The Trypanosomiases

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23 Current Chemotherapy of Animal Trypanosomiasis

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Introduction

Over most of sub-Saharan Africa, bovine trypanosomiasis continues to be controlled primarily by trypanocides. Only three compounds – isometamidium chloride, homidium (bromide and chloride) and diminazene aceturate – are available and all of these drugs have been on the market for over 40 years. A full account of the history and properties of these drugs was provided by Mulligan (1970) and later reviewed by Leach and Roberts (1981).

Isometamidium is principally used as a prophylactic drug and can provide up to 6 months' protection against tsetse challenge. Whilst homidium has limited prophylactic properties, it is primarily used as a therapeutic agent. Diminazene has only therapeutic properties. It is currently estimated that about 35 million doses of these drugs are used in Africa each year and this has remained relatively constant. There are estimated to be between 40 million and 60 million cattle at risk.

Over the past 40 years these three drugs have been primarily provided by a few large European pharmaceutical companies but recently several generic forms of these compounds from a wider range of companies have become available on the African market.

Although there is a consistent demand for trypanocides by African farmers, the total value of the market (about US$30 million) is not considered sufficient to justify investment by large pharmaceutical companies in the development and licensing of new animal trypanocides, the costs of which may exceed US$250 million for a single compound. The challenge, therefore, remains to make optimal use of the three relatively old compounds until new methods of treatment emerge, possibly through serendipitous cross-reactivity with new broad-spectrum anti-protozoal compounds such as those currently being developed for the treatment of malaria and cryptosporidiosis.

Perhaps the greatest risk to the future use of the existing three trypanocides is the development and spread of drug resistance to the point where they become ineffective over large areas of Africa, and so a large part of this chapter is devoted to this issue. However, other risks exist. One is that, because of the risk of drug resistance (real or perceived), the market will shrink and manufacture will become unprofitable. Secondly, the spread of generic products, some of which are of doubtful quality, may undermine farmers' confidence in trypanocides. Thirdly, it is possible that extensive vector control may remove the need for trypanocides over large parts of the

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existing market. Fourthly, there may be growing concerns over the toxicity of these compounds and the potential threat they pose to human health. Despite these risks, the three established compounds remain available and popular and their use is very well established across Africa. In many African countries they remain the most frequently used veterinary drugs. A list of the currently available trypanocidal drugs for use in domestic livestock is presented in Table 23.1 (modified from Peregrine, 1994).

Strategies for Trypanocidal Drug Usage

Current use of trypanocides for the control of African bovine trypanosomiasis is generally practised according to one of a number of defined treatment strategies, as described below.

Routine block treatments

These are generally carried out using prophylactic drugs, notably isometamidium chloride, at predetermined intervals based on the perceived duration of prophylaxis. All animals in a herd may be treated, or treatment may be targeted at a particular group of valuable or 'at-risk' animals. When routine block treatment with isometamidium is practised it is recommended that, once a year, the animals are separately treated with diminazene in order to delay the development of drug resistance following the concept of the 'sanative pair' (Whiteside, 1962).

Strategic block treatments

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

Monitoring and treatment of individual infected animals

Cattle are monitored using standard parasitological methods, e.g. wet blood film, haematocrit centrifugation technique or buffy coat technique. Treatment of infected animals is generally conducted using a therapeutic drug, usually diminazene aceturate.

Monitoring and treatment of clinical cases

This is similar to monitoring and treatment of individual infected animals, but not all infected animals are treated. Cattle are treated, usually with a curative trypanocide, only if the packed cell volume (PCV) falls below a predetermined threshold, or if clinical signs of trypanosomiasis are detected. The rationale for this strategy is the belief that cattle may acquire a degree of immunity or resistance to locally circulating strains of trypanosomes, and that treatment of animals with subclinical infections is unnecessary and may interfere with this process.

