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Drugs and Drug Resistance in African Animal Trypanosomosis: A Review

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Abstract: Trypanosomosis is the most serious animal health problem in sub-Saharan Africa and prevents the keeping of animals in over millions square kilometres of potentially productive land. Trypanocidal drugs belong to different chemical families and they are used quite intensively in veterinary medicine. Drug for control of animal trypanosomosis relies essentially on three drugs namely homidium salts, diminazene aciturate and isometamidium chloride. About thirty five million doses of trypanocidal drugs are used annually in the treatment of animal trypanosomosis in Africa. Most of these drugs are very old and utilized for a long period of time. Hence, treatment of trypanosomosis is complicated by development of drug resistance. Drug resistance has been reported in 17 countries of Africa. The exact mechanism how trypanosomal parasite develop resistant and the factors responsible for the development of drug resistance are yet to establish. In addition, it is very unlikely that new trypanocidal drugs will be released into the market in the near future. Therefore, it is essential to maintain the efficacy of the currently available drugs through proper utilization. The general features of trypanosomosis, drugs for the treatment and drug resistance in African trypanosomoses are briefly reviewed in this paper and measures to combat drug resistance especially at field level are also suggested.

Key words: Drug Resistance · Trypanocidal Drugs · Trypanosomosis

INTRODUCTION

Trypanosomosis is a complex debilitating and often fatal disease caused by species of unicellular parasite (trypanosome), which is found in the blood and tissues of vertebrate including livestock, wild life and people [1]. The disease can be transmitted between the hosts mainly by tsetse flies cyclically, or by other biting flies mechanically and it is widespread in African continent occupying 37 countries. The impact of the tsetse-associated disease extends in sub-Saharan Africa over 10 million km^2 (a third of the continent). Trypanosomosis in Africa costs livestock producers and consumers an estimated USD1340 million each year [2]. If lost potential in livestock and crop production are considered, then trypanosomosis is costing Africa an estimated USD 5 billion per year [3].

There is no effective vaccine against trypanosomes and in the absence of coherent environmentally friendly and sustainable vector control strategies, the control of trypanosomosis continues to rely principally on chemotherapy. About 35 million doses of drugs are used in Africa each year, with about 50-70 million animals at risk from trypanosomiasis [4, 5]. The development of every effective drug between 1940 and 1960 has considerably contribute to this large scale use of trypanocidal drugs. Since most of trypanocidal drugs have been in use for more than half a century, they can cause the appearance of the drug resistant strain of trypanosomes. Since there is no indication that new products will become available in the near future, it is of utmost importance that measures are taken to avoid or delay the development of resistance and to maintain the efficacy of the currently available drugs. Professionals and livestock owners must be well awared about drugs and drug resistance in trypanosomosis. Therefore, the objectives of this paper were to review about drugs currently used for the treatment of trypanosomosis and to give highlights on the current status and mechanisms and measures to combat drug resistance.

Drugs for the Treatment Animal Trypanosomosis: The compounds in common use for the treatment or prevention of animal trypanosomosis are diminazene

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derivatives, suramin, quinapyramine, homidium, isometamidium and pyrithidium [6, 7]. They are grouped either curative or prophylactic drugs or both depends on their use. Trypanocides are usually supplied in dry form as powder, granules or tablet, which have to be dissolved in sterile water for injections. The satisfactory treatment of trypanosomosis requires correctly administered trypanocidal therapy and supportive measures [8].

Curative Drugs

Diminazene Derivatives: Diminazene derivatives like diminazene aceturate have remarkable curative properties. Diminazene aceturate is very active, stable and easy to use and has very low toxicity. These advantages make it a practical and risk free trypanocides at least for cattle. It is prepared as a yellow powder and easily soluble in water. This solution can only be kept for two to three days. It is injected subcutaneously in cattle (slight local reactions possible) or intramuscularly (very rapid absorption) at a dose of 3.5 mg/kg live weight for treating *T. vivax* and *T. congolense* infections. Infections due to *T. brucei* can be treated in horse and cattle with the dose of 7mg/kg [9].

Diminazene derivatives bind to DNA and interfere with parasite replications. This class of drugs has tendency to accumulate in tissue, therefore half life is very long, which may lead to residual problems in food producing animals [10].

Suramin Sodium: Suramin is a white crystalline powder and soluble in cold water. It is practically the only ureic compound, the oldest, but still used. It is always used in treating the first stage of *T. brucie rhodensiense* and *T. brucei gambiense* infections. The minimum dose is 3-4 g/animal in 10% aqueous solution given intramuscularly or intravenously. The dose for horse is 10mg/kg body weight. Animal generally show good tolerance of the drug. It may be used both as a curative and prophylactic drug for horses, donkeys and dogs [6].

