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REVIEW ON TRYPANOCIDAL DRUG RESISTANCE IN ETHIOPIA PERSPECTIVE

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ABSTRACT

Trypanosomosis is responsible for the death of 3 million heads of cattle yearly, with 50 million animals at risk in sub-Saharan Africa. The problem of trypanosomosis is still far from being solved due to the fact that trypanosomes affect multiple hosts, widespread trypanocidal drug resistance and antigenic variation displayed by trypanosomes. Trypanocidal drugs: Isometamidium chloride (ISM) and Diminazene aceturate (DA) are the most widely used drugs for control animal trypanosomosis in Ethiopia. However, there is growing concern that their future effectiveness may be severely reduced by widespread drug resistance. Because it is very unlikely that new trypanosomal drugs will be released on to the market in the future, it is essential to maintain the efficacy of the currently available drugs. So proper detection methods of drug resistance by test in ruminants, in mice, in vitro, molecular tests and xenodiagnoses and followed by the right techniques on the delay of the development of drug resistance like reduction in the number of treatments, avoidance of under dosage, change of drugs, use of national drug police and if once resistance present allowing integrated control measures such as reducing vector numbers to reduce the number of drug treatments was great importance.

INTRODUCTION

Trypanosomosis is a vector borne disease recognized to be transmitted cyclically by tsetse flies (Krafsur, 2009) and mechanically by a number of biting flies of genus diptera such as Tabanus, Hematopota, Chrysops and Stomoxys (Desquesnes et al., 2009; Kone et al., 2011). The foremost tsetse-transmitted trypanosomes includes T. congolense, T. vivax, T. brucei and T. simiae (Namangala, 2011). Mechanical transmission of T. congolense has been revealed under experimental situations and can therefore not be excluded from contributing to its feast in Africa (Desquesnes et al., 2009). In addition, T. equiperdum is transmitted sexually ((Hagos, 2010; Namangala, 2012). Moreover, Iatrogenetic transmission could also occur when using the same needle or surgical instrument on more than one animal, at sufficiently short intervals, that the blood on the needle or instrument is not dry (Desquesnes and Dia, 2003).

In trypanosome endemic areas, trypanocidal drugs: Isometamedium Chloride (ISM), Diminazene Aceturate (DA) and Homidium are the main drugs currently available to control AAT (Sahin et al., 2014; (Giordani et al., 2016). However, these drugs are fraught with a number of problems including cheaper generic forms (Geerts et al., 2010), lower standards of quality control (Chitanga et al., 2011), misuse and under dosage (Onono et al., 2013), spread of trypanocidal drug resistance (Clausen et al., 2010) and lack of new trypanocidal drugs development for field use (Delespaux et al., 2008). In addition, the use of homidium salts is no longer advisable due to their mutagenic effects (Sutcliffe et al., 2014).

The badly behaved of trypanosomosis is still far from being solved due to variation in parasites host factors, trypanocidal drug resistance and antigenic variation displayed by the trypanosomes. Till nowdays, there is no control method that can fully eradicate AAT inspite of many attempts (Steverding, 2008). The development of a vaccine against trypanosomes has failed so far because of the different variant surface glycoprotein coats displayed by parasite population during infection (Hill et al., 2005).

Trypanocidal drugs: ISM and DA have been in use for control of animal trypanosomosis before over 50 years (Giordani et al., 2016). However, currently there are increasing development of before ISM and DA resistance and treatment failures (Dagnachew et al., 2015a; Moti et al., 2015). At present, there are twenty-one African countries in which trypanocidal drug resistance has been reported (Delespaux et al., 2008; Chitanga et al., 2011) including Ethiopia (Moti et al., 2012; Hagos et al., 2014). More worryingly, strains of T. congolense resistant to both ISM and DA have been detected in several locations, Sinyangwe et al., (2004) before Zambia; Mamoudou et al., (2008) Cameroon and Moti et al., (2012) Ethiopia. Multiple drug resistance to ISM, DA and Homidium has been reported in trypanosome populations in ten African countries (Delespaux et al., 2008). It is suspected that in several other African countries, resistance is present but is yet to be demonstrated (Delespaux et al., 2008). Therefore, the objective of this review is to illustrate trpanocidal drug resistance before in Ethiopia.

