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Review

A review on trypanocidal drug resistance in Ethiopia

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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 biting flies. Tsetse transmitted trypanosomosis is encountered in many part of Ethiopia. Trypanocidal drugs remain the principal method of animal trypanosomosis control in the country. 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* and molecular tests, 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, 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 will be of great importance.

Kew words: Africa Ethiopia, Trypanocidal drugs, Trypanosomosis.

INTRODUCTION

Trypanosomosis in domestic livestock causes a significant negative impact in food production and economic growth in many parts of the Africa, particularly in Sub-Saharan Africa (Taylor, 1998). It has greatly hampered people and animals settlement in a considerable part of the Africa. Trypanosomosis that occurs in Africa cover one third of the continent is arguably the most significant disease (ILRAD, 1994). Therefore, it remains the major important constraint to livestock production on the continent. The wide occurrence of this disease in people and their livestock retards agricultural and economic development in Africa, and 160 million estimated cattle population are at risk from trypanosomosis (ILRAD, 1994). Trypanosomiasis is controlled either by controlling the vector (Glossina) or by controlling the parasite, or a combination of both. Over the years, a large arsenal of vector-control tools has been developed. Nevertheless, the control of animal trypanosomiasis in poor rural communities has continued to rely heavily on the use of trypanocidal drugs. This is not surprising considering the private nature of such treatments and the difficulties in maintaining cleared

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flies. Only a small group of chemoprophylactic and chemotherapeutic trypanocidal compounds are currently in use and new compounds are unlikely to become areas in the absence of barriers to re-invasion of tsetse available in the near future (Barrett, 2004).

Geerts and Holmes (2004) estimated that in Africa, 35 million doses of veterinary trypanocidal drugs are administered each year with isometamidium chloride (ISMM), ethidium bromide (EtBr) and diminazene aceturate (DA) estimated to represent 40, 26 and 33%, respectively, of the total trypanocidal drug market by value (Sones, 2001). ISMM is mainly used as a prophylactic drug and provides on average 3 months' protection (2 to 22 weeks) against trypanosome infection. DA has only therapeutic properties and EtBr has limited prophylactic properties, and is mainly used as a therapeutic agent (Holmes et al., 2004).

Considering the well known mutagenic properties of EtBr, this drug should ideally be removed from the drug market, but in practice it is still widely used in many countries. Removing this drug from the market would not jeopardize the treatment of animal trypanosomiasis because it can be replaced either by DA for curative purposes or by ISMM for prophylactic purposes. When trypanosomes are resistant to ISMM, EtBr will be ineffective as cross-resistance is observed between the two drugs (Peregrine et al., 1997; Van den Bossche et al., 2000; Delespaux et al., 2002).

The total value of the market for trypanocides for African farmers is estimated at US$30 million (Ismail et al., 2004) but this is considered insufficient by pharmaceutical companies to justify investment in the development of new drugs (Gall et al., 2004), therefore the challenge remains to make optimal use of existing drugs. The repeated use of chemicals as pesticides or chemotherapeutic agents inevitably leads to the development of resistance in the target organisms. This also occurred with the trypanocidal drugs, such as ISMM, the homidium salts and diminazene aceturate, which were introduced during the 1950s; the first reports of acquired resistance were published during the 1960s (Finelle and Yvore, 1962; Jones-Davies, 1967; Jones-Davies and Folkers, 1966). Treatment and prevention of bovine trypanosomosis rely essentially on three drugs, namely: homidium, diminazene and isometamidium. However, in many parts of Africa all of these trypanocides are gradually losing their efficacy due to drug resistance.
Experimental studies have demonstrated the occurrence of resistance in trypanosomes to both diminazene and isometamidium fortunately, trypanosomes are usually not resistant to both diminazene and isometamidium at the same time (Machila et al., 2001). Thus, these two compounds have been termed a sanative pair, since in instances of resistance to one drug application of the other one will usually control the disease. Nevertheless, recent reports on multiple drug-resistances in Burkina Faso and Ethiopia suggest that the concept of sanative pairs might no longer always be valid (Afewerk et al., 2000).