In practice, a combination of these strategies may be used, such as routine or strategic block treatments together with monitoring and treatment of individual infected animals. The number of individual animal treatments given may then be used in deciding when to administer the next strategic block treatment. Routine and strategic block treatments are options frequently used in large-scale ranching situations, or under government or donor-funded control campaigns. However, there is an increasing trend towards the use of individual treatment on the basis of clinical signs alone, as livestock keepers become of necessity increasingly self-reliant in the diagnosis and treatment of bovine trypanosomiasis. Finally, where drugs are unavailable or beyond their financial means, many farmers resort to traditional ethnoveterinary medicines for the treatment of cattle diseases. However, as yet none has proven efficacy against bovine trypanosomiasis and, moreover, farmers rarely use this approach where they do have the option of the use of trypanocidal drugs.

Table 23.1. Currently available trypanocidal drugs for use in domestic livestock.

Drug	Trade names[a]	Dose (mg/kg)	Route	Use	Activity	Animal
Diminazene aceturate	Berenil® Many others	3.5–7	i.m.	T	*T. congolense* *T. vivax* (*T. brucei*) (*T. evansi*)	Cattle Small ruminants [Dogs] [Equidae]
Homidium chloride Homidium bromide	Novidium® Ethidium®	1	i.m.	T/P[b]	*T. congolense* *T. vivax*	Cattle Small ruminants Pigs [Equidae]
Isometamidium chloride	Samorin® Trypamidium® Veridium®	0.25–0.5 0.5–1	i.m. i.m.	T P	*T. congolense* *T. vivax* *T. brucei* *T. evansi*	Cattle Small ruminants Equidae Camels
Quinapyramine dimethylsulphate Quinapyramine dimethylsulphate:chloride (3:2 w/w)	Trypacide sulphate® Trypacide Pro-salt®	3–5 3–5[c]	s.c. s.c.	T P	*T. congolense* *T. vivax* *T. brucei* *T. evansi* *T. simiae*	Camels Equidae Pigs Dogs
Suramin	Naganol®	7–10 g per animal	i.v.	T (P)	*T. evansi*	Camels Equidae
Melarsomine	Cymelarsan®	0.25	s.c./i.m.	T	*T. evansi*	Camels

i.m., intramuscular; s.c., subcutaneous; i.v., intravenous; T, therapeutic; P, prophylactic.
( ), limited activity; [ ], small therapeutic index.
[a]Incomplete list.
[b]Prophylaxis observed in areas of low tsetse challenge.
[c]Dosage of sulphate.

Factors Influencing Drug Usage

Veterinary services in Africa are in a state of transition, notably contracting and consolidating in response to global economic forces and the process of structural adjustment. Privatization of veterinary services means a shift from project-driven to demand-driven campaigns and from external funding to cost recovery from the livestock owners themselves. Adequate access to animal health services is key to livestock development. Unfortunately the governments of most African countries now lack the funds and institutional capability required to provide adequate public veterinary services and the emerging private sector has been slow to fill the void. This is particularly so in terms of services to resource-poor farmers, which represent the least financially attractive end of the new market. Hence most rural livestock disease diagnosis and drug administration is now in the hands of farmers rather than professional animal health workers. Recent studies have indicated that currently more than 60% of treatments are given by livestock owners. The problem is compounded because many veterinary diagnostic laboratories are underused by fee-paying farmers and, in the absence of public support, quickly fall into disuse.

Functional and sustainable drug delivery in the post-privatization context requires that social structures involved in the relationship between village-level organizations and privatized or public veterinary services and research institutes need to be taken into consideration. Table 23.2 shows some of the principal stakeholders in the veterinary aspects of bovine trypanosomiasis control before and after privatization.

In spite of decreased farmer motivation for animal health interventions as a result of privatization, there is nevertheless a tendency among farmers to purchase and use drugs from a reliable and affordable supply rather than to dispense entirely with veterinary care. Trypanocidal drugs are clearly regarded as a priority by smallholder farmers and sales of these compounds have remained in the region of US$30 million/annum over the last decade. In several countries there have been attempts to provide trypanocides through government-supervised cost-recovery programmes, with farmers purchasing drugs through a local supply network. In others, where there has been less government supervision, informal markets in trypanocidal drugs have expanded, with many small-scale pharmacies selling products directly to farmers.

