Review On Anti Trypanosome And Resistance To The Drugs

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Summary: Trypanosomosis is a major constraint to livestock production in sub Saharan Africa and to a lesser extent in latine America and Asia. The distribution of the disease is influenced by the existence of tsetse and biting flies. Trypanocidal drugs remain the principal method of animal trypanosomosis control in most African countries. However, there is a growing concern that their future effectiveness may be severely curtailed by widespread drug resistance. Because it is very unlikely that new trypanocidal drugs will be released into the market in the future, it is essential to maintain the efficacy of the currently available drugs. So detection methods of drug resistance by tests in ruminants, in mice and in vitro assays followed by the right techniques on the delay of the development of drug resistance like reduction in the number of treatment is manadatory. And once resistance present allowing integrated control measures such as reducing vector numbers to reduce the number of drug treatments will be of great importance.

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1. Introduction

Trypanosomosis is a parasitic disorder of mammals caused by protozoa, belonging to the genus trypanosoma of the family trypanosomatidae. The parasite multiplies in the blood stream, lymphatic vessels, and tissues, including the cardiac muscle and central nervous system. The disease is characterized by anemia, progressive cachexia, and death (Itard, 1982).

Trypanosomosis remains the single most important animal disease in sub Saharan Africa, (D' Ieteren, 1993) and in Ethiopia it is found through out the country with exception of highlands where it is rare. The disease has serious effects on productivity and reduces the reproductive potential of the host (Williams, 1993). The distribution of the disease is influenced by the existence of tsetse and biting flies (Masake and Minja, 1996). The problems of trypanosomosis has increased extremely and still increasing due to a number of factors. The advance of tsetse flies into uninfested areas and the widespread of drug resistance have increased the magnitude of the problems (langridge, 1976, NLDP, 1997).

This devastating livestock disease can be controlled by various methods namely, control of the vector responsible for the transmission of the trypanosomosis, breeding trypanotolerant animals, and chemoprophylaxis and chemotherapy. Therapeutic drugs have been widely and practically used to control animal trypanosomosis over long periods of time. However, antitrypanosomal drug are facing serious problems that deter the purpose of application, of which development of resistance to the existing drugs is the most common.

Therefore, the objectives are indicated below:

- To review major drugs used in treatment and prevention of trypanosomosis.
- To brief on mechanisms of resistance development against anti trypnosomal drugs.
- To discuss contributing factors of resistance development and forward control options.

2. Pathogenesis of trypanosomosis

Metacyclic trypanosomosis are injected into the host by the fly during feeding then multiply at the subcutaneous site, provoking a local skin reaction called chancre (Radiostitis *et al*., 1994). According to Molyneux to and Ashford (1983), chancre is formed initially by infiltration of poly morphonuclear leukocyte then with macrophage and plasma cells. Within chancres, metacyclic parasites change to trypomastigate form and enter the blood stream directly or through the lymphatic. *T. vivax* usually multiplies rapidly in blood and evenly dispersed through out the cardiovascular system, where as *T. congolense* tends to aggregate in small blood vessels and capillaries of the heart, brain and skeletal muscular where a small portion of parasites enters the blood added capacity passing out the capillaries in to the interstitial tissues and serous fluids of body cavities where they continue to multiply (Radiostitis *et al*., 1994). Lymphadenopathy is the generalized enlargement of lymph node and spleenomegally develops in association with marked proliferation of lymphoid cells in the organ. Anemia is also one of the most sequels of trypanosomosis associated with

hemolysis of the red blood cells by mononuclear phagocytic system (Urquhart *et al*., 1996). Finally, trypanosomes also cause damage to tissue particularly to heart.

Trypanosomosis exerts its direct effect through loss in milk and meat production, morbidity and mortality, and indirect economic loss through the non-use of land and other resources due to the high prevalence of the disease (Smallow, 1997).

3. Control option of trypanosomosis

It has been indicated that the establishment of trypanosomosis requires interaction of the parasite; vector (tsetse and other flies) and the animals. The control options have also been directed to control the vectors, breeding of trypanotolerant animals and chemoprophylaxis and chemo therapy targeting the causative agent (Morezaria *et al*., 1996).

