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Summary

Trypanocidal drugs remain the principal method of animal trypanosomiasis control in most African countries. However, there is growing concern that their future effectiveness may be severely curtailed by widespread drug resistance. This document presents an overview of the current situation of resistance to drugs for the chemotherapy of trypanosomiasis in African livestock. Although the number of case reports on drug resistance is increasing, there is a lack of reliable data at the regional or national level on the true prevalence and impact of drug resistance.

In order to compare data on a temporal and spatial basis across Africa there is an urgent need for better standardization of tests for the detection of drug resistance. The advantages and disadvantages of the currently available assays are briefly reviewed and measures suggested to improve the situation.

Finally, some guidelines on delaying the development of drug resistance are proposed and measures which may be adopted to control drug resistance when it occurs are recommended. Although there is still a lack of knowledge about the mechanisms of resistance and the factors responsible for the development of drug resistance, urgent measures are needed to maintain the efficacy of the existing drugs. Based on experiences of the control of resistance to other drugs such as antimalarials, antibiotics and anthelmintics it is suggested that reliance on the "sanative pair" guideline might not be sufficient to control resistance to

trypanocides. This guideline needs to be accompanied by the following additional measures:

- *Reduction in the number of treatments.* The most efficient way to delay the development of drug resistance is to reduce the selection pressure caused by these drugs. Exclusive reliance on drugs for the control of trypanosomiasis, especially in areas of high challenge, and mass treatments at short intervals should be avoided. More attention should be given to integrated control measures involving the vector as well as the parasite.
- *Avoidance of underdosing.* Underdosing commonly occurs in the field and is an important cause of resistance development. Measures should be adopted to minimize underdosing. Better formulations of the existing prophylactic drugs may help to avoid subtherapeutic concentrations, which exert a strong selection pressure for resistant clones.
- *Quinapyramine should no longer be used in cattle.* Cross-resistance with the other available trypanocides has now been clearly demonstrated at the level of individual trypanosomes. The use of this drug in cattle is therefore contraindicated.

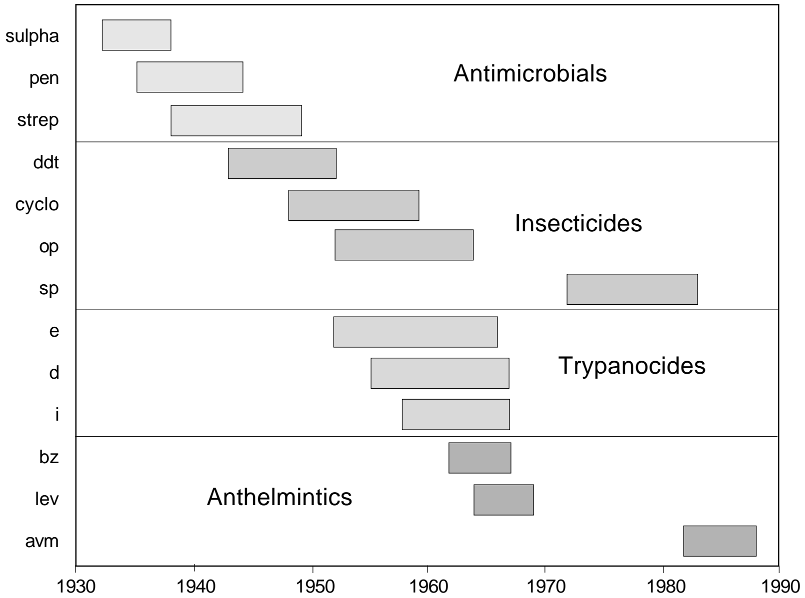
Introduction

Isometamidium, diminazene and the homidium salts have been in use for more than 35 years and it is estimated that about 35 million doses per year are currently used in Africa. These drugs remain popular with livestock owners and veterinarians because they are generally affordable and effective. 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.

The repeated use of chemicals as pesticides or chemotherapeutic agents inevitably leads to the development of resistance in the target organisms. Figure 1 clearly illustrates that resistance systematically occurs within approximately ten years following the introduction of antimicrobials, insecticides, trypanocides and anthelmintics to the market (Waller, 1994). This also occurred with the trypanocidal drugs, such as isometamidium chloride (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 and Folkers, 1966; Na'Isa, 1967; Jones-Davies, 1967). Quinapyramine was marketed earlier, but was withdrawn in 1976 because of resistance and toxicity problems. It was later reintroduced for use in camels and horses and may still be used in error in cattle in some locations.