The increasing practice of treatments being given by livestock owners is not without serious drawbacks. Most farmers do not have adequate knowledge on diagnosis and appropriate drug use even in areas of high prevalence of trypanosomiasis, and trypanocides are frequently used in

Table 23.2. Stakeholders in veterinary aspects of trypanosomiasis control.

Level	Pre-privatization	Post-privatization
National	Central veterinary research institutes Pharmaceutical companies	Government veterinary laboratories Private veterinary laboratories Pharmaceutical companies
Provincial	Regional veterinary laboratories	Private veterinarians
District	District veterinary office	Animal health assistants Farmers' associations NGOs
Village	Animal health assistants Extension workers	Women's groups Schools Churches Extension workers Pharmaceutical retailers
Farm	–	Smallholder farmers

the absence of diagnosis or used to treat conditions for which they are ineffective. Many surveys have shown that the amount spent by livestock keepers on trypanocides is not related to the prevalence of the disease. A further factor is that the choice between the use of therapeutic drugs and prophylactic drugs may be made on the basis of cost per dose, without a clear understanding by farmers of the advantages of prophylactic drugs in appropriate circumstances. Surveys in Zambia, for example (Van den Bossche *et al.*, 2000), have shown that despite farmers administering most of the trypanocide treatments there was little evidence of underdosing, though there was a strong tendency to use curative (diminazene) rather than prophylactic drug (isometamidium). Furthermore, the majority of trypanocidal treatments were given to clinically sick animals that were not necessarily infected with trypanosomes and, irrespective of the type of drug used, oxen and cows received the majority of treatments. This indicates that farmers prefer to treat their most productive animals as a priority.

Misuse or overuse of drugs is uneconomic and environmentally unsound and may lead to drug resistance and other problems. However, despite the widespread use of trypanocides, it has been estimated that over half of the cattle raised under trypanosomiasis risk are not treated with trypanocidal drugs.

Although farmers do administer a high proportion of treatments to their own animals, most owners seek advice from others on which medications to use. Many seek advice from animal health assistants and community-based animal health workers. These individuals, although without full professional veterinary training, represent an increasingly important cadre of animal health service providers. Projects to support private-sector community-based animal health workers to deliver veterinary drugs and advice to small-scale livestock farmers are being developed in several countries. In such schemes cattle owners themselves undertake control operations, but are coached by project field personnel and private veterinarians to ensure timeliness and correct treatment of cattle. Clearly, enabling these groups to make better diagnoses and treatment decisions can have a major impact on the health status of livestock of the rural poor.

Drug Resistance

Resistance to one or more of the trypanocidal drugs used in cattle has been reported in at least 13 countries of sub-Saharan Africa (Geerts and Holmes, 1998). Most of the currently available information on drug resistance is derived from case reports and does not give any indication of the prevalence of resistance in a region or a country. Very few systematic surveys have been carried out using a representative number of randomly selected trypanosome isolates. In the few surveys that have been conducted, the occurrence of drug resistance was found to be greater in those regions where drug use was more intensive. It was shown that resistance is widespread in some regions of Africa where drug pressure is high enough to select resistant strains. Since there is a lack of baseline data, however, it is not known whether the increasing number of resistance reports is due to a higher prevalence of resistance or simply to a growing interest in drug resistance by scientists.

How do trypanosomes develop resistance to trypanocidal drugs?