Current vector control methods may include ground spraying, aerial spraying, tsetse traps and attractants, pour-on techniques, sterile insect techniques (SIT) and biological control methods (Seifert, 1996). Trypanotolerance is the ability of an animal to survive, reproduce and be productive in an environment with trypanosomosis risk with out the necessity for trypanocidal drugs. It is characterized by control of development of anemia and the ability to acquire a better immune response when infected however, constraints to putting these three traits into practical use is difficult (D'Ieteren, 1993). Use of anti trypanosomal drugs, as curative and prophylactic treatment is the most common practice. Curative treatment is practiced areas where the incidence of trypanosomosis is known to be low or medium to cure individual infected animals (Dolan *et al.,* 1998). Chemoprophylaxis has been in widespread use in tropical Africa for many years (Leak, 1999).

It has been undertaken where trypanosome risk is so high that the health of the livestock cannot be maintained by the use of curative drugs (Dolan *et al*., 1998). These prophylactic drugs should be used with great cautions for specific purpose such as protection of cattle passing through tsetse- infested areas for slaughter (Itard, 1982).

4. Anti trypanosomal drugs

The use of trypanocidal drugs is the most widely accepted means of controlling the disease. In some African countries, sales of trypanocides account for more than half of the total sales of veterinary pharmaceutics (Feldmann and Hendrichs, 2001). Chemoprophylaxis of trypanosomosis has been taken as areal alternative in disease prevention. Since drugs vary in their effectiveness for different species of trypanosomes, the infecting organisms must be identified to select appropriate drugs and confirm the sensitivity of the trypanosomal strains. This is comparatively straight forward for most type of livestock but present some difficulty when the mass treatment of cattle is involved (Getachew, 1983).

Drugs currently recommended for chemotherapy of animals trypanosomosis come from only three chemical related groups. These are phenathridines, isomethamidium and homidium and the aromatic diamidine, diminazene. Only isomethamidium and homidium are recommended for prophylactics (Molyneux and Ashford, 1983) as indicated in table 1 below.

Though diminazene is considered a therapeutic drug because of its way of acting, it is also being applied more as a prophylactic. Diminazene aceturate offeres itself as a secoundary ''sanative'' trypanocides. It has no prophylactic actions but rather eliminates those trypanosomes strains which have become resistance of the primary prophylactic. After eliminating the resistance strain, the primary drug may be applied again. The intravenous application of isomethamidium chloride had not only therapeutic but also considerable prophylactic effect for about 4 weeks in Boran cattle in Kenya (Munstermann *et al*., 1992).

Depending up on the threat of infection (frequency of fly challenge), the treatment has to be repeated in regular intervals with high incidence of infection, prothidium may be applied in an interval of two months, diminazene aceturate may be applied twice a year in order to eliminate resistance strains. With high incidence of infection, antrycide-prosalt is applied every two months, or prothidium is applied every 15 weeks, and diminazene aceturate is applied once a year in order to control resistance. For medium incidence of infection, antrycide is applies every 3 months, or prothidium is applied every 5 months; resistance control everyith diminazene aceturate is dosed every 2 years. In case resistance is suspected against diminazene aceturate the dose can be increased 7.0-10.5mg/kg body weight (Seifert, 1996).

Quinapyramine sulphate has been used extensively. It is generally effective against *T. vivax*, *T. congolense, T. brucei* and *T. evansi* in all class of stock. Mild reaction evidenced by salivation, sloughing and muscular tremors is often observed. In animals under physical stress, as when long journey are undertaken, immediately before or after treatment, fatalities may occasionally occur but can be avoided y rest and watering on the day before and the day after treatment.