This document gives an overview of the current state of knowledge on trypanocide resistance, the current methods of its detection and how these may be improved, and more refined guidelines to delay the development of resistance and to control resistance when it occurs.

FIGURE 1
Evolution of resistance: year of commercial release of drugs or chemicals and first appearance of resistance in target organisms



Key:

Antimicrobials:
- sulpha = sulphonamides
- pen = penicillin
- strep = streptomycin

Insecticides:
- ddt = dicophane
- cyclo = cyclodienes
- op = organophosphates
- sp = synthetic pyrethroids

Trypanocides:
- e = ethidium
- d = diminazene
- i = isometamidium

Anthelmintics:
- bz = benzimidazoles
- lev = levamisole
- avm = avermectins

Source: adapted from Waller, 1994.

Chapter 1
Current situation of resistance against trypanocidal drugs

So far, resistance to one or more of the three trypanocidal drugs used in cattle has been reported in at least 13 countries in sub-Saharan Africa. In addition to the 11 countries (Burkina Faso, Chad, Côte d'Ivoire, Ethiopia, Kenya, Nigeria, Somalia, the Sudan, the United Republic of Tanzania, Uganda, Zimbabwe) reported by Peregrine (1994), the Central African Republic (Finelle and Yvore, 1962) and Zambia (Mubanga and Sinyangwe, 1997) should be included. 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. In eight of the 13 countries, multiple resistance has been reported. Most of the currently available information on drug resistance, however, is derived from limited numbers of case reports and does not give any indication of the prevalence of resistance in a region or a country as systematic surveys have not been conducted. There is also considerable variation in the criteria that have been used to diagnose drug resistance. The table summarizes the published reports in which a number of trypanosome isolates have been examined.

Very few authors provide information on the method of sampling (randomized or not). There is an urgent need for surveys in which representative numbers of trypanosome isolates are examined for drug resistance. Such surveys should be taken at random and use agreed methods of diagnosis. This type of survey should provide more reliable data on the true prevalence of drug resistance in regions and countries. In addition, risk analysis should help to identify the factors that influence sensitivity or resistance to trypanocidal drugs.

It is also important to stress that drug resistance is not an “all or nothing”

Surveys for drug resistance in trypanosomes

Country	Trypanosome species	No. of isolates		% of R isolates	Resist to	Reference
		exam.	resist			
Burkina	Tc	12	9	75	I	Pinder & Authié, 1984
Ethiopia	Tc	12	12	100	D	Codjia *et al.,* 1993
			11	92	I	
Ethiopia	Tc	10	10	100	D, H, I	Mulugeta *et al.,* 1997
Kenya	Tc	7	2	29	I	Gray *et al.,* 1993
Kenya/Somalia	Tv	7	6	86	I	Schönefeld, Röttcher & Moloo, 1987
			3	43	H	Ainanshe, Jennings & Holmes, 1992
			5	71	Q	
Nigeria	Tv	19	12	63	D, H, I	Ilemobade, 1979
Nigeria	Tb	12	2	17	D, I	Kalu, 1995
			1	8	I	
Sudan	Tc, Tv, Tb	12	5	42	H	Abdel Gadir *et al.,* 1981
Uganda	Tb	36	1	3	D, I	Matovu *et al.,* 1997
Zimbabwe	Tc	14	6	43	D	Joshua *et al.,* 1995

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

phenomenon and the degree of drug sensitivity and resistance varies considerably between individual trypanosomes. A further factor that can influence drug effectiveness is identified in the interesting observations of Sones and Holmes (1992), Silayo *et al.* (1992), Burudi *et al.* (1994) and Mamman *et al.* (1995a; 1995b), who reported differences in drug sensitivity according to the timing of treatment after infection and the concentration of trypanosomes in the blood.

