose that gives complete cure in 50 and 95% of the animals, respectively) (Eisler *et al*., 2001).

The advantage of the mouse assay is that it is cheaper than the test in cattle. However, there are several disadvantages with this method. Firstly, most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice (Holmes *et al.,* 2004). Secondly, although there is a reasonable correlation between drug sensitivity between mice and cattle, higher doses of drugs must be used in mice (normally ten times higher) in order to obtain results comparable to those from cattle, because of the vast different metabolic size. Thus, the curative dose for ruminants cannot be extrapolated from the assay results in mice. Thirdly, a danger further exists of selecting against particular trypanosome species, particularly in mixed infections. Fourthly, precise assessment of resistance requires a large number of mice

per isolate. Finally, it takes as long as 60 days to evaluate the drug sensitivity of an isolate (Sones *et al*., 1988).

2.6.3. In vitro assays test

In vitro tests can be used to detect resistance in *T. brucei* and *T. congolense* (Gray *et al*., 1993; Hirumi *et al*., 1993). However, the slow adaptation of trypanosomes to the experimental conditions is one of the major constraints of these tests (Clausen *et al*., 2000). The advantage with this technique is that large numbers of isolates can be examined and tests with metacyclic trypanosomes correlate well with field observations. However, in vitro cultivation of bloodstream forms is only possible using pre-adapted lines and not isolates directly from naturally infected animals (Hirumi *et al*., 1993). A potential problem associated with this lengthy time adaptation is the possible selection against trypanosomes that possess the phenotype of the original population. Further, in vitro assays are quite expensive and require good laboratory facilities and well-trained staff(FAO, 1998).

2.6.4. Molecular detection

Two molecular tools for the detection of ISM resistance in cattle have been developed. Recent studies using the amplified fragment length polymorphism (AFLP) technique allowed differentiating two isogenic clones of *T. congolense*, differing in their ISM-sensitivity phenotype. From these results, apolymerase chain reaction restriction fragment length polymorphism (PCRRFLP) test using the restriction enzyme *Mbo*II was developed and used to diagnose *T.congolense* resistant to ISM. This test is based on the polymorphism observed in the 381 bp fragment (in sensitive strains) or the 384 bp fragment (in resistant strains) of a putative gene presenting some homologies with an ABC transporter. Indeed, the gene in ISM-resistant strains of *T. congolense* has a conserved triplet insertion (GAA) coding for an extra lysine. The correlation of the *Mbo*II-PCR-RFLP tool with the standard mouse test was 85.7% for *T. congolense* isolates (N=30) collected from different areas of the tsetse fly belt (Delespaux *et al*., 2005). This number decreased to 60% (Delespaux *et al*., 2008) and 75% (Dayo, 2005) when the same test was used on 20 *T. congolense* strains originating from Ethiopia and Burkina Faso and 9 isolates from Zambia. In a recent study in Cameroon, the *Mbo*II-PCR-RFLP only identified 4 strains as resistant among 12 isolates confirmed to be resistant in the *in vivo* mouse test (Mamoudou *et al*., 2008). These results suggest the existence of an alternative mechanism to ISM resistance.

Another test, *Sfa*NI-PCR-RFLP, based on the polymorphism of a 677bp fragment of the*Tb*AT1 gene allowed the distinction between ISM-resistant and ISM-sensitive strains of *T. brucei* (Afework *et al*., 2006).