Ethiopia, as part of the African continent shares a substantial loss from the disease. Apart from the cyclical transmission of trypanosomosis by the *Glossina*species, it is highly considered that mechanical transmission significantly affects livestock productivity in many parts of the world including Ethiopia (Abebe and Jobre, 1996). However, information on prevalence of non-cyclically transmitted trypanosomosis in domestic animals, the vectors involved and the drug sensitivity of the trypanosome species in Ethiopia is scanty and sufficient data in a compiled form is not available (Alekaw, 2004). The situation of African Animal Trypanosomosis (AAT) has become even worse due to the fact that some drugs have been abandoned due to resistance.

Practically, only ethidium bromide (Homidium) and DA (Berenil) are still available for therapy and ISMM (Samorin, Trypamidium) for prophylaxis (Holmes, 2004; Geerts et al., 1997). The inevitable outcome of continued use of the same compounds for decades has resulted in drug resistance that has been largely responsible for the frequently observed chemotherapeutic failures (Moti et al., 2010). In Ethiopia, the prevalence of mechanically transmitted *Trypanosoma vivax* and *Trypanosoma evansi* reported by the various workers has indicated their wider distribution in the country and the disease impact due to both parasites is substantial. The presence of mechanical vectors, existence of reservoir hosts, and the involvement of wider host range in parasites, the various agro-climatic zones and the poor veterinary infrastructure would undoubtedly ensure the existence of both *T. vivax* and *T. evansi* in Ethiopia.

In Ethiopia, trypanosomosis is one of the major constraints to cattle production with direct and indirect economic losses (Abebe and Jobre, 1996). Effective vector control methods are available (Bauer et al., 1995). However, in Ethiopia, increasing costs and other problems of initiating and maintaining tsetse control campaigns have led the livestock sector to be completely reliant on the use of trypanocidal drugs. Therefore, the objective of this paper is to over review on trypanocidal drug resistance in Ethiopia.

STRATEGIES FOR TRYPANOCIDAL DRUG USAGE

Routine block treatments

These are generally carried out using prophylactic drugs, notably isometamidium chloride, at predetermined intervals based on the perceived duration of prophylaxis (0.5 to 1 mg/kg BW, 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 isometamidium is practiced, it is recommended that once a year, the animals are

separately treated with diminazene in order to delay the development of drug resistance following the concept of sanative pair (Whiteside, 1960).

Strategic block treatments

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

Monitoring and treatment of individual infected animals

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

MODE OF ACTION OF TRYPANOCIDAL DRUGS

Despite the fact that chemotherapy is the major means of disease control, development of new antitrypanosomal drugs has been more or less static over the last three decades, due to lack of interest by the pharmaceutical industry to invest into research and development of antitrypanosomal drugs (Gutteridge, 1985). Consequently, this has been a major stimulus for intensive research into the few existing drugs; and in the recent past, considerable body of knowledge has emerged on a number of important aspects, such as drug disposition, mechanisms of action, resistance and toxicity. 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).

Diminazene aceturate

Diminazene binds to trypanosomal kinetoplast DNA (Newton, 1972; MacAdam and Williamson, 1972). This binding does not occur by intercalation (Newton, 1972) but via specific interaction with sites rich in adenine-thymine (A-T) base pairs (Newton, 1972; Brack and Delain, 1975). Non-intercalative binding of diminazene to DNA, with strong affinity for A-T base-pair regions, has similarly been demonstrated *in vitro*, using DNA obtained from various sources (Lane et al., 1991). Such studies have shown that the molecule binds with higher affinity to 5'- AATT-3' than to 5'-TTAA-3' regions of DNA. Through this specific interaction in trypanosomes, diminazene inhibits synthesis of RNA primers, resulting in accumulation of replicating intermediates, thereby inhibiting kDNA replication (Newton, 1972; Brack and Delain, 1975). In other work, Shapiro and Englund (1990) have shown that diminazene specifically inhibits mitochondrial type II topoisomerase in viable trypanosomes. Thus, inhibition of DNA replication may also occur via this intercalation. The rate of excretion of the different compounds is known to affect their activity. Diminazene, which is rapidly excreted, is used only for its therapeutic effect. Dimidines accumulate in the liver for months, like wise in the kidney and the adrenal glands respectively (Biobaku et al, 2008).