Quinapyramine Sulphate: Quinapyramine methyl sulphate is sold in the form of white powder that dissolves easily in water. It is prescribed as a curative drug for cattle and small ruminants and is given subcutaneously as a 10% aqueous solution at dose 5mg/kg. It is used to treat *T. evansi* infectious in dromedaries at a standard dose of 2g/ adult. From 1950, until recently it was used in all the African countries, giving excellent result for cattle trypanosomosis (especially *T. congolense*); it was slightly less successful against *T. vivax*. It causes appreciable systematic reactions and intramuscular injection cause painful local reactions leading to discomfort or lameness. In pig with *T. simiae* infections heavy doses (12.5-35mg/kg) can be used as curative treatment. Trypanosomes resistant to this compound should be treated with dimenazene [6].

Homidium Salts: Homidium salts are effective against *T. vivax* infections in cattle but less so against *T. congolense* and *T. brucei*. Their limited and protective activity in cattle depends on severity challenge and may last three to five weeks. Homidium resistant trypanosome can be controlled by diminazene or isometadium [11]. It is given to cattle in one or 2.5% solutions at the rate of one mg/kg. Novidium, which is a mixture of homidium chloride and bromide, has the same action as ethidium. It can also be used in *T. brucie* infections in dogs at the rate of 3-5mg/kg [6].

Prophylactic Drugs: Prophylactic treatment should be given with a great caution because of the constant risk of creating resistant trypanosome strain. The possibilities of prophylactic drug treatment under traditional African livestock management condition are therefore strictly limited. Only three drug share a sufficiently long lasting effect to be used in practice, these are isometamidium, prothidium and quinapyramine prosalt [6].

Isometamidium: Isometamidium is a phenanthridine aromatic amidine with a narrow therapeutic index which has been marketed for both a prophylactic and a therapeutic trypanocidal agent. Isometamidium chloride is used as curatively at lower dosage rates and prophylactically at higher dosage rates. It is usually prepared as red powder easily soluble in water. It is used in a one or two percent aqueous solution and administered by deep intramuscular injection at the rate of 0.25-1mg/kg, depend on drugs resistant risk. Strain of trypanosomes resistant to isometamidium and other phenanthridine appear frequently, but they remain susceptible to diminazene aceturate. It is given to the animal at dose rate of 0.51mg/kg and it will be protected for two to four months depending on the extent infections risk. Dromedaries appear to be more sensitive to this drug than other animals [6].

Pyrithidium Bromide: Pyrithidium bromide introduced in 1956, has been widely used in East Africa and is given as 2 to 4% solutions at the rate of two mg/kg body weight, subcutaneously or by deep intramuscular

injections [11]. Pyrithidium is basically used for prophylaxis in cattle. Its protective effect was 3 to 5 months depending upon tsetse fly challenge; trypanosomes rapidly developed resistance to this synthetic hybrid, showing cross-resistance to both quinapyramine and homidium in mass treatment; resistant strains were usually sensitive to diminazene. It can be used both as a curative and prophylactic drug for horses, donkeys and dogs [6]. The mechanism of killing has been unclear and controversial. It has long been known to cause loss of the mitochondrial genome, named kinetoplast DNA (kDNA), a giant network of interlocked minicircles and maxicircles. However, the existence of viable parasites lacking kDNA (dyskinetoplastic) led many to think that kDNA loss could not be the mechanism of killing.

Quinapyramine Prosalt: Quinapyramine prosalt is mixture of salts of a sulphate and a chloride, it is almost insoluble and is deposited in the subcutaneous connective tissue from which it is slowly absorbed into circulatory system. This property gives the prosalt a prophylactic effect varying between two to three months depending on the degree of risk of infections. The prosalt is prepared as a suspension is distilled water at a rate of 3.5g/15ml water. The resulting ml/kg suspension is administered at the rate of 5ml/100kg live weight. In regions where *T. evansi* is endemic, horses and dromedaries can be protected for 2-3 month with quinapyramine prosalt, but this causes serious local reactions, especially in horse [6]. Generally, quinapyramine is highly active against *T. congolense, T. brucei, T. vivax* and *T. evansi* and therapeutic levels quickly. The largest action of quinapyramine is protein synthesis inhibition, displacing magnesium ion (mg^{2+}) and polyamine from cytoplasmic ribosome's and condensation of kinetoplast DNA leading to an extensive loss of ribosome [11].