Selection by drugs essentially takes place during asexual multiplication in the animal or human host, though there is some evidence that, during passage through the tsetse fly, genetic exchange (sexual recombination) may occur at least in *Trypanosoma brucei*. The genetic structure of a parasite population (clonal or panmictic) is an important parameter influenced by the transmission intensity, and this in turn might influence the rate of development of drug resistance. This has been suggested by studies on drug resistance in *Plasmodium*, which have shown that parasite populations in infected hosts are polyclonal with differ-

ing drug sensitivities between the clones making up the population. It is likely that a similar situation occurs in trypanosome infections.

Therefore drug resistance in trypanosomes is likely to occur under the same circumstances as for many other parasites, i.e. under large-scale drug use, by using inadequate dosing and by using correct dosing with drugs that are slowly eliminated from the body. In the past the development of drug resistance in trypanosomes was mainly ascribed to their exposure to subtherapeutic concentrations of trypanocidal drugs. Although this is certainly an important aspect, the intensity of drug pressure (i.e. the treatment frequency and the degree of exposure of the parasite population) is probably even more important. The immunocompetence of the host also appears to play an important role, since it has been clearly shown that drug resistance develops more rapidly in trypanosomes present in immunosuppressed mice than in normal mice. Furthermore, some trypanocidal drugs such as ethidium are well-known mutagenic compounds and might induce mutations, the most resistant of which would be selected under drug pressure. 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*, the effects of this phenomenon should not be underestimated. Finally, the phenomenon of cross-resistance has been well established. For instance, quinapyramine usage has been shown to induce resistance to isometamidium, homidium and diminazene (Ndoutamia *et al.*, 1993).

Mechanisms and genetics of resistance to trypanocides

Isometamidium

The trypanosome kinetoplast is the primary site of isometamidium accumulation. The main mode of action of the drug is the cleavage of kDNA–topoisomerase complexes. The mechanism of resistance to isometamidium is less clear. Several workers have shown that accumulation of isometamidium is significantly lower in resistant populations than in sensitive ones. It remains to be shown whether this is due to a decreased number of protein transporters of isometamidium in the plasma membrane or to changes in the balance between influx and efflux. The role of nucleoside transporters in resistance to isometamidium by *Trypanosoma congolense* remains to be examined but changes in these transporters have been associated with resistance to arsenical drugs in *T. brucei*. Changes in mitochondrial electrical potential have also been demonstrated in isometamidium-resistant *T. congolense*.

Homidium salts

Homidium chloride and especially homidium bromide or ethidium are still widely used as trypanocidal drugs, though they are known mutagenic compounds. Their mechanism of action is not well understood but it has been shown that the drugs interfere with glycosomal functions, the function of an unusual AMP-binding protein, trypanothione metabolism and the replication of kinetoplast minicircles. The mechanism of resistance by trypanosomes to these drugs is unknown but there are indications that it is similar to that described for isometamidium.

Diminazene

Diminazene probably exerts its action at the level of the kinetoplast DNA but other targets cannot be excluded. As for other trypanocides, the molecular basis of resistance to diminazene in trypanosomes is not clear. The accumulation of diminazene has been shown to be markedly reduced in arsenical-resistant *T. b. brucei* due to alterations in the nucleoside transporter system (P2). However, there might be other resistance mechanisms.

The genetic stability of drug resistance remains uncertain but recent reports from Ethiopia based on cloned populations have shown that drug-resistance phenotypes had not altered over several 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. The same is true for the genetics of drug resistance in trypanosomes. There are strong indications that several genes are involved in resistance to isometamidium. However, the mono- or polygenic nature of drug resistance, its stability over time, and the dominance or recessiveness of the gene(s) involved need to be further examined, because of the implications for the effective control of drug resistance.

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

Several authors have observed a loss of virulence and/or a loss of fitness in drug-resistant trypanosomes but this has not been a consistent finding from the limited number of studies that have been conducted. 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.