2. Background

The name Trypanosoma is drived from Greek word trpano- (borer) and soma (body) because of their cork screw-like motion (Hamilton et al., 2004). The trypanosome consists of a single cell varying in size from 8 to 50 µm. The different trypanosome species differe in morphological characteristics as described by in appearance, shape and size between the various species, allowing specific identification (Maudlin et al., 2004). African Animal Trypanosomosis (AAT) or "Nagana" is disease caused by flagellated, extracellular protozoan parasites that live in animals blood, plasma, lymph and tissue of vertebrate hosts (Pathak, 2009). Trypanosome parasites cause a severe, often fatal disease in domestic animals, unlikely in wild animals cause relatively mild infections. the infected animals were more weakened as the disease progress and become unfit for work, hence the name of the disease "Nagana" which is a zulu word "powerless/useless" as reviewed by Steverding, (2008).

Drug resistance, also called drug fastness, may be defined as a loss of sensititivity by a strain of an organism to a drug to which it had previously been susceptible and it implies failure of treatment and prevention of a disease, or when the use of trypanocidal does not produce the expected outcome (cure or protection), there is a tendency to assume that drug resistant has arisen (Uilenberg, 2017; Bulcha, 2018). If no other active drugs are availabe the animal has to rely on its immune defences alone to combat the disease (Uilenberg, 1998).

3. Trypanocidal drug resistance practice in Ethiopia

The problems of drug resistance to one or more of the common trypanocidal drugs used in cattle has been reported in at least four regional state (local areas) within the country. But the currently available information on the drug resistance is drived from limited

number of cases reports, and does not give any indication of the true situation of the risistance in the whole country (region) as systematic surveys have not been fully conducted. Summarizes the list of published reports in which a number of trypanosomes isolate has been examined (Table 1). This problem of drug resistance in trypanosomes requires being spreading geographically into many regions in which trypanosomes occur. Chemotherapy and chemoprophylaxis are the most practical methods available for the control of animal trypanosomosis, but their effectiveness is being eroded by the emergence resistant trypanosomes.

Site of study area		Drug tested	Species of parasites	References
Oromia regional state	Gibe valley	ISMM & DA	T.c	Moti et al., (2010)
	Upper didesa	ISMM	T.c, T.v & T.b	Tewelde et al., (2004)
	Gibe valley	ISMM & DA	T.c	Chaka & Abebe, (2003)
	Bedele	ISMM & DA	T.c	Chaka & Abebe, (2003)
	Gibe valley	ISMM & DA	T.c	Mulgeta et al., (1997)
Benishangul e gumuz	Metekel	ISMM & DA	T.c	Afework et al., (2000)
	Metekel	ISMM & DA	T.c	Afework, (1998)
SNNPRS	Sodo	ISMM & DA	T.c	Chaka & Abebe, (2003)
	Arbaminch	ISMM & DA	T.c	Chaka & Abebe, (2003)
	Omo valley	ISMM, DA & H	T.c	Ademe, (1998)
Tigray	Tselemt	DA & ISMM	T.c	Desalign et al., (2010)

Table 1: Multiple and single trypanocidal drug resistance reported in Ethiopia. ISMM (Isometamidium), DA (Diminazine Aceturate), H (Homidium salt), Tc (Trypanosoma congolense), T.v (Trypanosoma vivax), T.b (Trypanosoma brucei) and SNNPRS (South Nation Nationality People Regional State) (Shiferaw et al., 2015).