Chapter 2

Pathogenicity of drug-resistant parasites and the impact on livestock productivity

Whether or not drug-resistant trypanosomes are less pathogenic than susceptible ones remains a controversial issue. Several authors (Berger, Carter and Fairlamb, 1995; Mutugi, Boid and Luckins, 1995; Silayo and Marandu, 1989; Stephen, 1962; Whiteside, 1962) have observed a loss of virulence and/or a loss of fitness in drug-resistant trypanosomes. Transmission by tsetse flies, however, does not appear to affect the drug sensitivity of trypanosomes and drug-resistant strains remain resistant after passage through tsetse flies. This was shown by several workers in the past and has been confirmed more recently (Moloo and Kutuza, 1990; Peregrine, Gray and Moloo, 1997). Recent studies at the International Livestock Research Institute (ILRI), however, using four populations of *Trypanosoma congolense*, ranging from extremely sensitive to strongly resistant to ISMM, found no differences in virulence between them (ILRI, 1996). Only the most resistant one showed a reduced viability, i.e. it took longer to establish parasitaemia than the other three. The loss of fitness in other drug-resistant parasites is a well known phenomenon and is probably also present in trypanosomes. Well-designed experiments in trypanosome naive definitive hosts using significant numbers of resistant and sensitive isolates should provide valuable data on this controversial but important topic.

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 (Codjia *et al.,* 1993). Rowlands *et al.* (1994a; 1994b) followed more than 300 East African zebu calves from birth to three years of age, together with their dams, in this region (between 1986 and 1992). During most of the period, animals that were parasitaemic and had a packed cell volume (PCV) below 26 percent, or animals with clinical signs of trypanosomiasis were treated with diminazene aceturate at 3.5 mg/kg, although resistance against this drug was known to occur. Some effects of trypanosome infection on the growth rate of parasitaemic calves were observed, but these were temporary. The authors concluded that regular trypanocidal therapy might have helped to maintain health and productivity of the young cattle. Although calf mortality was rather high, growth rates compared favourably with those in other village-managed systems in Africa. Similarly, reasonable 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 (1986 to 1992) (Rowlands *et al.,* 1994b). There was, however, an impact of trypanosome infections on the incidence of abortion. Over 8 percent of pregnancies resulted in abortion or stillbirths and there was a significant increase in the rate of abortion associated with cases of parasitaemia detected during the last trimester of pregnancy.

A benefit-cost analysis was carried out by Itty *et al.* (1995) to evaluate the financial and economic returns generated by cattle raised in this area (over 500 cattle belonging to nine herds from 1986 to 1989). The latter authors showed that, despite the high level of trypanosomiasis risk, the high prevalence of drug-resistant *T. congolense* and a moderately high average number of diminazene treatments per year (2.2 and 3.2 for animals aged 12 to 24 and > 24 months, respectively) cattle production was able to generate attractive economic returns for herdowners. The financial analysis (ten-year projections) showed a net benefit-investment ratio varying from 1.1 to 2.4 for the nine herds and an internal rate of return between 12 and 30 percent. This case study shows that profitable cattle production is possible in a problem area with high prevalence of drug-resistant *T. congolense*. Similar studies should be carried out in other regions with different host genotypes and under different management conditions.

Chapter 3
Mechanisms and genetics of resistance to trypanocides

ISOMETAMIDIUM

In 1990, Shapiro and Englund suggested that the main mode of action of ISMM was the cleavage of kDNA-topoisomerase complexes. This explanation was supported by Wells, Wilkes and Peregrine (1995) 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, 1993). 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* remains to be examined, although changes in these transporters have been associated with resistance to arsenical drugs in *T. brucei* (Carter and Fairlamb, 1993; Carter, Berger and Fairlamb, 1995; Ross and Barns, 1996). 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).

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 (Peregrine, Gray and Moloo, 1997).

DIMINAZENE

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 (Peregrine and Mamman, 1993). Similarly the molecular basis of resistance to diminazene in trypanosomes is not clear. Carter, Berger and Fairlamb (1995) 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 (Zhang, Giroud and Baltz, 1992).

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 (including diminazene) *T. 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. Hayes and Wolf (1990) 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, Ouellette and Borst, 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.

Chapter 4
Detection of drug resistance

Several methods have been described to identify drug resistance in trypanosomes (reviewed by Peregrine, 1994; 1996). At present, three types of technique are commonly used to identify drug resistance: 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. The advantages and disadvantages of each of the different techniques are briefly summarized in the following sections.

TESTS IN RUMINANTS

Tests in ruminants provide direct information from studies in ruminants using recommended doses of trypanocide. The tests commonly consist of infecting a group of cattle or small ruminants with the isolate under investigation and later, when the animals are parasitaemic, treating them with various levels of trypanocide. The animals are then regularly monitored over a prolonged period (up to 100 days) to determine the effective dose (ED), i.e. the dose that clears the parasites from the circulation, and the curative dose (CD), i.e. the dose that provides a permanent cure (Sones, Njogu and Holmes, 1988). For these studies, the cattle or small ruminants must be kept in fly-proof accommodation or in a non-tsetse area in order to eliminate the risk of reinfection during the study. A variation of this technique was used by Ainanshe, Jennings and Holmes (1992) in Somalia to examine a group of isolates from a district. Blood from a group of infected cattle was inoculated into a single recipient calf, which was monitored, and later, when it was parasitaemic, treated with trypanocide at the recommended dose. A breakthrough infection, indicative that one of the inoculated trypanosome populations was drug-resistant, was inoculated into 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 into a single calf were resistant.