Strains of *T. congolense* and *T. vivax* may readily develop a level of resistance against quinapyramine sulphate and it has been recorded that such resistance strains may also develop to homidium and isomethamidium chloride (Getachew, 1983). Phenanthridines group, homidium compared (both chloride and bromide) have been in field use in some

territories for almost as long as quinapyramine. The drugs are effectives in *T. vivax* and *T. congolense* in all classes of stock and are usually well tolerated. Severe general reactions have only occasionally been seen in horses. The drugs may cause local reactions and should be administered by deep intramuscular injection taking care avoid leakage into subcutaneous tissues. Strains of *T. congolense* resistance to the phenanthridium compound have been found in all tsetse- infected area of Ethiopia (Pegram and Scott, 1976).

Isomethamidium chloride is a very active trypanocidal compound against *T. vivax* and *T. congolense* infections and, at higher dosage is potent prophylactic. The drug is particularly effective against strains of trypanosomes that have not been repeatedly exposed to chemotherapeutic agents. But some field strains of *T. congolense* which are resistance to homidium may also be resistance to isomethmidium at dosage of 0.8mg/ kg whilst some remains sensitivity to doses of 0.25 and 0.5 mg/ kg.

Of the diamidine group, diminazene aceturate is generally effective against *T. vivax, T. congolense* and *T. brucei*. Although this is the least toxic of current trypanocide drugs and is well tolerated by some classes of livestock, symptoms of general malaise may be observed after administration to horses. However, local reactions in the horses are sometimes severe and may be accompanied by abscess formation, necrosis and sloughing. It is advisable to divide the dose and treat the injection site by massage twice a day for two or three days. Camels are sensitive to this drug and doses of 7 mg/kg may prove fatal. Diminazene aceturate was also found to stay in animals body for some long period. Recent studies have confirmed the occurrence of field strains of *T. congolense* and *T. vivax,* in cattle, which were resistance to twice the recommended curative dose of 3.5mg/kg. This resistance seems more marked and troublesome in respect to *T. vivax* than it is in *T. congolense*. *T. brucei* has also shown variation in sensitivity to this drug. Suramin is effective against *T. evansi*, *T.brucei* and *T. equiperdium*. Two or three treatments given at weekly intervals are required to cure and the drug must be administred intravenously. Laminitis may be seen in horses during treatment and to minimize this risk treatment should be spread over a longer period.

Table1. drugs used in the control of tsetse- transmitted trypanosomosis in domestic animals (source: pegram and scott. 1976; seifert, 1996)

Drug	Proprietary preparations	Host	Dosage (mg/kg)	Indications (trypanosomes)
Curative drugs				
Diminazene Aceturate	Berenil	Cattle	3.5(im)	*T.vivax, T.congolense, T.brucei*
Homidium Chloride	Novidium Ethidium ‘’C’’	Cattle, horses	1.0-2.0(Im)	*T.vivax,T.congo,T.brucei*
Homidium Bromide	Ethidium Bromide	,,		,,
Isomethamidium Chloride	Somarin Trypamidium	Cattle, horses	0.25-1.0(im)	,,
Suramin	Naganol	Camels Horses, dogs	7-10 (iv)	*T.brucei*
Prophylactic drugs				
Isomethamidium Chloride	Somarin Trypamidium	Cattle	1-2.0(im)	*T.vivax,T.congo,T.brucei*
Quinopyramine- suramin complex	,,	Pigs	2.2-4.4 curative 7.4 sc	*T.simiae*
Pyrithidium Bromide	Prothidium Boots	Cattle	2.2-5 im	*T.vivax, T. congolense*

For T. *evansi* in camels 5.0 gm suramin iv or 2.0gm quinopyramine sulphate sc per 1000 ib animals.

5. Resistance to Trypanocidal drugs

No new trypanocidal drugs were produced for treating domestic animals for over 50 years. The use of same drugs over such long period have resulted in the widespread development of drug resistance strains of trypanosome (Peregrine, 1994).