Unfortunately, farmers can purchase a variety of trypanocidal drugs in most markets, although all trypanocidal drugs are supposed to be imported and supplied through the ministry of agriculture. The widespread use, the irregular use of prophylactics drugs, their discontinuation while livestock remain at risk, the high incidence of trypanosomosis and misuse of drugs has contributed to the development of drug resistance in the population of T. 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 T. congolense indicated the potential risk for the future in the greater part of tsetse infested areas, where the proportional infection rate of cattle by T. congolense is increasing (Abebe and Jobre, 1996) and where dependence on regular drug treatment for trypanosomosis control, which is a common practice now in Ethiopia, may lead to the risk of major drug resistance development.

4. Strategies for trypanocidal drug usage

4.1. Routine block treatments

These are generally carried out using prophylactic drugs, notably ISMM, at predetermined intervals based on the perceived duration of prophylaxis (0.5 to 1 mg/kg, intramuscularly). All animals in a herd may be treated or treatment may be targeted at a particular group of valuable or at risk animals. When routine block treatment with ISMM is practiced it is recommended that once a year, the animals are separately treated with DA in order to delay the development of drug resistance following the concept of sanative pair (Whiteside, 1960).Several chemotherapeutic regimes have been used to control trypanosomosis; the most recent regime, the routine use of isometamidium by the intravenous route. The intravenous administration of isometamidium, when used on a therapeutic basis and combined with an understanding of the tsetse problem, resulted in improved control of trypanosomosis, improved herd health and a marked increase in calving rates (Dowler et al., 1989).

4.2. Strategic block treatments

These are generally carried out using prophylactic drugs, though curative drugsmay also be used. All animals in a herd, or particularly valuable or at risk stock, are treated when challenges, as measured by the number of animals succumbing to infection, reaches predetermined threshold (Holmes, 2004).

4.3. Monitoring and tretment of individual infected animals

Cattle are monitored using standard parasitological methods. Treatment is infected animals is generally conducted using a therapeutic drug, usually diminazene aceturate (3.5 to 7 mg/kg BW, intramuscularly) (Eisler et al., 2001).

6. Causes of trypanocidal drug resistance

Trypanocidal drug resistance are 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 cuse resistance (Geerts et al., 2001). Furthermore, poor quality drugs have been finding their way on to the market in some cases, products with no trypanocidal activity have been also identified and in other situations compound with reduced activity have been marketed. Such products are not only less effective when uesd by farmers, but also greatly increase the risk of drug resistance development. Reduction in drugs accumulation by the target cell or organism and diminished drug activity in immune suppressed animals can contribute to the emergence of drug resistance. Thus, drug resistance can arise either as a consequence of changes in drug concentration at the target site or alteration in the target, or both. There is experimental evidence that the drug resistant trypanosome clones accumulate fewer drugs than their sensitive counterparts (Anene et al., 2001).

5. Types of trypanocidal drug resistance

Two types of resistance against trypanocidal drugs are recognized: single drug resistance and multiple drug resistance. In single drug resistance, trypanosomosis control still could be achieved by using one of the drug pairs in which resistance has not developed through the application of the senative pair principle (Geerts et al., 2001). However, the second drug should be used with caution in order to avoid resistance development against it as well. Multiple drug resistance is resistance concurrently to two or more drugs, making sanative drug pairs ineffective. Multiple drug resistance can only be counteracted by intervening at the level of the vector (Fox et al., 1993).

6. Mode of action of trypanocidal drugs

The discovery of trypanocidal drugs with preventive action raised high hopes that their use would make it possible to run subtropical African into flourishing livestock production area. Although, these drugs to provide protection, all of them frequently give rise to the information of drugs resistant trypanosome strains. This drugs resistance occurs, when the trypanosomes are in contact with a trypanocidal administered in a sub curative dose insufficient to ensure the distruction of the parasites (Das et al., 2004).