Drug Resistance in Trypanosomoses: Drug resistance in trypanosomoses has been a continuing problem in the treatment of trypanosomosis and it is particularly common with *T. congolense*. Resistance is developed more ready to prophylactic drugs. Curative drugs are usually rapidly eliminated and the risk of resistance developing after their use are not great, unless treatment have to be repeated frequently, as in area where the incidence of infection is high [12].

Trypanocidal drugs have been used for more than 50 years, often badly and trypanosomes have become resistant to them in many areas. Dose that are too small only kill some trypanosomes; other survive and becomes resistances to drugs [13]. 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 (selective pressure), cross-resistance or sometimes by mutagenesis [14].

The development of resistance to therapeutic agent has been well documented for antibiotics, anthelmintics and insecticides. Thus, it is not surprising that drug resistance has also emerged to three commonly used trypanocides, given their long use (isometamidium, homidium and diminazene) [15]. Figure 1 depicts year of commercial release of drugs or chemicals and first appearance of resistance in target organisms.

So far, resistance to one or more of the three more commonly used trypanocidal drugs used in cattle has been reported in at least 17 countries in sub-Saharan Africa (Burkina Faso, Chad, Ivory coast, Ethiopia, Kenya, Mali, Somalia, Sudan, Tanzania, Uganda, Zimbabwe, Zambia, Mozambique, Cameroon, Nigeria, Guinea and Central African Republic) (Fig.2) [16]. In eight of the 17 countries, multiple resistances have been reported. This is probably an underestimation of the true situation, because in several countries surveys for resistance have not yet been carried out or cases of resistance have not been published. Table 1 shows the *Trypanosoma* species and type of drugs in which their resistance has been reported [15].

Mechanism of Drug Resistance: 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 spite of the length of time these drug have been available and widespread interest in drug resistance, relatively little work has been done on how these drug are taken up by trypanosomes and the processes that are changed when drug resistance emerges. Progress is being made in elucidating the role of nucleoside transporters in resistance to trypanocidal drugs [17]. Furthermore, changes in the mitochondrial electrical potential have been demonstrated in isometamidium resistant trypanosomes. As the mitochondrial electrical potential is closely linked with the rate of isometamidium uptake seems to be a good indicator of the degree of drug resistance. Measuring the mitochondrial electrical potential might a rapid indication of the degree of drug resistance. It could be carried out

Table 1: Drug resistant trypanosomes species in African countries

S/n	Country	Trypanosome species	No. of Isolates		% of Resist isolates	Resist to
			Examine	Resistance		
1	Ethiopia	*T. congolense*	12	12	100	D
			11	9	8.81	
			10	10	100	D, H, I
2	Burkina	*T. congolense*	12	9	75	I
3	Kenya	*T. congolense*	7	2	29	I
4	Kenya/Somalia	*T. vivax*	7	6	86	I
				3	43	H
5	Nigeria	*T. vivax*	19	12	63	D, H, I
		T. brucei	12	2	17	D, I
				1	8	I
6	Sudan	*T. congolense*, *T. vivax*, *T. brucei*	12	5	42	H
7	Uganda	*T. brucei*	36	1	3	D, I
8	Zimbabwe	*T. congolense*	14	6	43	D

D= diminazene; H = homidium bromide (ethidium); I = isometamidium [15]

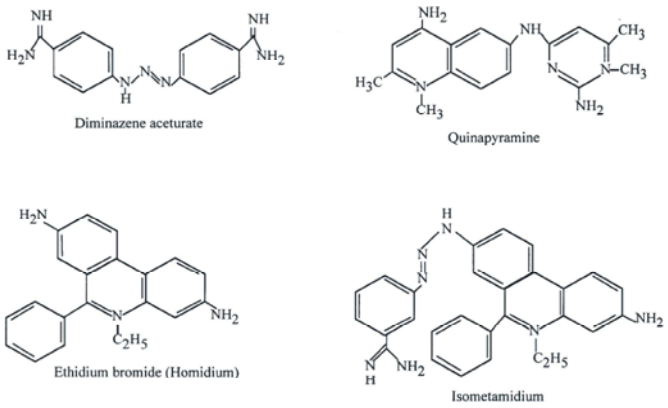
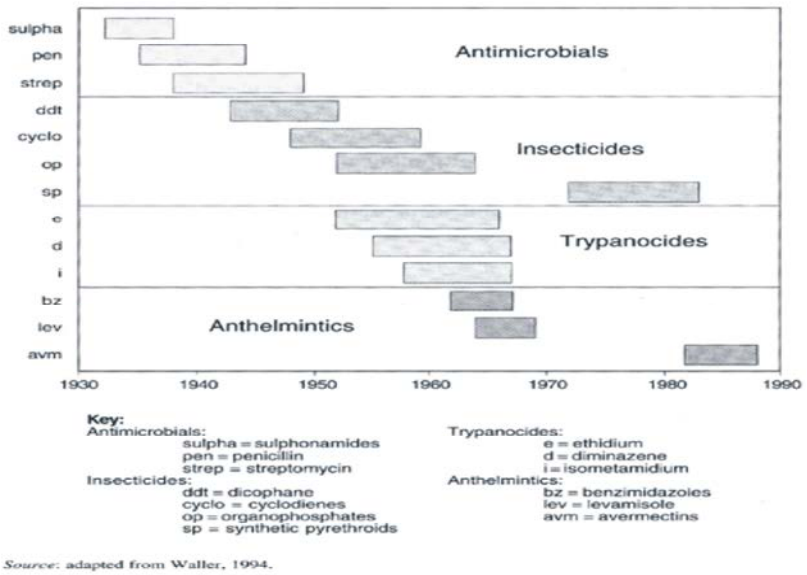