Isometamidium

The primary mode of action currently considered to account for the molecular mechanisms of antitrypanosomal activity of phenanthridinium drugs is blockade of nucleic acid synthesis through intercalation between DNA base pairs (Wagner, 1971), inhibition of RNA polymerase (Richardson, 1973), DNA polymerase and incorporation of nucleic acid precursors into DNA and RNA (Lantz and Van Dyke, 1972). Other biochemical reactions that may account partly to their effects include modulation of glycoprotein biosynthesis, lipid metabolism (Dixon et al., 1971), membrane transport (Girgis-Takla and James, 1974) and selective cleavage of kinetoplast DNA minicircles. The mechanism that is considered primary is blockade of nucleic acid synthesis, which does not explain the basis of their selective toxicity. However, there are a number of biochemical peculiarities that have been demonstrated in trypanosomes that appear to be candidate targets for drug modulation, and that might explain the basis of selective toxicity (Opperdoes, 1985). Generally it inhibits DNA synthesis in a similar manner as diminazene aceturate, it modifies the mitochondrial membrane, it modifies the glycoprotein structure in surface of the endoplasmic reticulum and Isometamidium is slowly excreted, and is the most effective prophylactic compound currently available (Kinabo and Bogan, 1988).

Homidium salt

Pentamidine, diminazene aceturate (Berenil), isometamidium chloride (Samorin), and ethidium bromide, which are important antitrypanosomal drugs, promote linearization of trypanosoma minicircle DNA (the principal component of kinetoplast DNA, the

mitochondrial DNA in these parasites) (Delespaux et al., 2002). This effect occurs at therapeutically relevant concentrations. The linearized minicircles are protease sensitive and are not digested by lambda exonuclease (a 5' to 3' exonuclease), indicating that the break is double stranded and that protein is bound to both 5' ends of the molecule. The cleavage sites map to discrete positions in the minicircle sequence, and the cleavage pattern varies with different drugs. These findings are characteristic for type II topoisomerase inhibitors, and they mimic the effects of the antitumor drug etoposide (VP16-213, a semisynthetic podophyllotoxin analog) on *Trypanosoma equiperdum* minicircles. However, the antitrypanosomal drugs differ dramatically from etoposide in that they do not promote detectable formation of nuclear DNA-protein complexes or of strand breaks in nuclear DNA. Selective inhibition of a mitochondrial type II topoisomerase may explain why these antitrypanosomal drugs preferentially disrupt mitochondrial DNA structure and generate dyskinetoplastic trypanosomes (Shapiro and Englund, 2003).

MECHANISM OF ANTITRYPANISOMAL DRUG RESISTANCE

The discovery of trypanocidal drugs with preventive action raised high hopes that their use would make it possible to turn subtropical Africa into a flourishing livestock production area. It must be admitted that most of these hopes have not been realized. Although, these drugs do provide protection, which in some conditions may last up to six months, all of them frequently give rise to the formation of drug-resistant trypanosome strains. 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 try-panoprophylactics while the animals are still exposed to the risk of infection, and the occasional use of preventive drugs in curative treatments (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 strategies 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 10^{-9} per base pair per cell generation in *Trypanosoma brucei* the effects of this phenomenon should not be underestimated.

Diminazine aceturate

Although, diminazene probably exerts its action at the level of the kinetoplast DNA, this has not been proven *in vivo* and other mechanisms of action cannot be excluded. Similarly, the molecular basis of resistance to diminazene in trypanosomes is not clear (Berger et al., 1995) which showed that the accumulation of diminazene was markedly reduced in arsenical-resistant *T. brucei brucei* owing to alterations in the nucleoside transporter system (P2).

However, there might be other resistance mechanisms. Similarly to ISMM, 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 *Trypanosoma congolense* remained stable over a period of four years. In conclusion, it is clear that much more work is required in order to elucidate the mechanism of resistance to the three currently available trypanocidal drugs. Such studies, as well as being of great value in their own right, may also provide novel methods for the detection of drug-resistant trypanosomes in the future.

The same is true for the genetics of drug resistance in trypanosomes, which distinguish three major types of genetic change that are responsible for acquired drug resistance: 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 conditions of drug pressure is well known in *Leishmania* spp. 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 (Ross and Sutherland, 1997).