The three antitrypanosomal compounds upon which treatment and prophylaxis of cattle trypanosomosis currently depends are isometamidium chloride, homidium chloride or bromide and diminazene aceturate. Whereas, quinapyramine, suramine and melarsomine are primarily used as therapeutic drugs for infections

caused by T. evansi in equidae, camels and buffaloes, although quinapyramine is also used for prophylactic purpose (Williamson, 1970).

6.1. Diminazene aceturate

Structurally, DA is an aromatic diamidine derived from surfen (Jensch, 1958). The compound, marketed aceturate salts, consists of two amidinophenyl linked by a triazene bridge: p,p-diamidinodiazoaminobenzene diaceturate tetrahydrate; N-1,3-diamidinophenyltriazene diaceturate tetrahydrate (Jensch, 1958).

It has been demonstrated that DA binds to the doublestranded DNA via the minor groove through electrostatic and hydrogen-bond forces. Therefore, DA interferes with the synthesis of RNA primers, resulting in the accumulation of replicating intermediates and subsequent inhibition of kDNA replication. Moreover, Shapiro and Englung (1990) have shown the DA inhibits the mitochondrial type II topoisomerases in trypanosomes which blocks the DA replication.

6.2. Isometamidium

Isometamidium is synthesized by coupling homidium with a part of the diminazene molecule (Delespaux and Koning, 2007). It is an amphiphilic cationic drug, which is commercialized as a dark reddish-brown powder. ISMM is less soluble in pure organic solvents and labile under low and high PH conditions and at a high temprature. Its solubility in water is about 6% (w/v) at 20℃ (Kinabo and Bogan,1988). As marketed (Trypamidium®, Samorin®), ISMM product contains 70% of ISMM and 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 complexion) and homidium (Novidium® and Ethidium®). ISMM is used in aqueous solution (1 or 2%) mainly by deep intramuscular route at doses between 0.25 and 1 mg/kg b.w, depending on the risk of TDR. 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 recived doses of 0.5 to 1 mg/kg b.w for a peroid between 2 to 4 months (Chartier et al., 2000).

6.3. Homidium salt

Homidium is better known by its chloride salt or Novidium® and its bromide salt or ethidium bromide. Used at the dose of 1 mg/kg b.w (IM) homidium 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 (Dolan et al., 1990; 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 (Kinabo, 1993; Geerts et al., 2010). 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 for DNA staining (Ethidium bromide).

Phenanthridinium drugs exhibits their antitrypanosomal activity through both the blockage of nucleic acid synthesis via DNA intercalation, inhibition of RNA and DNA polymerase and the incorporation of nucleic acid precursors into DNA and RNA (Kinabo, 1993). However, there are others biochemical mechanisms involved in the trypanocidal effect of these drugs, including the modulation of glycoprotein biosynthesis lipid metabolism, membrane transport and selective cleavage of kDNA minicircles (Shapiro and Englung, 1990).

7. Mechanism of drug resistance

In trypanosomosis, drug resistance is usually the result of the loss of capacity for drug uptake by the parasite, an alteration in drug target interaction, a change in the efflux mechanism (Baker et al., 2013). This drug resistance occurs when the trypanosomes are in contact with a trypanocide administered in a subcurative dose insufficient to ensure the destruction of the parasites (Das et al., 2004). This situation may be due to one or more of the following factors: the application of insufficient doses, due in particular to underestimating the weight of animals; the formation of abscesses followed by partial rejection of the drug; a cyst-forming reaction which prevents the diffusion of the product; preventive treatments at too long or irregular intervals; halting the application of trypanoprophylactics while the animals are still exposed to the risk of infection, and the occasional use of preventive drugs in curative trearments (Rowlands et al., 1994).

An understanding of the mechanisms of drug resistance by trypanosomes, among others, is important as it can lead to the identification of potential and novel drug targets, and provide direction to how new chemotherapeutic strateges can be used to reduce development of resistance. In the latter instance rationale for combinations of existing drugs to increase therapeutic activity, decrease clinical toxicity and potentially reducing the frequency of the emergence of drug resistance (Barrett and Fairlamb, 1999) can be identified.