There have been few studies to assess accurately the impact of drug-resistant trypanosomes on livestock productivity but it is generally assumed that uncontrolled infections will have a severe impact on both survival and productivity. A useful study to assess the impact of drug-resistant trypanosomes on the productivity of the local cattle was carried out in the Ghibe valley, Ethiopia, by scientists from the International Livestock Research Institute (ILRI) in the early 1990s and a high prevalence of multiple drug resistance was reported. The study showed that profitable cattle production was possible in a problem area with high prevalence of drug-resistant *T. congolense* and cattle production was able to generate attractive economic returns for herd owners (Itty *et al.*, 1995). Similar studies should be carried out in other regions with different host genotypes and under different management conditions.

Detection of drug resistance

Three types of techniques are commonly used to identify drug resistance: tests in ruminants, tests in mice and *in vitro* assays. Standardized protocols for the tests in animals have been developed, which should allow better comparisons of data on a temporal and spatial basis (Eisler *et al.*, 2001), but none of these tests is ideal. Other tests are still in the phase of development or validation. The advantages and disadvantages of each of the different techniques are briefly summarized below.

Tests in ruminants

These tests 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 they are parasitaemic, treating them with various dosages of trypanocide. It is preferable to use at least three animals in each group, because it has been shown that results obtained after inoculation and treatment of one animal are not always reliable. The animals are regularly monitored over a period of 100 days to determine the efficacy of standard drug doses in terms of their ability to provide a permanent cure. 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 (Table 23.3). A variation on this technique is to inoculate blood from several different infected cattle into a single recipient calf. 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 grow equally well and that sensitive isolates might overgrow resistant ones when inoculated together.

Table 23.3. Standardized protocols for testing trypanocidal drug resistance in mice and cattle (Eisler *et al.*, 2001).

	Single-dose test in mice[a]	Multi-dose test in mice[a]	Test in calves[b]
Number of groups of animals			
Treatment groups per drug	1	5	1
Control groups	1	1	not necessary
Number of animals per group	6	6	3 to 6
Inoculum			
Number of trypanosomes	10^5	10^5	10^5
Route of administration	i.p.	i.p.	i.v.
Drug dosages (mg/kg bw)			
Isometamidium chloride	1	0.01, 0.1, 0.5, 3.0, 20	0.5
Diminazene aceturate	20	1.0, 3.0, 10, 20, 60	3.5
Homidium bromide/chloride	ND	ND	1
Drug administration			
Time	24 h post inoculation	24 h post inoculation	1st peak parasitaemia
Route	i.p.	i.p.	i.m.
Parasitological examination			
Method	Tail blood wet smear	Tail blood wet smear	Buffy coat
Frequency	2 ×/week[d]	2 ×/week[d]	3 ×/week
Duration of follow-up	60 days	60 days	100 days
Interpretation of results for treated animals			
Isolate sensitive	At least 5/6 cured[e]	Probit or logit analysis of number of mice cured at each dose	3/3 cured
Isolate resistant[c]	Less than 5/6 cured		Less than 3/3 cured

[a]Mice should be weighed to an accuracy of 1 g and body weights (bw) should not vary by more than 10%; strain, sex and age of mice should be recorded.
[b]Calves must be kept in a fly-proof stable or other environment non-endemic for trypanosomosis.
[c]Resistance of individual stabilates in mice should not be extrapolated to cattle.
[d]When *T. brucei* are being tested, the frequency of parasitological monitoring should be increased to three times a week for the first 2 weeks.
[e]At least five out of six control mice must become parasitaemic; if not, the test must be repeated.
ND: not yet determined; i.m., intramuscular; i.p., intraperitoneal; i.v., intravenous.