5.1 **Mechanism of resistance to the anti trypanosomal drugs varies based on the type of the drugs and their mechanism of action**.

a. Isomethamidium

In 1990 Shapiro and England suggested that the main mode of action of ISMM is the cleavage of k-DNA- topoismerase complexes. This explanation was supported by Wells *et al*. (1995) that showed the kinetoplast is the primary site of ISMM accumulation. The mechanism of resistance to ISMM, however, is lesser clear; decreases levels of drug accumulation have been observed in drug resistance population of *T. congolense* (Sutherland *et al*., 1991) and later found indirect evidence of an increased efflux of drug form resistance trypanosomes (Sutherland, and Holmes, 1993). Recently, Mulugeta *et al*. (1997) showed that the maximal up takes 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 transport in resistance to ISMM by *T. congolense* remains to be examined, Carter *et al.*, 1995; Ross and Barns, 1996. It is also demonstrated that resistance *T. congolense* changes its mitochondria electrical potential in ISMM (Wilkes *et al*., 1997).

b. 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. However there are indications that it is similar to that described for ISMM (Peregrine *et al*., 1997).

c. Diminazene aceturate

Although diminazene probably exerts its action at the level of the kinetoplast DNA, and other mechanisms of action can not be excluded (peregrine and Mamman, 1993). Similarly, the molecular basis of resist to diminazene in trypanosomes is not clear. Carter *et al*. (1995) showed that the accumulation of diminazene was markedly reduced in arsenical-resistant *T. brucei* owing to alterations in the nucleaosides transporter system however; there might be other resistance mechanisms. Similarly to ISMM, contradictory reports have also been published on the stability of resistance 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.

The mechanism of resistance to the three currently available trypanocidal drugs has not been well addressed requiring much work to provide novel method for the detection of drug resistance trypanosomes in the future. Hayes and wolf, 1990 have distinguished three major types of genetic changes that are responsible for acquired drug resistance mutations of amplifications of specific genes directly involved in a protective pathway; mutations in genes that regulates stress response processes and need to alter expression of large number of proteins; and gene transfer. Gene amplification under conditions of drug pressure has been demonstrated in trypanosomes, but untile now there is no evidence that this occurs a mechanism of drug resistance (Ross and Sutherland, 1997). The current possibilities to insert or delete genes will certainly lead to a better insight in to the resistance mechanisms. Other aspects, such as the stability of drug resistance, its mono- or poly genic nature, dominant or excessiveness is also need to be examined because of their far- reaching impact on the control of resistance.

5.2 **Contributing Factors for Development of Drug resistance**

Table 2. Cross resistance and sanative pair of anti trypanosomal drugs

Trypanosomes Directly resistance	Cross resistance Curative dosage				Higher dosage		
	Berenil 3.5(mg/kg)	Antrycide (5.0(mg/kg)	Ethidium 1.0(mg/kg)	Samorin 0.5(mg/kg)	Berenil 5.7(mg/kg)	Ethidium 2.0(mg/kg)	Samorin 2.0(mg/kg)
Berenil	R	O	O	O	R	O	O
Antrycide	+	R	+	+	+	+	O
Ethidium	O	+	R	+	O	R	O
Samorin	O	+	+	R	O	+	R

NB: R: direct resistance, O= no cross resistance, += cross resistance
Source: (Getachew, 1983)

The development of drug resistance occurs with time under the following conditions:

1. Under dosage which occurs for a variety of reasons such as under estimation of animal body weight, over diluted solutions of trypanocides, deliberate under dosing or incorrectly calculated dose volume.

2. Too long intervals between prophylactic treatment beyond the manufacturers order

3. Heavy challenges of tsetse flies and frequent application of trypanocides.

4. Illegal and unprofessional handling of drugs by smugglers, traders and pharmacies.

5. Formation of abscess on cyst at the site of injection.

6. Incorrect injection.

An incorrect strategy of drug use (Leak, 1999).

Trypanosomes resistance to a particular drug may also resist other trypanocides. This cross-resistance frequently occurs between chemically related drugs, but it is also observed for drugs that are chemically very different (Itard, 1982). Major hazard to field schemes is because the number of drugs available is very limited, drugs are chemically related and the development of direct resistance to some drugs is often accompanied by cross- resistance to other drugs (table.2).