Further constraints to this technique are that not all populations might grow equally well and that sensitive isolates might overgrow resistant ones when inoculated together (Sones, Holmes and Urquhart, 1989). However this is not a consistent observation (Burudi *et al.,* 1994).

A useful indication of the level of resistance can be obtained from studies in ruminants (and mice) by recording the length of time between treatment and the detection of breakthrough populations of trypanosomes. The shorter the period, the greater the level of resistance (Ainanshe, Jennings and Holmes, 1992; Williamson and Stephen, 1960).

The advantages of studies in ruminants are that most trypanosome isolates of cattle are able to grow in these hosts and that the data obtained are directly applicable to the field.

The disadvantages are the long duration (a follow-up of 100 days is necessary to allow the detection of relapses) and the cost (purchase and maintenance of the animals are expensive). Furthermore, if only one isolate per animal is tested, it is usually impractical and too expensive to examine a large number of isolates.

TESTS IN MICE

After expansion of an isolate in a donor mouse, groups of five or six mice are inoculated with trypanosomes. Twenty-four hours later, or at the first peak of parasitaemia, each group except the control group is treated with a range of drug doses. Thereafter, the mice should be monitored three times a week for 60 days.

The ED50 or ED95 (the effective dose that gives temporary clearance of the parasites in 50 or 95 percent of the animals, respectively) can be calculated, as can the CD50 or CD95 (the curative dose that gives complete cure in 50 or 95 percent of the animals, respectively). Sones, Njogu and Holmes (1988) 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 trypanosome strains.

The advantage of the mouse assay is that it is cheaper than the test in cattle. There are several disadvantages, however: i) most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice; ii) although there is reasonable correlation between drug sensitivity data in mice and in cattle, higher doses of drug must be used in mice (normally ten times higher) in order to obtain comparable results to those obtained in cattle because of the vast difference in metabolic size (Sones, Njogu and Holmes, 1988); iii) precise assessment of the degree of resistance needs a large number of mice per isolate, which makes it a labour-intensive test – identification of a discriminatory dose, above which an isolate should be considered as resistant, could drastically reduce the number of mice and the amount of work to be carried out; and iv) it takes as long as 60 days to evaluate the drug sensitivity of an isolate.

IN VITRO ASSAYS

Since the review of Kaminsky and Brun (1993) further progress has been made in the field of *in vitro* assays to determine the drug sensitivity of trypanosomes. These authors advised the use of metacyclic or bloodstream forms instead of procyclic forms in such assays. However, 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 trypanosomes correlate well with field observations. However there are several disadvantages. *In vitro* cultivation of bloodstream forms is only possible using preadapted lines and not using isolates directly from naturally infected animals (Hirumi, Hirumi and Peregrine, 1993). A simplified axenic culture system has been developed by these authors, but further research is still necessary to study the correlation with field data. A potential problem associated with this lengthy time of adaptation is the possible selection against trypanosomes that have the phenotype of the original population. *In vitro* assays are expensive to perform and require good laboratory facilities and well-trained staff.

If better techniques can be developed in order to adapt isolates more rapidly to grow *in vitro*, these assays may become more popular, especially in those laboratories where culture facilities are already established.

TRYPANOCIDAL DRUG ELISAs

As an alternative to the tests mentioned above, the use of trypanocidal drug enzyme-linked immunosorbent assays (ELISAs) in combination with parasite detection tests has given promising results for the detection of resistant trypanosomes. A competitive ELISA which allowed the detection of small amounts of isometamidium in serum of cattle was first described by Whitelaw *et al.* (1991). This technique was further improved by Eisler *et al.* (1993) and Eisler, Elliott and Holmes (1996) and has been validated in cattle under experimental and field conditions (Eisler, 1996; Eisler *et al.,* 1994; 1996; 1997a). The test is both sensitive, detecting subnanogramme concentrations, and specific. It allows the monitoring of drug levels over extended periods and the evaluation of factors influencing drug disappearance rates from the plasma.