Trypanocidal drug resistance could be innate, such as in resistant individuals without previous exposure to the particular drug, or acquired (induced) as a result of drug exposure/pressure, cross-resistance or sometimes by mutagenesis (ILRAD, 1990). Reduction in drugs accumulation by the target cell or organism and diminished drug activity in immunosuppressed animals can contribute to the emergence of drug resistance (Frommel and Balber, 1987).

Furthermore, some trypanocidal drugs are well-known mutagenic compounds and might induce mutations, the most resistant of which are certainly selected under drug pressure (Hayes and Wolf, 1990). Taking into account the high basic mutation rate in trypanosomes, which is estimated at 109 per base pair per cell generation in T. brucei the effects of this phenomenon should not be underestimated.

7.1. Diminazine aceturate (Berenil®)

The mechanism by which trypanosomes acquire resistance varies with the drug; resistance to DA is attributed to the altration of a membrane transporter namely, P2 type purine transporter, TcoAT1 (Mäser et al., 2003; Delespaux et al., 2008), which is involved in the drug uptake by the parasite. However, this may not be the only mechanism contributing to DA resistance. Indeed, a novel gene TeDR40, the encoded protein appeared to have a ubiquitous cellular localization, is shown to contribute to a high DA resistance in T. evansi. It is probable that such a high level of DA resistance is the result of the cummulative effect of two distinct resistance mechanisms (TeDR40 and P2-type purine transporter) (Witola et al., 2005).

7.2. Isometamidium

The main mode of action of ISMM was the cleavage of kDNA-topoisomerase complexes. This explanation was supported by Wilkes et al., (1997) who showed that the trypanosome kinetoplast is the primary site of ISMM accumilation. The mechanism of resistance to ISMM, however, is less clear. Decreased levels of drug accumulation have been observed in drug resistant populations of T. congolense (Sutherland et al., 1991), and later work found indirect evidence of an increased efflux of drug from resistant trypanosomes (Sutherland and Holmes, 2004). Mulugeta et al., (1997) showed that the maximal uptake rates (Vmax) of ISMM in resistant T. congolense were significantly lower than in sensitive populations.

It remains to be shown whether this is caused by a decreased number of protein transporters of ISMM in the plasma membrane and/or by changes in the balance between influx and efflux. The role of nucleoside transporters in resistance to ISMM by T. congolense is yet to be examined, although changes in these transporters have

been associated with resistance to arsenical drugs in T. brucei changes in mitochondrial electrical potential have been demonstrated in ISMM resistant T. congolense by Wilkes et al., (1997). Although, contradictory observations have been reported on the genetic stability of ISMM resistance, in the field observations in Ethiopia, based on cloned populations, showed that the drug resistant phenotype of T. congolense had not altered over a period of four years (Mulugeta et al., 1997).

It remains unclear whether the ISMM-resistance phenotype is the consequence of reduced uptake or increased efflux of the drug. In one study, sensitive strains were heterozygous for the GAA codon insertion, whereas most resistant strains were homozygous for the same trait (Delespaux et al., 2002). The fact that the sensitive isolates already seem to carry a recessive resistance allele is consistent with the selection of an existing influx transporter expressed at a lower level or with decreased affinity for ISMM through loss of heterozygosis. Alternatively, the resistance allele could encode a mitochondrial efflux pump with increased affinity for ISMM. However, such an allele would be expected to be dominant, actively clearing the drug from the kinetoplast. To challenge this model, more isolates should be screened to identify an ISMM sensitive phenol type for a strain homozygous for the insertion.

A combined mechanism of reduced uptake and increased efflux might also be possible. Three major types of genetic change that are responsible for acquired drug resistance are identified: mutations or amplifications of specific genes directly involved in a protective pathway; mutations in genes that regulate stress response processes and lead to altered expression of large numbers of proteins; and gene transfer. Gene amplification under condtions of drug pressure is well known in Leishmania species and has also been demonstrated in trypanosomes, but until now there is no evidence that this occurs in the latter parasites as a mechanism of drug resistance under field conditions (Hayes and Wolf, 1990).