6. Detection of Trypanocides

Several methods have been described to identify drug resistance in trypanosomes. Three types of techniques are commonly used to identify drug resistance: test in ruminants, tests in mice, and in vitro assays. None of these is, however an ideal test and other tests are still in the phase of development or validation (Peregrine, 1996).

6.1 Test in ruminants:

Test in ruminants provides direct information using recommended doses of trypanocides in ruminants. The test consists of infecting a group of ruminants with the isolates under investigation and then regularly monitored over a prolonged period up to 100 days to determine the effective dose ED, ie. The dose that clears the parasites from the circulation, and the curative dose CD, ie the dose that provides a permanent cure (Sones *et al*., 1988). For this study the cattle or small ruminants must be kept in fly- proof accommodation or in a non- tsetse area. A variation of this technique was used by Ainashe *et al*. (1992) in which blood from a group of infected cattle was inoculated into a single recipient calf, which was monitored, and then later treated with trypanocides at the recommended dose. A break through infection indicates that one of the inoculated in to groups of calves and mice to determine the level of drug resistance. This technique is useful in situations where laboratory facilities are very limited but it only allows a qualitative assessment and does not indicate how many of the isolates inoculated in to single calf were resistance.

Its advantages are that most trypanosome isolates of cattle are able to grow in these hosts and the data obtained are directly applicable to the field but it requires long duration a follow up of 100 days and it is costly. Furthermore, if only one isolates per animal is tested; it is usually imported impractical and too expensive to examine a large number of isolates.

6.2 Tests in mice

After expansion of isolates in a donor mouse, groups of five or six mice are inoculated with trypanosomal. Twenty four hours later, or at the first peak of parasiteamia, each group except the control group is treated with a range of drug doses. Therefore, the mice should be monitored three times a week for 60 days. The ED50 or ED95 can be calculated, as can the CD50 or CD95. Sones *et al*. (1998) used groups of five mice which allowed an easy calculation of ED80 and CD80 values one out of five mice was not cleared or cured. These figures should be compared with those obtained using reference sensitive trypanosomes strains. It is cheaper than the test in ruminants, however most *T. vivax* isolates, and also some *T. congolense* isolates, don't grow in mice and higher dose of drug must be used in mice normally ten times higher in order to obtain comparable results to these obtained in cattle (Sones *et al*., 1988). Precise assessment of the degree of resistance needs a large number of mice per isolates. It takes as long as 60 days to evaluate the drug sensitivity of isolates (Sones and Holmes, 1992).

6.3 in - vitro assays

It takes up to 40 to 50 days of in vitro incubation to generate metacyclic trypanosomes (Gray *et al.,* 1993). The advantage of this technique is that large numbers of isolates can be examined; tests with metacyclic trypanosome correlate well with field observations. However, there are several disadvantages. In vitro cultivation of blood stream is only possible using readopted lines and not using isolates directly from naturally infected animals (Hirumi *et al*., 1993). A potential problems associated with this lengthy time of adaptation is the possible selection against trypanosomes that have the phenol type of the original population. In vitro assays are expensive to perform and require good laboratory facilities and well- trained staff.

As an alternative test the use of trypanocidal drug enzyme-linked immunosorbent assays ELISA in combination with parasite detection tests has given promising results for the detection of resistance trypanosomes. Whitelaw *et al*., (1991) developed a competitive ELISA, which allows the detection of small amounts of ISMM in serum of cattle. The test is both sensitive, detecting sub nano gramme concentrations, and specific. It allows the monitoring of drug levels over extended periods and the evaluations of factors influencing drug dis appearance rates from the plasma.

7. Impact of Anti trypanosomal resistance

It is unclear whether drug resistance trypanosomes are less pathogenic than susceptible ones or not. Several authors Silayo and Marandu, 1989; Berger *et al*., 1995; Mutugi *et al*., 1995 have observed a loss of values and/or a loss of fitness in drug resistance trypanosomes. Transmission by tsetse flies does not appear to affect the drug sensitivity of trypanosomes and drug resistance strains remains resistance after passage through tsetse flies (Moloo and Kutuza, 1990). The loss of fitness in other drug resistance parasite is well known phenomenon and is probably also present in the trypanosomes. The long–term occurrence of *T. congolense* resistance to diminazene, isomethamidium and homidium in the Ghibe valley of Ethiopia (Mulugeta *et al*., 1997) indicated the magnitude of the problem. The epidemiology of drug resistance populations of trypanosomes is dynamic; once established the incidence progressively spread with in the population, once drug resistance is established in a herd the resistance trait is known to be stable for a long time and such stocks can spread to wider areas and different species of animals through animals movement and/ or the spread of tsetse populations (Mulugeta *et al*., 1997).