Concerning DA-resistance diagnosis, a PCR-RFLP test using the restriction enzyme *Bcl*I was also developed by the ITM. Studies made on 26 resistant *T. congolense* strains coming from various geographic areas and previously characterized for their resistance/sensitivity to DA into mice, have concluded that the *Bcl*I-PCR-RFLP technique is a powerful tool for diagnosing the presence or absence of *T. congolense* resistance to DA (Delespaux *et al*., 2006). The test is based on a single nucleotide permutation (G to A) observed in the DA-resistant strains that can be easily detected via *Bcl*I restriction of theamplicon. This single point mutation confers a Val306 to Ile306 permutation in the*TcoNT10* gene. There is a statistical correlation between the presence of this mutation and the *in vivo* resistance phenotype but it is not the mutation itself that is affecting the transport of DA within the trypanosome. Concerning *T. brucei,* a conserved set of six point mutations was described in the *TbAT1* gene of melarsoprol-resistant strains (Mäser *et al*., 1999). Notwithstanding the crucial role of *TbAT1* gene in high levels of resistance to melarsoprol, others factors such as action of the high-affinity pentamidine transporter (HAPT1), the low-affinity pentamidine transporter (LAPT), aquaporines or ABC transporters could be involved (Bridges *et al*., 2007).

2.7. Guidelines on the Delay of the Development of Trypanocidal Drug Resistance

Drug resistance in trypanosomes is likely to occur under certain circumstances such as i) under large-scale drug use; ii) by using inadequate dosing; and iii) by usingcorrect dosing with drugs that are slowly eliminated from the body. Furthermore, some trypanocidal drugs are well-known mutagenic compounds and might induce mutations, the most resistant of which are certainly selected under drug pressure. Taking into account of these factors different measures can be proposed in order to reduce the chance of drug resistance. Of these the most important measures are use of the correct dose, changing of drugs, sanative treatment, increased dosage, repeative treatment and use of combined drugs. In addition to these, care must be taken to avoid fake drugs and good quality assurance must be implemented(Greets and Holmes, 1998).

2.7.1. Use of the correct dose

Under dosing is one of the major causes of resistance development. Sub-therapeutic drug concentrations exert a strong selective pressure for the emergence of resistant clones that pre-exist in the trypanosome population. Unfortunately, under dosing occurs very frequently farmers have the tendency to underestimate the weight of their animals when they have to treat them since farmers or unskilled persons in many countries of Africa are administering drugs due to absence of strict rules about the utilization of veterinary drugs (Geerts and Holmes, 1998).

2.7.2. Changes of drugs

Changing drugs or alterative use of drugs in different time may reduce the chance of drug resistance. For example one group of chemical can be used for prophylactic purpose and the other can be applied for curative (Uilenberg, 1998).

2.7.3. Be aware of fake drugs

Drugs are either fake in their composition or are faked by dilution of the original products or by substitutions by an ordinary component apparently a like coffee or charcoal for ethidium, potassium permanganate for isometamidium. For isometamidium, one must pay attention to the information given on the packages (the examples of a shell found on a fake product labeled "for veterinary use" and in general carefully check the logo of firms. Use known products and be regular customer to trust worthy supply service (Leeflang, 1978).

2.7.4. Sanative treatment

The concepts of sanative treatment is the use of a pair of trypanocides which are chemically unrelated and therefore, unlikely to induce cross resistance (Rowlands *et al*., 1994). Diminazene and homidium, or diminazene and isometamidium can be used in the field as sanative combinations. These pairs when strategically employed can be used to maintain herd productivity in the field without the development of resistance to either of the compounds (Anene *et al*., 2001).

2.7.5. High dose and repeated treatment regimen

High dose treatment offers the best opportunity for eliminating infections with trypanosomes which express high degree of resistance to drugs. However, it must be appreciated that the scope for increased drug dosage is highly dependent on the relationship between the maximal tolerated dose and the minimal dose required to treat cure. This is a major limitation to high dose treatment with trypanocides as the margin of safety of most of them is usually quite narrow, trypanocidal drug toxicity being quite common. So this technique is helpful in the utilization of drugs with wide safety of margin. Studies on the efficacy of repeat treatments of *T. congolense* infections with diminazene aceturate indicate that such regimen may be useful especially if administered at 48 or 96 hour intervals. This tends to support the suggestion that the efficacy of trypanocides depends not only on the concentration of the drug to which the parasites are exposed but, also on the length of exposure. But this may not be true for all (Anene *et al*., 2001).