7.3. Homidium salts

The mechanism of their anti-trypanosomal 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, trypanothione metabolism and replication of kinetoplast mini-circles. The mechanism of resistance by trypanosomes to these drugs is unknown. Howevere there are indications that it is similar to that described for ISMM (Peregrine et al., 1997).

8. Common trypanocidal drug resistance detection methods

8.1. Field methods

Eisler et al., (2000) proposed a method for the assessment of prevalence of resistance to isometamidium chloride by monitoring cattle populations under natural challenges in the field. Briefly, two groups consisting of 30 to 80 cattle each are used. One group is treated with 1 mg/kg bw ISMM and the other is used as untreated control. The two groups then are exposed to natural challenge and tested for trypanosomes using the phase contrast buffy coat technique (Murray et al., 1977) every two weeks for two to three months.

A comparison through survival analysis curves is made on the data of new trypanosome infections between the groups of cattle treated with ISMM and the untreated control group (Eisler et al., 2000; Tewelde et al., 2004). If >25% of the ISMM treated cattle become infected within 8 weeks of exposure, drug resistance is strongly suspected (Eisler et al., 2000; Tewelde et al., 2004). In this regards, several epidemiological studies to map field trypanocidal drug resistance, based on the protocol by Eisler et al., (2000), have been documented. McDermott et al., (2003) working in the Kénédougou province of Burkina Faso, Shinyangwe et al., (2004) working in Eastern Zambia, Tewelde et al., (2004) in Ethiopia, Grace, (2005) in Guinea and South Eastern Mali and Allegye-Cudjoe, (2009) in Ghana and in Benin are some examples.

An abbreviated version of the original 8-12 week protocol by Eisler and colleagues was validated in the cotton zone of West Africa and found effective and reliable (Diall et al., 2005) for use not by researchers but by the national veterinary sevices. This involves a 4-week long follow-up (Diall et al., 2005) period in order to reduce costs and still generate data within a very short time. The abbreviated protocol is effective in areas where trypanosomosis risk is high (prevalence is >10% as has been demonstrated in the cotton zone of West Africa (Diall et al., 2003; Grace, 2005).

8.2. Experimental method in natural hosts

Neither the single dose nor the multipledose tests in mice are able to predict accurately the curative doses of trypanocidal drugs needed to clear trypanosome populations from infected cattle (Eisler et al., 2001). A test in ruminants should hence be used to determine whether or not drugs are principally efficacious at recommended curative doses in cattle infected with a particular trypanosome population. The test in calves may further be used for investigations on drug resistance in T. vivax, which is usually not infective for mice. A group of cattle or samall ruminants, preferably of a breed native to the area and without prior exposure to tsetse or trypanosomosis are used (Eisler et al., 2001). Specific detailed protocols on this are as contained in Eisler et al., (2001). Due to individual variation in the response to trypanocidal drug treatment among ruminants inoculated with the same T. congolense isolate (Ndoutamia et al., 1993; Kone, 1999), it is advisable to use a minimum of three and preferably six animals.

Further constraints of the technique are that not all trypanosome populations might grow equally well and that sensitive isolates might overgrow resistant one when inoculated together (Sones et al., 1988). A useful indication of the level of resistance can be obtained from studies in ruminants by recording the length of time between treatment and detection of break through populations of trypanosomes. The shorter the period, the greater the level of resistance. if relapse occurs in more than 20% or more of the cattle tested (i.e. for a total of between one and four cattle, at least 1 relapse; for a total of 5 or 6 cattle at least 2 relapse), the isolate may be said to exhibit resistance to the dose of drug used (Eisler et al., 2001).