Tests in mice

Tests in mice can be used as a single-dose test or as a multi-dose test. In the latter case the objective is to obtain more detailed information by determining the CD_{50} or CD_{80} values (curative dose that gives complete cure in 50% or 80% of the animals) for a given trypanocidal drug. In the case of a single-dose test, a large number of trypanosome isolates is tested at a single discriminatory dosage – 1 mg/kg for isometamidium and 20 mg/kg for diminazene (Eisler *et al.*, 2001) – with the objective of characterizing the geographical area of origin of the isolates in terms of the extent of drug resistance, rather than in-depth characterization of individual isolates. The details of both tests are presented in Table 23.3. The advantage of the mouse assay is that it is cheaper than the test in cattle. There are several disadvantages, however. Firstly, most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice. Secondly, although there is reasonable correlation between drug sensitivity data in mice and in cattle, higher doses of drug must be used in mice in order to obtain results comparable to those from cattle because of the vast difference in metabolic size. The results in mice cannot be directly extrapolated to calculate the curative dose to be

used in cattle. Thirdly, precise assessment of the degree of resistance requires a large number of mice per isolate. This makes it a rather labour-intensive test. Finally, it takes as long as 60 days to evaluate the drug sensitivity of an isolate.

In vitro *assays*

For the *in vitro* evaluation of drug sensitivity procyclic, metacyclic or bloodstream forms of trypanosomes can be used. The advantage of *in vitro* assays is that large numbers of isolates can be examined. However, there are several disadvantages. The use of metacyclic and bloodstream forms is considered more reliable than the use of procyclic forms. Tests with metacyclic trypanosomes correlate well with field observations, but it may take up to 40 to 50 days of *in vitro* incubation to generate metacyclic trypanosomes. *In vitro* cultivation of bloodstream forms is only possible using preadapted lines and not using isolates directly from naturally infected animals. A simplified axenic culture system has been developed, 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. In contrast to *T. brucei*, it is very difficult to cultivate *T. congolense*. If techniques can be improved to adapt trypanosome isolates to grow *in vitro* more rapidly, these assays may become more popular, especially in those laboratories where culture facilities are already established. An interesting alternative is the drug incubation *Glossina* infectivity test (DIGIT), in which the trypanosomes are exposed to the drug *in vitro* for a short time and thereafter fed to tsetse flies to check whether or not they develop into metacyclic forms (Clausen *et al.*, 1999). This technique distinguishes resistant from sensitive isolates and does not require experimental animals, but it does require a ready supply of teneral tsetse flies from an artificially reared colony.

Trypanocidal drug-ELISAs

As an alternative to the tests mentioned above, the use of trypanocidal drug-ELISAs in combination with parasite detection tests has given promising results for the detection of resistant trypanosomes. A competitive ELISA allowing the detection of small amounts of isometamidium in serum of cattle (Eisler *et al.*, 1996) has been validated in cattle under experimental and field conditions. 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 i.m. injection of isometamidium in cattle. 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. Observations showed that the presence of trypanosomes in animals with an isometamidium concentration of > 0.4 ng/ml suggests resistance; the higher the drug level detected the greater the degree of resistance that could be inferred. 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 and diminazene.

Block treatment studies and longitudinal parasitological data

Analysis of data on the frequency of infections after block prophylactic drug treatment can give clear indications of drug resistance but comparisons with untreated cattle in the same environment are required in order to confirm the level of challenge and efficacy of the drug treatment. Alternatively, longitudinal parasitological data may be used to compare trypanosome incidence and prevalence in herds under a therapeutic drug regimen, using PCV to distinguish new from recurrent infections.

Potential new tests for the detection of resistance

All of the available tests for drug resistance have significant disadvantages and none is ideal. There is an urgent need to develop cheap reliable tests that can be used easily in developing countries. None of the recent new tests have fulfilled these essential criteria but some show potential.

For example, it has been known that the rate of isometamidium accumulation in *T. congolense* is a good indicator of the degree of drug resistance and since the mitochondrial electrical potential 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 based on these findings. If such a test could be carried out using a small number of trypanosomes directly on whole blood samples, it might provide a rapid indication of the level of resistance of a given trypanosome isolate. Some progress has also been made in identifying genetic markers for resistance. These might be developed into reagents for the identification of resistant trypanosomes using PCR in the future.