A study to assess the impact of drug- resistance trypanosomes on the productivity of the local cattle was carried out in the Ghibe valley, Ethiopia, where high prevalence of multiple – drug resistance was reported (Codjia *et al*., 1993). (Rowlands *et al*., 1994a; 1994b) followed more than 300 east African zebu calves from birth too three years of age together with their dams, in this region between 1986 and 1992. During most of the period, animals that were parasiteamic and had a packed cell volume PCV below 26% or animals with clinical signs of trypanosomosis were treated with diminazene aceturate at 3.5 mg/kg. Calf mortality was high, low growth, levels of reproduction in terms of calving interval and age at first calving were maintained under regular trypanocidal therapy in the cows that were monitored over the same period, (Rowlands *et al*., 1994b). Impact of trypanosome infections on the incidence of abortion was over 8 percent of pregnancies associated with cases of parasitamia detected during the last trimaster of pregnancy (Itty *et al*., 1995).

8. Current situation of resistance against Trypanocidal drugs in Africa

So far resistance to one or more of the trypanocidal drugs used in cattle has been reported in at least 13 countries in sub-saharan Africa including Burkina Faso, Chad, Cote D' Ivoire, Ethiopia, Kenya, Nigeria, Somalia, the Sudan, the United Republic of Tanzania, Uganda, Zimbabwe, the Central African Republic, and Zambia (Peregrine, 1996). In several countries surveys for resistance have not yet been carried or cases of resistances have not been published. In 8 of 13 countries, multiple- drug resistance has been reported.

Table 3. Published reports drug resistance trypanosomes

Country	Trypanosome species	No of isolates		% R. isolates	Resistance to
		exam	resist		
Burkina	TC	12	9	75%	I
Ethiopia	TC	12	10 11	100 92	D I
Ethiopia	TC	10	10	100	D,H,I
Kenya	TC	7	2	29	I
Kenya/somalia	TV	7	6 3 5	86 43 71	I H Q
Nigeria	TV	19	12	63	D,H,I
Nigeria	TB	12	2 1	17 8	D,I I
Sudan	TC,TV,TB	12	5	3	H
Uganda	TB	36	1	3	D,I
Zimbabwe	TC	14	6	43	D

NB=*TV=T.vivax, TC=T.congolense, TB= T.brucei*
I= isomethamidium, H= homidium, Q= quinopyramine, D=Diminazene
(Source= Geerts and Holmes, 1998)

There is an urgent need for surveys in which representative numbers of trypanosome isolates are examined for drug resistance. It is also important to stress that drug resistance is not an ''all or nothing'' phenomenon and the degree of drug sensitivity and

resistance varies considerably between individually trypanosomes.

8.1 **Situation of Anti- trypanosomal drug resistance in Ethiopia**

Previous studies have shown the prevalence of drug resistance is widespread in cattle herd of Ethiopia. Some of the published works are summarized on table 4 below.

Table 4. Published articles on trypanocidal drug resistance in Ethiopia

Origin isolates	Species	Host	Reference
Didessa/Anger	*T.congolense*	Mice	Pegram and scott,1976
Ghibe	*T.congolense*	Cattle, mice	Codjia *et al*., 1993
Ghibe	*T. congolense*	Cattle, mice	Mulugeta *et al*.,1997
Kindo koysha	*T. congolense*	Cattle	Adem and Abebe, 2000
Metekel	*T.congolense*	Cattle, mice	Afework *et al*., 2000
North Omo	*T.congolense*	Donkey	Assefa and Abebe, 2001
Arbaminch, Sodo bedella, Ghibe	*T.congolense*	Cattle,mice	Chaka and Abebe, 2003
Didessa	*T.congolense*	Cattle	Tewelde *et al*., 2004

9. Guidelines to delay development of Drug resistance

The most important guidelines to delay the development of drug resistance were considered to be: use of the'' sanative pair'' of drugs (isomethamidium or ethidium and diminazene); and avoidance of the exposure of trypanosomes to sub therapeutic drug concentrations (Boyt, 1986).