The available data indicate that there is a considerable individual variation after intramuscular injection of ISMM in cattle (Eisler, 1996). One interesting finding has been that the drug disappears more rapidly in animals challenged and becoming infected with drug-resistant trypanosome isolates than in those challenged but protected against infection with sensitive trypanosomes (Eisler *et al.,* 1994). Observations showed that the presence of trypanosomes in animals with an ISMM concentration of > 0.4 ng/ml suggests resistance; the higher the drug level detected the greater the degree of resistance that could be inferred (Eisler *et al.,* 1997a). Further research is necessary, however, in order to confirm these results in a larger number of animals. Similar drug ELISAs have been developed for the detection of subnanogramme amounts of homidium bromide (Murilla, 1996) and a similar test for diminazene is in development.

The advantage of the ISMM ELISA is that large numbers of sera can be tested within a relatively short time. The ELISA may also provide information on drug usage in an area of investigation. The disadvantage is that further studies are required to confirm the correlation between protection against tsetse challenge with various trypanosome populations and the ISMM concentration in the serum. It is not yet possible to draw firm conclusions on the sensitivity or resistance of a trypanosome population at the level of the individual animal. The ELISA should, however, give some indication of the resistance situation at the level of the herd. A further disadvantage is that, while the ELISA may indicate the level of drug

withstood by a trypanosome population, it does not provide information about the level required for protection.

LONGITUDINAL PARASITOLOGICAL DATA

Longitudinal parasitological data can be used to detect resistance problems, although their use is not suitable as a routine test. Rowlands *et al.* (1993) showed that the application of a computer model to parasitological data collected over a long period on a monthly basis allowed the incidence of new infections to be distinguished from recurrent infections. This analysis showed that the prevalence of diminazene-resistant infections in the Ghibe valley, Ethiopia, increased from 6 percent in 1986 to 14 percent in 1989.

The advantage of these kinds of data is of course that they are directly applicable to the field. The disadvantages, however, are that: i) the true prevalence of drug-resistant infections seems to be underestimated; ii) it is retrospective by at least six months; and iii) the technique is quite expensive, if a longitudinal study is not carried out for other purposes (Peregrine, 1996).

NEW TESTS (IN DEVELOPMENT) FOR DETECTION OF RESISTANCE TO ISMM

Since it has been shown that the rate of ISMM accumulation in *T. congolense* is a good indicator of the degree of drug resistance and since the mitochondrial electrical potential (MEP) appears to be closely linked with the rate of drug uptake, it might be possible in the near future to develop a quantitative *in vitro* test to evaluate the MEP (ILRI, 1996; Wilkes *et al.,* 1997). If such a test could be carried out using a small number of trypanosomes, it might provide a rapid indication of the level of resistance of a given trypanosome isolate. It is hoped that this test could be conducted on whole blood samples and would not, therefore, suffer from the same disadvantages as other *in vitro* tests referred to earlier. An alternative approach in the longer term may be made to identify genetic markers for ISMM resistance, which might be developed into reagents for the identification of resistant trypanosomes using polymerase chain reaction (PCR).

Unfortunately, it will take several years before such a test is validated and becomes available to potential users. Standardization of the existing tests should, therefore, receive high priority, especially of the assays in mice and in the definitive hosts, because these can be carried out in less well-equipped laboratories. Such standardization should allow the establishment of a resistance monitoring system capable of comparing data on a temporal and spatial basis across Africa and avoiding the current lack of reliable data in the field of drug resistance of trypanosomes.

Chapter 5

Guidelines on the delay of the development of drug resistance

The factors responsible for the development of resistance to antitrypanosomal compounds are not well known. The exposure of parasites to subtherapeutic drug concentrations (owing to underdosing) has been considered as the most important factor for the development of resistance (Whiteside, 1960; 1962; Boyt, 1986). Boyt suggested that the evolution of drug resistance in trypanosomes is fundamentally different from resistance in insects, helminths or micro-organisms. For the latter, it is generally accepted that resistance genes are present in a very small proportion of the population and that these pre-existing resistant individuals are selected by drug pressure. Boyt suggested, however, that in a manner similar to antigenic variation, chemoresistance is another example of the remarkable ability of the trypanosome to adapt defensively in the face of unfavourable changes appearing in its environment. However, no concrete evidence has been brought forward to show that resistance is indeed an adaptation rather than a selection process. Nevertheless it is possible to take drug-sensitive clones and induce drug resistance rapidly by repeated underdosing and passage. Furthermore, Osman, Jennings and Holmes, (1992) showed that this occurs much more rapidly in immunosuppressed hosts (mice). Similar observations were also reported by Hess *et al.* (1997), who showed that malnourished children (malnutrition adversely affects the immune status) were at a higher risk from treatment failure resulting from drug-resistant *Plasmodium falciparum* than well-nourished children. These findings serve to illustrate the importance of immune responses in drug clearance of parasites. The type and degree of host immunity – either generalized (regulation of the overall level of infection) or specific immunity (each clone within the infection is regulated independently) –