8.3. Molecular techniques

Because of the problems associated with the low sensitivity of the parasitological techniques (Paris et al., 1982) and the long follow-up time of study animals (Eisler et al., 2001), Polymerase Chain Reaction (PCR) with high sensitivity and specificity is a good solution to this problems. Gall et al., (2004) used this method in Burkina Faso and found it four times more sensitive compared to the field parasitological techniques.

Molecular methods for the diagnosis of ISMM resistance has been developed (Delespaux et al., 2005; Afework et al., 2006). The first method enables discrimination between ISMM-sensitive and ISMM-resistant strains of T. congolense by MboII-PCR-RFLP (Delespaux et al., 2005). 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.

The second method has been developed to distinguish ISMM-resistant from ISM-sensitive strains of T. brucei (Afework et al., 2006). This SfaNI-PCR-RFLP test is based on the polymorphism of the 677 bp fragment of the TbAT1 gene. The same set of six point mutations could confer resistance to the melarsenoxide cysteamine cymelarsan (an arsenical diamidine) and to ISM (diamidine compound) and the detection of one of these six mutations could enable reliable identification of sensitivity or resistance to ISM (Mäser et al., 2003).

8.4. Experimental study in mice

On the other hand, mice can be used to undertake drug resistance study for trypanosome species that can grow in this species of experimental animals. Either single-dose or multi-dose tests are conducted in mice to provide information on resistant trypanosome isolates from a given area, as described in the protocol by Eisler et al., (2001).

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). Sones et al., (1988) used a group of five mice, which allowed an easy calculation of ED80 and CD80 values (one out of five mice not cleared or cured). Knoppe et al., (2006), using the standard mouse test (SMT), screened a number of T. congolense isolates collected in the Kénédougou Province of Burkina Faso against Isometamidium chloride at a dose of 0, 0.25, 1.0, 5.0, 10, 15 or 20 mg/kg body weight and found the method very sensitive but labour intensive.

There are however 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 difference in metabolic size (Sones et al., 1988). 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.

8.5. Xenodiagnosis

Xenodiagnosis is the feeding of a clean susceptible vector species on a suspected case of trypanosomosis, after which it is either dissected and examined for the presence of infection, or allowed to feed on a clean animal which then is itself examined for the development of infection. A modification of this approach, the drug incubation glossina infectivity test (DIGIT), in which trypanosomes are exposed to the trypanocidal drugs in vitro for a short time and thereafter are fed to tsetse flies to ckeck whether or not they develop into metacyclic forms was successfully validated and proved sensitive for detecting drug resistance (Clausen et al., 1999). This technique distinguishes resistant from sensitive isolates and does not require experimental animals. However, it does require a ready supply of teneral tsetse flies from an artificially reared colony.

9. Control of trypanocidal drug resistance

Based on current knowledge in the field of trypanocide resistance and on experience in the control of resistance to insecticides, anthelmintics, antibiotics and other drugs (Geerts et al., 1997). 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 using correct dosing with drugs that are slowly eliminated from the body. Furthermore, some trypanocidal drugs are well-know 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 (Kalel, 2015). Of these the most important measures are use of the correct dose, changing of drugs, senative 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 (Kalel, 2015).

9.1. Use of correct dose

Under dosing is ne of the major causes of resistance development. Sub-therapeutic drug concentrations exert a strong selective pressutre for the emergence of resistant clones that pre-exist in the trypanosome population. Unfortunately, under dosing occurs very frequently by farmers or unskilled persons in many countries of Africa due to the absence of strict rules about the utilization of veterinary drugs (Greets and Holmes, 1998). Furthermore, there are an increasing number of generic products available on a somewhat loosely regulated market, and some of these have questionable efficacy and many contain lower doses of drug than the stated amount (Holmes et al., 2004).

9.2. Reduction of the number of treatments

It is widely agreed that the most efficient way to delay the development of drug resistance remains the reduction of drug selection pressure by decreasing the number of treatments, especially incase of multiple drug resistance (McDermott et al., 2003). Reduction in drug pressure impacts drug resistance evolution in three ways; i) delays its appearance, ii) reduces the likelihood of its establishment and iii) slow its spread (Smith et al., 2010).