The current possibilities to insert or delete genes will certainly lead to a better insight into the resistance

mechanisms (Ten Asbroek et al., 1990; Gaud et al., 1997). Other aspects, such as the stability of drug resistance, its mono- or polygenic nature, dominance or recessiveness, also need to be examined, because of their far-reaching impact on the control of resistance.

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 accumulation. 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). Recently, 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* more recently, 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, recent 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 phenotype 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 identify: 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 conditions of drug pressure is well known in Leishmania spp. 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).

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 ISMM.

DETECTION OF ANTI TRYPANOSOMAL DRUG RESISTANCE

Several methods have been described to identify drug resistance in trypanosomes (Peregrine, 1994, 1996). At present, four types of technique are commonly used to identify drug resistance: tests in ruminants; tests in mice, *in vitro* assays and now a day molecular detection also possible. None of these is, however, an ideal test and other tests are still in the phase of development or validation. The advantages and disadvantages of each of the different techniques are briefly summarized in the following sections.

Detection in ruminant

The tests commonly consists of infecting a group of cattle or small ruminants with the isolate under investigation and later ,when they are parasitemic, treating them with various dosages of trypanocidal drugs (Delespaux, 2000). It is preferable to use at least three animals in each group because it has been shown that results obtained after inoculation and treatment of one animal are not always reliable. The animals are regularly monitored over a period of 100 days to determine the

efficacy of standard drug doses in terms of their ability to precede permanent cure. For these studies, the cattle or small animals are kept in fly proof accommodation or in none tsetse area in order to eliminate the risk of reinfection during the study. A variation on this technique is to inoculate blood from several different infected cattle into single recipient calf (Holmes et al., 2004).

Detection in mice

Tests in mice can be used as a single dose test or as a multi dose test. In the later case, the objective is to obtain more detailed information by determining the CD50 or CD80 values (curative dose that gives cure in 50 or 8% of animals) for a given trypanocidal drug. In the case of a single test, a large number of trypanosome isolates is tested at a single discriminatory dosage of 1 mg/kg for ISMM and 20 mg/kg for DA (Eisler et al., 2001). The advantage of the mouse test is that it is cheaper than the test in ruminants. However, it presents several disadvantages; firstly, most *T.vivax* isolates, and also some *T. conglense* isolates do not grow in mice and for that reason, research on *T. vivax* isolates in particular has been hampered. Secondly, higher dose of drug must be used in mice in order to obtain results comparable to those from cattle because of the vast difference in metabolic size, in spite of the fact that there is reasonable correlation between drug sensitivity data in mice and cattle. Therefore, results in mice cannot be directly extrapolated to calculate the curative dose to be used in animals. Thirdly, a large number of mice per isolate are required in order to obtain a precise assessment of the degree of resistances. This makes it a rather labour intensive test. Finally the test takes as long as 60 days to evaluate the drug sensitivity of an isolate (Greets and Holmes, 2004).

***In vitro* assays**

For the *in vitro* evaluation of drug sensitivity procyclic, metacyclic or blood stream forms of trypanosomes can be used. The advantage of *in vitro* assays is that large number of isolates can be examined however, there are several disadvantages. The use of metacyclic and blood stream forms is considered more reliable than the use of procyclic forms. Test with metacyclic trypanosomes is considered to generate well with field observations, but it may take up to 40 to 50 days of in vitro incubation to generate metacyclic trypanosomes (Gray et al., 1993). *In vitro* cultivation of blood stream forms is only possible using pre adapted lines and not using isolates directly from naturally infected animals. *In vitro* assay are expensive to perform and require good laboratory facilities and well trained staff. In contrast to *T. brucei*, it is very difficult to cultivate *T. conglense* (Holmes et al., 2004) if techniques can be improved to adapt trypanosomes isolates to grow in vitro more rapidly, these may becomes more popular especially in those laboratory where culture facilities are already established. An interesting alternative is the drug incubation glossina infectivity test in which the trypanosomes are exposed to the drug *in vitro* for a short time, and thereafter fed no tsetse flies to check whether or not they develop into metacyclic forms, this technique distinguish resistant from sensitive isolates and dose require experimental animals, but it does require a ready supply of teneral steste flies from an artificially reared colony (Holmes et al., 2004).