might also influence the rate of development of drug resistance, as was shown for *Plasmodium* (Hastings, 1997). This aspect needs to be examined thoroughly for trypanosomes.

How do trypanosomes develop resistance to trypanocidal drugs? Selection by drugs takes place during asexual multiplication in the animal or human host. During the passage through the tsetse fly genetic exchange (sexual recombination) may occur, at least in *T. brucei* (Jenni *et al.,* 1986; Tait and Turner, 1990), and is strongly suspected in *T. congolense* given the high degree of genetic diversity observed (G. Hide, personal communication). The genetic structure of a parasite population (clonal or panmictic) is an important parameter that is influenced by the transmission intensity, which in turn might influence the rate of development of drug resistance as is suggested by recent research on drug resistance in *Plasmodium* (Paul *et al.,* 1995). Similar to *Plasmodium* (Thaitong, 1983), populations of trypanosomes in infected animals are polyclonal with different sensitivities to antitrypanosomal drugs (Peregrine *et al.,* 1991; Mutugi, Boid and Luckins, 1995). Therefore drug resistance in trypanosomes is likely to occur under the same circumstances as for *Plasmodium* and many other parasites, that is: 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 (White, 1992). 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 *T. brucei* (Valdes *et al.,* 1996), the effects of this phenomenon should not be underestimated. Figure 2 summarizes some important factors influencing the development of drug resistance in trypanosomes.

Up to now the most important guidelines on the avoidance or delay of the development of drug resistance were considered to be: i) use of the "sanative pair" of drugs (ISMM or ethidium and diminazene); and ii) avoidance of the exposure of trypanosomes to subtherapeutic drug concentrations (Whiteside, 1960; Boyt, 1986). It is clear, however, that the application of these guidelines may not be sufficient to maintain the

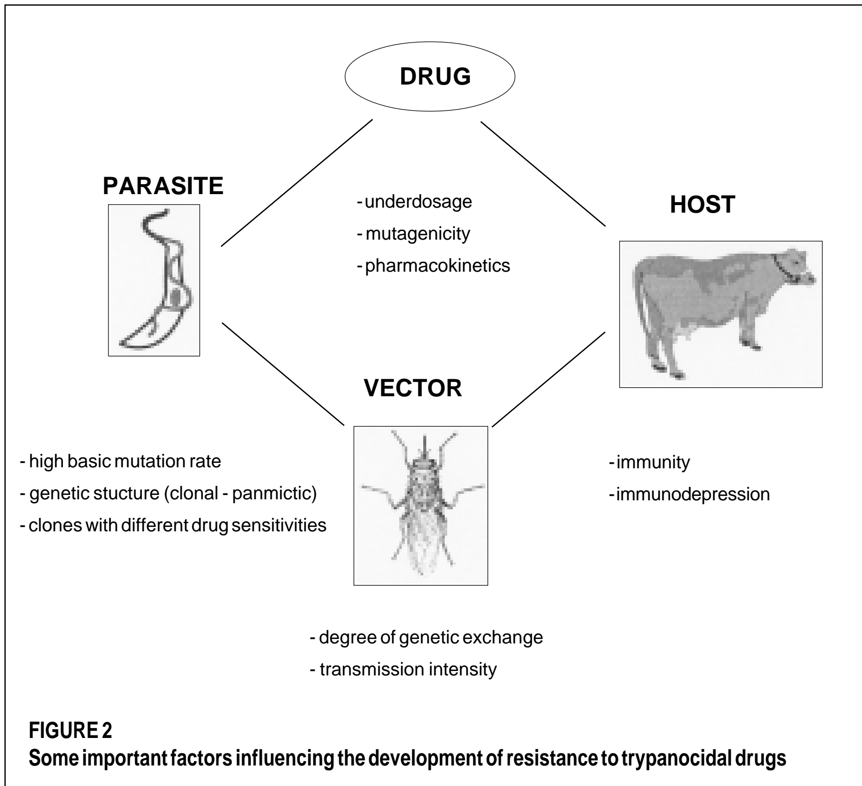


FIGURE 2
Some important factors influencing the development of resistance to trypanocidal drugs

efficacy of the existing drugs, especially since they lack recommendations concerning a reduction of the treatment frequency.