Fig. 1: The structures of the four most commonly used drug in the chemotherapy and chemoprophylaxis of animal trypanosomosis in Africa



Source: adapted from Waller, 1994.

Fig. 2: Evolution of resistance: year of commercial release of drugs or chemicals and first appearance of resistance in target organisms [15].

Table 2: Cross-resistance between trypanocidal drugs

	Cross resistance to								
	At curative doses					At increased doses			
Trypanosomes resistant to	QP	HM	PB	IM	DA	HM	PB	IM	DA
QP	+	+	+	+	+	+	±	-	-
HM	+	+	+	+	-	+	+	-	-
PB	+	+	+	+	-	+	+	-	-
IM	+	+	+	+	-	+	+	-	-
DA	+	-	-	-	+	-	-	-	+

QP = Quinapyramine; HM = Homidium;, PB = Pyrithidium bromide;, IM = Isometamidium;, DA = Diminazene aceturate.+ = resistant; - = not resistant; ± = some strains resistant [1].

Fig. 3: African countries with reported resistance to trypanocidal drugs. A star indicates that resistance to trypanocidal drugs has been reported in animal trypanosomes in that country [16].

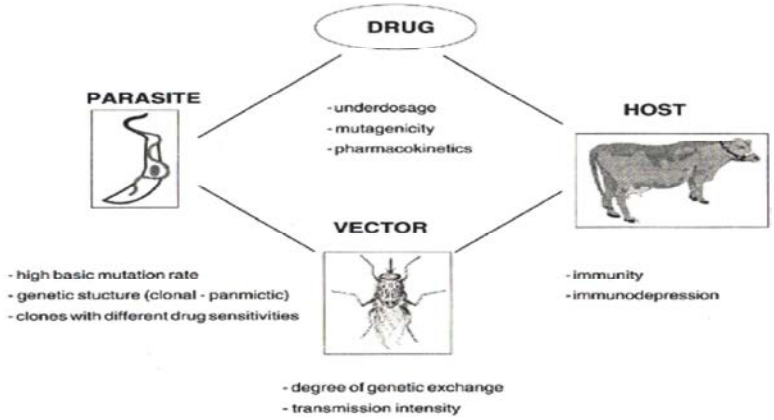


Fig. 4: Some factors influencing the development of resistance to trypanocidal drugs [4].

using small number of trypanosomes directly isolated from the blood of infected animals. Interesting work is also going on to identify genetic markers for isometamidium resistance which might be developed later on into reagents for the identification of resistant trypanosomes using polymerase chain reaction [15].

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 drug-resistant trypanosome clones accumulate fewer drugs than their sensitive counterparts [14].

Cross and Multiple Drug Resistance: With development of resistance to one compound, trypanosomes may show resistance to compound of the same series and may also to those of other of series thus, in the phenanthridium series, resistance to pyrithidium bromide leads to resistance to isometamidium and homidium. There is also cross resistance between quinapyramine and phenanthridium series. So sometimes, one strain of trypanosome may be resistant to many drugs (multiple drug resistant), a situation that constitutes a particularly grave threat to livestock production and health in Africa. In contrast, a number of experimental drug sensitivity studies with suramin and diminazene *in vitro* and in rodents have demonstrated that acquisition of resistance to suramin does not confer resistance to diminazene, suggesting that cross resistance may not exist between these drugs [14]. Table 2 shows the cross-resistance among the five compounds in use for the treatment of tsetse-transmitted trypanosomosis in livestock.