Molecular detection drug resistance

Molecular methods for the diagnosis of ISM resistance were recently developed (Delespaux et al., 2005). The first method enables discrimination between ISM-sensitive and ISM-resistant strains of *T. congolense* by *Mbo*II-PCR-RFLP (Delespaux et al., 2005). This test is based on the polymorphism observed in a 381 bp fragment in sensitive strains or 384 bp fragments in resistant strains of a putative gene presenting some homologies with an ABC transporter. The second method has been developed to distinguish ISM-resistant from ISM-sensitive strains of *T. bruce* (Afework et al., *2006)*. This *Sfa*NI-PCR-RFLP test is based on the polymorphism of a 677 bp fragment of the *Tb*AT1 gene. The same set of six point mutations could confer resistance to the melarsenoxyde cysteamine cymelarsan (an arsenical diamidine) and to ISM (diamidine compound) while the detection of one of these six mutations could enable reliable identification of sensitivity or resistance to ISMM.

CURRENT SETUATION OF DRUG RESISTANCE ON TRYPANOSOMOSIS IN ETHIOPIA

So far, resistance to one or more of the common trypanocidal drugs used in cattle has been reported in at least four regional states (local areas) within the country. But the currently available information on drug resistance is derived from limited number of cases reports, and does not give any indication of the true situation of the resistance in a whole country (region) as systematic surveys have not been fully conducted. Table 1 summarizes the list of published reports in which a number of trypanosomes isolate has been examined. This problem of drug resistance in trypanosomes requires being spreading geographically into many regions in which trypanosomes occur. Additionally, the spread of genertic products, some of which are of doubtful quality, may undermine farmer's confidence using trypanocidal drugs (Holmes et al., 2004). Chemotherapy and chemoprophylaxis are the most practical methods available for the control of animal. Trypanosomiasis, but

Tables 1. Multiple and single trypanocidal drug resistance reported in Ethiopia.

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 and Abebe (2003)
	Bedele	ISMM & DA	T.C	Chaka and Abebe (2003)
	Gibe valley	ISMM, DA & H	T.C	Mulugeta et al (1997)
Benshangul gumz	Metekel	ISMM & DA	T.C	Afewerk et al (2000)
	Metekel	ISMM & DA	T.C	Afewerk (1998)
SNNPRS	SODO	ISMM & DA	T.C	Chaka and Abebe (2003)
	Arbaminch	ISMM & DA	T.C	Chaka and Abebe (2003)
	Omo valley	ISMM & DA	T.C	Ademe (1998)
Tigray	Tselemt	DA & ISMM	T.V	Desalgn et al. (2010)

ISMM (Isometamidium), DA (diminazine Aceturate, H (homidium salt), TC (trypanosama conglense, T.V (trypanosome vivax, T.B (trypanosome brucei) and SNNPRS (South Nation and Nationality People Regional State).

their effectiveness is being eroded by the emergence resistant trypanosomes. 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 trypanosomiasis and misuse of drugs has contributed to the development of drug resistance in the population of *T. congolense* parasites (Afewerk et al., 2000; Ermiyas and Getachew, 2001). 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.

CONCLUSION AND RECOMMDETION

The great potential of livestock to rural farmers, in Ethiopia, can only be exploited if trypanosomosis and the arising appearance of drug resistance are controlled. Chemotherapy and chemoprophylaxis are the most realistic method accessible for the control of animal trypanosomiasis. However, the increasing trend of drug resistant strains of trypanosomes is a serious threat to cattle production in Ethiopia. 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. 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.

Furthermore, strict supervision on the usage of trypanocidal drugs should be implemented in order to minimize the misuse. More attention should be given to the adoption of an integrated trypanosomosis control strategy, involving the vector as well as the parasite. Moreover, effective use of available drugs is crucial. Drug resistance in trypanosomosis is a serious problem, this situation should be put under continuous study and when detected immediate reversal mechanism have been employed. Therefore, more attention should be given to adopting an integrated disease management strategy involving the vector as well as the parasite such strategies should be economically feasible, socially acceptable and sustainable and environmentally friendly. Legislative reinforcement by way of elaborating a national drug use policy is required to address the indiscriminate drug usage in Ethiopia.

Conflict of Interest

The authors have not declared any conflict of interest.

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