Based on current knowledge in the field of trypanocide resistance and on experience in the control of resistance to insecticides, anthelmintics, antibiotics and other drugs (Boray, Martin and Routh, 1990; Bergogne-Bérézin, 1997; Geerts, Coles and Gryseels, 1997; Routh, 1993) the following recommendations are proposed in order to delay the development of resistance.

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 the drugs,

i.e. 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 (Whiteside, 1960; 1962). 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.* (1995), who administered ISMM six to seven times a year and ethidium up to11 or 12 times a year, might be able to control the resistance problem temporarily, but are no solution in the long term. Furthermore, frequently repeated trypanocidal drug treatments have been associated with toxicity problems (Stevenson, Munga and Dolan, 1993; Eisler *et al.,* 1997b). 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 must 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; Routh, 1993). It is therefore strongly recommended that in high tsetse challenge areas control of trypanosomiasis should not rely solely on drugs but that an integrated approach should be adopted using vector control, to reduce the tsetse challenge, along with reduced frequency of drug dosing. Where such measures have been adopted the results have been impressive (Fox *et al.,* 1993; Peregrine *et al.,* 1994). In situations in West and Central Africa the use of trypanotolerant livestock and drugs may be appropriate in areas of high tsetse challenge (Diall *et al.,* 1992).

USE OF THE CORRECT DOSE

Underdosing is one of the major causes of resistance development. Subtherapeutic drug concentrations exert a strong selective pressure for the emergence of resistant clones that pre-exist in the trypanosome population. Unfortunately, underdosing occurs very frequently. Farmers have the tendency to underestimate the weight of their animals when they have to treat them (Besier and Hopkins, 1988). Sometimes generic products are used, which have a reduced efficacy, as has been shown in the field of anthelmintics (Van Wyk *et al.,* 1997), while anecdotal evidence

suggests that it also occurs with trypanocides. Given the fact that in many countries unskilled persons are allowed to administer drugs, errors easily occur in calculating the correct doses for the treatment of the animals. Packaging of isometamidium as a one-dose treatment – similar to diminazene – would undoubtedly help to reduce this problem. In addition, as the drugs are relatively expensive there is a temptation to overdilute the drug and hence underdose.

Data on the pharmacokinetics of ISMM in goats (Kinabo and McKellar, 1990) suggest that following intramuscular administration the bioavailability of ISMM in that species is very low, approximately half that in cattle under similar circumstances (Eisler, 1996). If this is confirmed, higher doses might be used in goats than in cattle, although caution is required because of the low therapeutic index of ISMM. Similar observations were made for benzimidazoles and levamisole, the dosage of which should be 1.5 to 2 times higher in goats than in sheep (Hennessy, 1994).

The use of improved formulations of existing drugs is another possible way to avoid subtherapeutic concentrations. Controlled release devices which provide more stable drug concentrations and a sharper cut off at the end of the release period might have particular advantages in this respect. The polymer devices containing ISMM or ethidium as described by Geerts *et al.* (1997) are a step in this direction.

AVOIDING EXPOSURE OF THE WHOLE PARASITE POPULATION TO A DRUG

Unlike human sleeping sickness, animal trypanosomiasis is commonly controlled with mass treatments which can be highly successful 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 the proportion of the trypanosome population exposed to the drug and the lower the proportion *in refugia* (i.e. the proportion of trypanosomes present in the fly population or in other hosts), the higher the selection pressure. The percentage of the total parasite population that is exposed to the drug at the time of treatment might, thus, have an impact on resistance development. It has been shown by computer models that

leaving 20 percent of the herd untreated significantly decreases the rate of development of anthelmintic resistance (Barnes, Dobson and Barger, 1995). Although no experimental data are available for trypanocide resistance, experiences with other drugs and pesticides indicate that systematic mass treatments hasten the development of resistance. Therefore, in well monitored situations 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 (Scott and Pegram, 1974). A similar approach is currently being used in South Africa to control anthelmintic-resistant *Haemonchus contortus* in sheep (Van Wyk, Malan and Bath, 1997). Instead of carrying out systematic mass treatments, only those sheep that are strongly anaemic are treated. The identification of animals not able to cope with *H. contortus* was done by clinical appraisal of the colour of the ocular mucous membranes using a colour chart (FAMACHA[R]). This allowed a significant reduction in the number of treatments without an increase in the mortality rate. The identification of trypanosome-infected animals by farmers and veterinarians would be greatly assisted by the development of reliable and cheap pen-side diagnostic tests. The need for such tests is widely recognized and they remain an important goal for the research community