Guidelines to Delay the Development of Drug Resistance

Until now the most important guidelines to avoid or to delay the development of drug resistance were considered to be to use of the 'sanative' pair of drugs (isometamidium or ethidium and diminazene) and to avoid the exposure of trypanosomes to subtherapeutic 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 any recommendation is lacking 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 the following recommendations are proposed in order to delay the development of resistance.

Reduction of 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. decrease 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, 1962). It is therefore strongly recommended that control of trypanosomiasis should not rely solely on drugs and 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.

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. Given the fact that in many countries drugs are often administered by unskilled persons, errors may easily occur in calculating the correct doses for the treatment of the animals. Farmers have the tendency to underestimate the weight of their animals when they have to treat them. Furthermore there is an increasing number of generic products available on a somewhat loosely regulated market, and some of these have questionable efficacy and many contain lower doses of drug than the stated amount. This has now been shown to be the case for many drugs for veterinary and human use as well as trypanocides.

Avoiding exposure of the whole parasite population to a drug

In contrast to the approach to human sleeping sickness, mass treatments are commonly used to control animal trypanosomiasis and can be highly successful over many years for example in ranch cattle (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* (e.g. the trypanosomes present in the fly population or in other hosts), the higher the selection pressure. The percentage of the total parasite population, which is exposed to the drug at the time of treatment, might thus have an impact on resistance development. Therefore in well-monitored situations there is a strong case for limiting treatment to individual clinical cases; this is also desirable on grounds of minimizing drug residues, avoiding potential toxicity and reducing costs. In such situations drug resistance problems can be minimized and acquired immunity encouraged (Scott and Pegram, 1974).

Banning the use of quinapyramine in cattle

Quinapyramine was widely used in cattle in Africa during the period 1950–1970. In 1976 it was withdrawn from sale for cattle use because of problems with toxicity and resistance development. However, it is still available for use in camels and it is likely that it is still used in cattle in some situations in Africa where both species exist in proximity to the margins of tsetse belts. 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 isometamidium, homidium and diminazene was expressed at the level of the individual trypanosome and could be transmitted by tsetse flies. This confirms the results of Whiteside's (1962) earlier field studies. Therefore, the use of quinapyramine as a trypanocide in cattle is completely contraindicated.

Guidelines for Action when Drug Resistance is Detected

Single drug resistance

Based on the examination of a representative number of trypanosome isolates from a given area, using tests in mice or in ruminants (see earlier section), the frequency of expression of resistance to a single trypanocidal drug can be calculated. The following guidelines for action (Table 23.4) based on the frequency of expression of trypanocidal drug resistance were proposed following a meeting of international experts in Nairobi in 1999 under the auspices of the EU Concerted Action programme entitled Integrated Control of Pathogenic Trypanosomes and their Vectors.

Multiple drug resistance

Resistance to both isometamidium and diminazene, if present at the level of individual trypanosomes, may be demonstrated by testing cloned populations in mice or in ruminants. If resistance to both isometamidium and diminazene is present at the level of individual trypanosomes similar guidelines to those recommended for cases of $> 60\%$ frequency of drug resistance should be followed (Table 23.4).

Recommendations on the use of isometamidium prophylaxis

Classically, regular prophylactic treatment with isometamidium has been advocated as a means of prevention of bovine trypanosomiasis. However, recent concerns about the development of drug resistance have led to the recommendation that the number of treatments should be minimized to reduce the exposure of the parasite population to the drug. In view of these considerations, the following guidelines on the use of isometamidium prophylaxis are proposed.

Table 23.4. Guidelines for action based on frequency of drug resistance.