Impact of Drug Resistance: It is essential to assess not only the distribution of drug resistance, but also the constraint it imposes on effective control. To date, few studies have accurately assessed the impact of drug-resistant trypanosomes on livestock productivity, although it is generally assumed that uncontrolled infections will have a severe impact on both survival and productivity. A useful recent study to assess the impact of drug-resistant trypanosomes on the productivity of the local cattle was carried out in the Ghibe valley, Ethiopia, where a high prevalence of multiple drug resistance was reported. In the study, calf mortality was rather high, incidence of abortion was increased and the financial and economic returns were also affected [14].

Detection of Drug Resistance: Several methods have been described to identify drug resistance in trypanosomes [16]. At present, three types of technique are commonly used to identify drug resistance in trypanosomes such as tests 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 [15].

Measures to Combat Drug Resistance in the Field: 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-known mutagenic compounds and might induce mutations, the most resistant of which are certainly selected under drug pressure. Taking into account of these factors different measures can be proposed in order to reduce the chance of drug resistance. Of these the most important measures are use of the correct dose, changing of drugs, sanative treatment, increased dosage, repeative treatment and use of combined drugs. In addition to these, care must be taken to avoid fake drugs and good quality assurance must be implemented [15].

Use of the Correct Dose: Under dosing is one of the major causes of resistance development. Sub-therapeutic drug concentrations exert a strong selective pressure for the emergence of resistant clones that pre-exist in the trypanosome population. Unfortunately, under dosing occurs very frequently. Farmers have the tendency to underestimate the weight of their animals when they have to treat them since farmers or unskilled persons in many countries of Africa are administering drugs due to absence of strict rules about the utilization of veterinary drugs [15].

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 [1, 5].

Sanative Treatment: The concepts of sanative treatment is the use of a pair of trypanocides which are chemically unrelated and therefore, unlikely to induce cross resistance [18]. Diminazene and homidium, or diminazene and isometamidium can be used in the field as sanative combinations. These pairs when strategically employed

can be used to maintain herd productivity in the field without the development of resistance to either of the compounds [14].

High Dose and Repeat Treatment Regimen: High dose treatment offers the best opportunity for eliminating infections with trypanosomes which express high degree of resistance to drugs. However, it must be appreciated that the scope for increased drug dosage is highly dependent on the relationship between the maximal tolerated dose and the minimal dose required to treat cure (the therapeutic index). This is a major limitation to high dose treatment with trypanocides as the margin of safety of most of them is usually quite narrow, trypanocidal drug toxicity being quite common. So this technique is helpful in the utilization of drugs with wide safety of margin. Studies on the efficacy of repeat treatments of *T. congolense* infections with diminazene aceturate indicate that such regimen may be useful especially if administered at 48 or 96 hour intervals. This tends to support the suggestion that the efficacy of trypanocides depends not only on the concentration of the drug to which the parasites are exposed but, also on the length of exposure. But this may not be true for all trypanocidal drugs [14].

Use of Combined Drugs: The rationale for the use of two or more of existing drugs in combinations to increase therapeutic activity, decrease clinical toxicity and potentially reducing the frequency of the emergence of drug resistance. A complex can be used at high enough dosage without the attendant toxic reactions to cure resistant strains [14].

Beware of Fake Drugs: Fake trypanocides may be sold in Africa. The drugs are either fake in their composition or are faked by dilution of the original products or by substitutions by an ordinary component apparently a like (Nere powder for diminazine (berenil), coffee or charcoal for ethidium, potassium permanganate for isometamidium. For isometamidium, one must pay attention to the information given on the packages (the examples of a shell found on a fake product labelled “for veterinary use” and in general carefully check the logo of firms. Use will known products and be regular customer to trust worthy supply service [20].

Quality Assurance of Trypanocidal Drugs: In recent years, a further issue has arisen associated with the liberalization of veterinary drug supply and market. The growing problem of poor quality drugs finding their way on to the market in some cases, products with no trypanocidal activity have been identified and in other situations compound with reduced activity have been marketed. Such products are not less effective when used by farmers, but also greatly increase the risk of drug resistance developing (especially when under dosing also allows the survival for the heterozygote resistant trypanosomes). Unfortunately, quality control on pharmaceutical products used in the developing world is frequently inadequate and there is already considerable evidence that the problem is widespread for a variety of pharmaceutical products [21].

CONCLUSION

Despite limited number of trypanocidal drugs, they are more widely used to control the disease. Drug resistance poses a potential treat to control measures. The exact mechanism how trypanosomal parasite develop resistant and the factors responsible for the development of drug resistance are yet to establish. In addition, it is very unlikely that new trypanocidal drugs will be released into the market in the near future. Therefore, it is essential to maintain the efficacy of the currently available drugs through proper utilization.

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