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 cattle 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 quinapyramine was the suggested cause of the multiple drug-resistance problem in the Ghibe valley of Ethiopia referred to earlier. Ndoutamia *et al.* (1993) 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. This confirms the results obtained in earlier field studies by Whiteside (1962). The use of quinapyramine as a trypanocide in cattle is, therefore, completely contraindicated.

Chapter 6
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 become necessary.

RESISTANCE AGAINST A SINGLE DRUG

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

Once resistance is present, it is unwise to increase the dose of the drug. Although some temporary benefits might be obtained, such an action would inevitably increase the selection pressure and, thus, the level of resistance. The use of a double dose of diminazene (two normal doses with an interval of eight or 24 hours between them) only slightly improved the therapeutic efficacy for resistant *T. congolense* (Silayo *et al.,* 1992). Similarly, although the intravenous administration of ISMM enhanced the therapeutic activity of the compound as compared with the intramuscular injection, it was not effective in eliminating resistant parasites (Sutherland *et al.,* 1992).

MULTIPLE DRUG RESISTANCE AT THE LEVEL OF INDIVIDUAL TRYPANOSOMES

If multiple resistance is expressed at the level of the individual trypanosome, chemotherapy can become increasingly ineffectual. To counteract multiple resistance in such a case, intervention at the level of the vector is required. Peregrine *et al.* (1994) showed that in the Ghibe valley, Ethiopia, multiple drug-resistant trypanosome infections (at the clonal level) could be controlled effectively using an integrated approach involving tsetse fly control (targets) and chemotherapy of clinically sick animals (using diminazene). The relative density of the main vector, *Glossina pallidipes*, fell from an average of 1.9 flies per trap per day before the introduction of tsetse control to 0.4 flies per trapper day during the first year of the control. Simultaneously, the apparent prevalence of *T. congolense* infections fell from approximately 30 percent before the tsetse control programme to ± 5 percent one year after the start of the control programme. The apparent prevalence of diminazene-resistant infections decreased by about 75 percent during the same period. Although this experiment had to be interrupted as a result of political instability in the region, it showed that such an approach can be successful. A similar level of success was also reported by Fox *et al.* (1993) at the Mkwaja Ranch in the United Republic of Tanzania using a deltamethrin dipping programme to overcome the problem of drug resistance. Interestingly, cattle productivity increased significantly and the cost of the dip was more than offset by savings on trypanocidal drugs and oxytetracycline, and thereby overall treatment costs were reduced by 50 percent.

Conclusions

Because it is very unlikely that new trypanocidal drugs will be released on to the market in the near future, it is essential to try to maintain the efficacy of the currently available drugs. The most important and most efficient measure is to adopt an integrated disease management strategy.

Furthermore, better data (instead of case reports) are required on both the true prevalence of trypanocide resistance and its impact on the productivity of livestock. In order to allow a reliable comparison of the data on a temporal and spatial basis, it is of crucial importance that tests for drug resistance are carried out across Africa according to standardized protocols. This is the case for antimalarials, antibiotics, anthelmintics, etc. for which standardized tests have been established. There is also a need for better understanding of the ways that farmers and veterinary assistants are using trypanocides. There are indications, for instance, that diminazene is used more and more frequently, whereas the use of ISMM is decreasing. The criteria farmers use to decide whether or not to treat an animal and when they select a particular trypanocide are not known.

In order to understand the phenomenon of drug resistance, more research is needed into the mechanisms and the genetics of resistance. Computer models that allow the prediction of the efficacy of certain measures to delay resistance are also very useful tools as shown by Cross and Singer (1991), Hastings (1997) or Barnes, Dobson and Barger (1995) in the field of malaria and anthelmintic resistance respectively. Finally, there is an urgent need for better surveillance. Currently it is not known whether the increase of the number of resistance reports is owing to a higher prevalence of resistance or simply to a growing interest in drug resistance by scientists. Collection of baseline data through well functioning monitoring systems is essential in order to allow the right measures to be taken at the right time.

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