2.7.6. Avoidthe use of quinapyramine in cattle

Quinapyramine was widely used in cattle in Africa during the period 1950 to 1970. In 1976, it was withdrawn from sale for cattle use because of problems with toxicity and resistance development. It is still available foruse in camels, however, and it is likely that it is still mistakenly used in cattlein some situations in Africa(FAO, 1998). Ndoutamia *et al.* (1993) showed that, after artificial induction of resistance to quinapyramine in *T. congolense,* multiple resistances to ISM, homidium and diminazene was expressed at the level of the individual trypanosome and could be transmitted by tsetse flies.

2.7.7. Quality assurance of trypanocidal drugs

In recent years, a further issue has arisen associated with the liberalization of veterinary drug supply and market. The growing problem of poor quality drugs finding their way on to the market in some cases, products with no trypanocidal activity have been identified and in other situations compound with reduced activity have been marketed. Such products are not less effective when used by farmers, but also greatly increase the risk of drug resistance developing (especially when under dosing also allows the survival for the heterozygote resistant. Unfortunately, quality control on pharmaceutical products used in the developing world is frequently inadequate and there is already considerable evidence that the problem is widespread for a variety of pharmaceutical products (Shakoor *et al*., 1997).

2.8. Guidelines on the Management of Drug Resistance Once Present

The measures already mentioned are important in the delay of the development of resistance. Once resistance is present, however, other interventions become necessary.

2.8.1. Resistance against a single drug

When resistance to DA, ISM or homidium is present, the use of the other drug of the sanative pair is still possible. The second drug should be used with caution in order to avoid resistance development here again. Integrated control measures, such as reducing vector numbers to reduce the number of drug treatments, will be of great importance. Administration of various drugs to which the different subpopulations are sensitive, will eliminate the whole trypanosome population (Mulugeta *et al.,* 1997)

Once resistance is present, it is unwise to increase the dose of the drug. Although some temporary benefits might be obtained, such an action would inevitably increase the selection pressure and, thus, the level of resistance. The use of a double dose of diminazene (two normal doses with an interval of eight or 24 hours between them) only slightly improved the therapeutic efficacy for resistant *T. congolense* (Silayo *et al.,* 1992). Similarly, although the intravenous administration of ISM enhanced the therapeutic activity of the compound as compared with the intramuscular injection, (Sutherland *et al.,* 1992).

2.8.2. Multiple drug resistance at the level of individual trypanosomes

If multiple resistances are expressed at the level of the individual trypanosome, chemotherapy can become increasingly ineffectual. To counteract multipleresistances in such a case, intervention at the level of the vector is required. Peregrine *et al.* (1994) showed that in the Ghibe valley, Ethiopia, multipledrug-resistant trypanosome infections could be controlled effectively using an integrated approach involving tsetse fly control (targets) and chemotherapy of clinically sick animals. The relative density of the main vector, *Glossina pallidipes*, fell from an average of 1.9 flies per trap per day before the introduction of tsetse control to 0.4 flies per trapper day during the first year of the control. Simultaneously, the apparent prevalence of *T. congolense* infections fell from approximately 30 percent before the tsetse control programme to ±5 percent one year after the start of the control programme. The apparent prevalence of diminazene resistant infections decreased by about 75percent during the same period.

3. Conclusion And Recommendations

In conclusion livestock production has a great potential to rural farmers in Africa. It can be well exploited if trypanosomosis and drug resistance are controlled very well. Trypanocidal drugs are the most realistic means to control animal trypanosomosis. However, the increasing trends of drug use and drug resistance are a serious problem to cattle production in sub Saharan Africa including Ethiopia. Since there are no new products will become available in the near future, it is of utmost important to maintain the efficacy of the currently available drugs.

Based on the above conclusion the following recommendations are forwarded;
  ➢ Strict supervision on the usage of trypanocidal drugs should be implemented.
  ➢ More attention should be given to the adoption of an integrated trypanosomosis control involving the vector and the parasite.
  ➢ Professionals and livestock owners should be well aware of about drugs and drug resistance in trypanosomosis.

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