It is clear, however, that the application of these guidelines may not be sufficient to maintain the efficacy of the existing drugs, especially since they lack recommendations concerning reduction of the treatment frequency. Based on current knowledge in field of trypanocide resistance and on experience in the control of resistance Ao insecticides antihelmentics, antibiotics, and other drug Geerts *et al*., 1997 the following recommendations are proposed in order to delay the development of resistance;

i. Reducing the number of treatments by integrating drug usage with other control measures

It is widely agreed that the most efficient way to delay the development of drug resistance remains the reduction of selection pressure by drugs, ie decreasing the number of treatments. This is of particular importance in areas of high tsetse challenge which are commonly associated with reduced periods of chemoprophylaxis. In such situations the treatment frequency is commonly increased and drug resistance often emerges as a constraint to further drug usage. Very intensive drug treatment schedules, as described by Stevenson *et al*. (1993), who administered ISMM six to seven times a year and ethidium up to 11 or 12 times a year might be able to control the resistance problem temporarily, but are no solution in the long term. Further more, frequently repeated trypanocidal drug treatments have been associated with toxicity problems (Eisler *et al.,* 1997).

This kind of approach inevitably increases the selection pressure in the absence of any measures such as the use of the sanative pair to counteract the development of resistance and may lead to increased levels of drug resistance. It has been shown in other areas that there is a strong correlation between the treatment frequency and the rate of development of resistance (Conder and Campbell, 1995). It is therefore strongly recommended that in high tsetse challenges areas control of trypanosomosis should not rely solely drugs but an integrated approach should be adopted using vectors control to reduce the tsetse challenges, along it reduced frequency of drug dosing (Fox *et al*., 1993; peregrine *et al*., 1991). In west and central Africa the use of trypanotolerant livestock and drugs may also be appropriate in areas of high tsetse challenge.

ii. Use of Correct Dose

Underdosing is one of the major causes of resistance developments. Sub-therapeutic drug concentration exerts a strong selective pressure for the emergence of resistance clones that pre-exist in trypanosomes population. Unfortunately, under dosing occurs very frequently; farmers have the tendency to underestimate the weigh of their animals when they to treat them (Besier and Hopkins, 1998). Given the fact that in many countries unskilled persons are allowed to administer drugs, errors easily occurs in calculating the doses for the treatment of the animals. Packaging of isomethamidium as one-dose treatment similar to diminazene would undoubtedly help to reduce this problem. In addition, as the drug are relatively expensive there is a temptation to over dilute the drug and hence under dose. Data on the pharmacokinetics of ISMM in goats have suggested that following intra muscular administration, the bioavailability of ISMM in that species is very low, approximately half that in cattle under similar circumstances (Eisler *et al*., 1996). If this is confirmed, higher doses might be used in goats than in cattle. The use of improved formulation of existing drugs is another possible way

to avoid sub- therapeutic concentration; the polymer devices containing ISMM or ethidium as described by (Geerts *et al*., 1997) are step in this direction.