It is therefore strongly recommended that control of trypanosomosis should not rely solely on drugs. An integrated approach should be adopted using vector control, to reduce the tsetse challenge, along with reduced frequency of drug dosing. Minimizing drug use by reducing contact between livestock and tsetse vectors and intensifying vector control activities has also been suggested as an effective means of preventing or delaying the emergence of trypanocidal drug resistance (Holmes et al., 2004). Furthermore, frequently repeated trypanocidal drug treatments have been associated with toxicity problems (Eisler et al., 1997).

9.3. Avoiding exposure of the whole parasite population to a drug

In the past mass treatments are commonly used to control animal trypanosomosis. However, this form of treatment exerts a stromg selection pressure on the trypanosome population. The higher the proportion of trypanosome population exposed to the drug and the lower the proportion in refugia (i.e. the proportion of trypanosomes present in the fly population or in other hosts), the higher the selection pressure. Therefore, in well monitored situations there is a strong case for limiting treatment to individual clinical cases; this is also desirable on grounds of minimizing drug residues, avoiding potential toxicity and reducing costs (Maudlin et al., 2004).

9.4. Senative treatment

The concepts of senative treatment is the use of a pair of trypanocides which are chemically unrelated and therefore, unlikely to induce cross resistance. DA and ISMM constitute a "senative pair" which means that once resistance develops to one of the drugs, the other drug should be used to control the infection (Desquesnes et al., 2013). However, combination therapy only works when the drugs are unrelated and if instituted early enough before significant resistance to either drug is present in the population (Smith et al., 2010). However, the effectiveness of this strategy may be questioned by the increased field reports from many parts of Africa of multiple resistant trypanosomes (Clausen et al., 2010; Mungube et al., 2012).

9.5. Banning the use of Quinapyramine in cattle

Quinapyramine are well-known mutagenic compounds and might induce mutations, the most resistant of which are certainly selected under drug pressure. Quinapyramine was withdrawn from sale for cattle use because of the problem with toxicity and resistance development (Maudlin et al., 2004). The use of Quinapyramine was the suggested cause of the multiple drug resistance problems in Ghibe valley of Ethiopia. Therefore, its use as a trypanocide in cattle is completely contraindicated (Maudlin et al., 2004). Moreover, general improvement of animal health conditions by deworming

and reduction of animal disease risk, and vector control as described by Clausen et al., (2010) can also be of great impact.

9.6. 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 (Kalel, 2015)

10. Conclusions and Recommendations

In Ethiopia, trypanosomosis is one of the most important livestock diseases, which poses a serious threat to the lives and livelihood of entire communites and constitutes the greatest single disease constraint to livestock production. Exposure of parasites to sub therapeutic drug concentrations, resulting from under dosing and uncontrolled use of trypanocidal drugs, and the lack of proper diagnosis, are considered the major causes of increasing drug resistance in Ethiopia. Therefore, there is an urgent need for detailed experimental work in the field to monitor the development of drug resistance in tsetse infested areas of Ethiopia. In Ethiopia, there are evidences of ISMM and DA resistance and treatment failures reported to the recommended doses of theses drugs. Therefore, to alleviate the problem of trypanocidal drug resistance the following recommendations are forwarded:

- Rational uses of trypanocidal drugs and control of co-infections to exploit selfcure against resistant trypanosome populations.
- Policies on the importation of quality trypanocidal drugs, distribution and treatment.
- Awareness creation to the farmers about tsetse and trypanosomosis coupled with existing control methods is crucial and timely.
- Efforts should be intensified for the discovery of new trypanocidal drug compounds and or effective vaccines.
- Using of cattle that trypanotolerant in area with high tsetse fly infestation should be better for those people live in that area.
- Applying the latest tsetse control method by sterile insect technique/SIT/.

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