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

Unlike human sleeping sickness, animal trypanosomosis is commonly controlled with mass treatment which can be highly successive over many years in ranch cattle for example (Trail *et al.*, 1985). However, this form of treatment exerts a strong selection pressure on the trypanosome population. The higher proportion of the trypanosome population exposed to the drug and the lower proportion in refugia ie the proportion of trypanosome present in the fly population or in other hosts, the higher the selection pressure. Study on pesticides indicate that systematic mass treatments hasten the development of resistance therefore, in well monitored situation there may be a case for limiting treatment to individual clinical cases. In such situations, drug resistance problems can be minimized and acquired immunity encouraged (Pegram and Scott, 1976). A similar approach is currently being used in south Africa to control antihelmentics resistance Hemonchus contortus in sheep (Vanwyk *et al*., 1997).

iv. Ban on the 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 use because of problems with toxicity and resistance development. It is still available for use in camels, however, and it is likely that it is still mistakenly used in cattle in some situations in Africa. The use of quinopyramine was suggested cause of the multiple drug resistance problems in the Ghibe valley of Ethiopia refered earlier. Ndoutamia *et al.* (1993) has showed that after artificial induction of resistance to quinapyramine in *T. congolense*, multiple- resistance to ISMM, homidium and diminazene was expressed at the level of the individual trypanosome and could be transmitted by tsetse flies.

10. Guidelines on the control 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 are necessary;

- The best method for avoiding drug resistance is the alternative use of two drugs or more, which are not chemically related this is mainly important in resistance against single drug. Examples; Berenil and Trypamidium may be used alternatively. Berenil for resistance to Ethidium, Prothidium, or Isomethamidium. Isomethamidium for resistance to quinapyramine methyl sulfate or Berenil (Itard, 1982) and also either removing animals from affected area, or
- Changing the drug or increasing the drug level, but not with phenanthridines.
- Correct assessment of the tsetse risk.
- Use of drug in correct sequences.
- Conducting drug sensitivity tests before initiating scheme, and establishing national drug use policy (Getachew, 1983).

But integrated control, measures such as reducing vector numbers to reduce the number of drug treatments will be of great importance both in resistance against single drug and multiple- resistance associated with mixed infectious. Peregrine 1994 has showed that in the Ghibe valley, Ethiopia, multiple – drug resistance trypanosome infections, could be controlled effectively using an integrated approach involving tsetse fly control targets and chemotherapy of clinically sick animals using diminazene. It indicated the apparent prevalence of *T. congolense* infectious fell from 30 percent $\pm$5 percent one year before and after the tsetse control program, the apparent prevalence of diminazene resistance infectious decreased by about 75% during the same period. A similar level of success was also reported by (Fox *et al*., 1993) in the united republic of Tanzania using a deltamethrin- dripping program to over come the problem of drug resistance (Geerts and Holmes, 1998). Administration of various drugs to which the different sub- populations are sensitive, will eliminate the whole trypanosome population (Mulugeta *et al*., 1997). Selection pressures thus the level of resistance. Similarly, although the intravenous administration of ISMM enhanced the therapeutic activity of the compound as compared with intramuscular injections; it was not effective in eliminating resistance parasite (Sutherland *et al*., 1992).

11. Conclusion and recommendations

Trypanosomosis is one of the most prevalent disease which caused both direct and indirect loss in livestock production and productivity. There has been various control measures of trypanosomes such as control of vectors, trypanotolerant breed and more practically use of anti trypanosomal drugs. There are limited numbers of these drugs; diminazene aceturate, isomethamidium chloride, homidium salts, quinapyramine, etc used as curative and prophylactic drugs. Because of the use of this limited numbers of drugs overlong period of time resistance has been developed to most of these drugs. Factors contributing to resistance development are multiple; because it is very unlikely that new trypanocidal drugs will be released onto the market in the near future and there is growing concern that their future effectiveness may be severely curtailed. So it is essential to try to maintain

the efficacy of the currently available drugs that requires integrated approach.

Based on above conclusion, the following points are therefore recommended;

- The limited number of anti trypanosomal drugs available should be used after proper identification of trypanosome and performing sensitivity to the drugs.
- The drugs should also be applied in correct dose, correct route, for correct duration and to the right animal.
- Further research should be done to reveal the mechanisms of resistance to trypanocides to alleviate the problems of drug resistance.
- Use of sanative pair might not be sufficient to control resistance trypanosomes and should be applied with cautions not to cause multiple drug resistance.
- The frequency of application of the drugs should be reduced by integrating other means of trypanosomosis control.

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