Frequency of drug resistance in trypanosome infections of cattle	Guidelines for action
Absent	• Use 'sanative pairs' • Minimize use of routine block treatments • Minimize drug use by vector control and/or by decreasing vector–host contacts
1–30%	• Use 'sanative pairs' • Target trypanocide treatment on clinical cases • Investigate drug usage patterns • Introduce vector control (targets, traps and/or bait techniques) • Monitor situation over a wider area and over time
31–60%	• Use 'sanative pairs' and monitor the efficacy of treatment • Strengthen other aspects of integrated disease control to minimize impact of intercurrent infections with other pathogens • Intensify vector control • Improve management/nutrition • Introduce trypanotolerant genotypes if feasible
> 60%	• Restrict use of trypanocides to clinical cases • Use zero grazing and/or fly-proof housing where appropriate, e.g. in smallholder dairy systems • Intensify vector control • Consider change from cattle to other types of livestock

- Avoid the use of continuous isometamidium prophylaxis and minimize the frequency of routine isometamidium block treatments.
- Consider the use of prophylaxis only in cattle exposed to heavy challenge for a defined period, e.g. transhumance or high seasonal challenge.
- Never administer isometamidium more frequently than every 3 months.

When routine block treatment with isometamidium is practised it is recommended that, once a year, the animals are separately treated with diminazene in order to delay the development of drug resistance following the concept of the 'santative pair' (Whiteside, 1962).

Quality Control of Trypanocidal Drugs

The increasing availability of generic trypanocides in Africa has created new problems over the quality assurance of these products. Recent pilot studies have indicated wide discrepancies in the quality of many of the products currently being sold to livestock owners.

Whilst some products are plainly fraudulent and possess no trypanocidal activity, the most common problem is variability in the content of active ingredient. This is partly caused by the lack of agreed specifications for these products but also reflects differences in batch quality and poor product control in the manufacturing or packaging plant. Whilst some products contain more than the stated quantity of active ingredient, many others contain less than that stated and it is these latter products that could lead to serious underdosing of livestock and both inefficacy of treatment and the resultant enhanced risk of the development of drug resistance. There is an urgent need to establish regional testing laboratories in Africa for trypanocides and other veterinary pharmaceutical products. These should be supported by international laboratories, which can provide back-up standardization and verification facilities.

Conclusions

Since it is unlikely that new trypanocidal drugs for treating animal trypanosomiasis will be released on to the market in the near future, it is essential to try to maintain the efficacy and supply of the currently available drugs. The most important and most efficient measure to achieve this is to adopt an integrated disease management strategy so that trypanocides are used within the broader context of trypanosomiasis control. In many respects the current privatization of veterinary services is mitigating against this policy but if more area-wide tsetse control is successfully implemented under the Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), which was launched in 2001, there is an opportunity for a more managed level of integrated control of animal trypanosomiasis to emerge.

In the meantime farmers should be supported in their use of trypanocides and measures adopted that assist this process. These include the provision of better data on the true prevalence of trypanocidal resistance and its impact on the productivity of livestock. In order to address the issue of true prevalence and 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 as described in this chapter and elsewhere (Eisler *et al.*, 2001). These methods should be promoted as routine monitoring tools in all areas where trypanocidal drugs are used.

In view of the increased trafficking in trypanocides in some regions of Africa, the proliferation of generic products and the pilot studies, which have identified substandard products, there is a need for drug quality control measures to be introduced along with an effective licensing system for trypanocides. Regional laboratories should be established which fulfil the criteria of technical competence, impartiality and acceptability to all stakeholders. Dialogue with the pharmaceutical industry should be stimulated as part of the solution to the problem of quality assurance, since the safeguarding of the long-term efficacy of products is of importance to the industry as well as to the users.

The frequent observation that curative trypanocides are administered without an accurate diagnosis of trypanosomiasis being made prior to treatment highlights the lack of practical and affordable field tests for trypanosomiasis. There is an urgent need to assist livestock owners in this regard and thereby reduce the inappropriate use of trypanocides.

Other priorities for future research include studies on the population dynamics of drug-sensitive and drug-resistant trypanosomes and determination of the risk factors that bring about drug resistance. There is also a need for more research on the genetic basis of drug resistance both to facilitate the development of markers for drug resistance and to model the development and spread of drug resistance in trypanosome populations. The relationship between the level of tsetse challenge and the rate of emergence of drug resistance in trypanosome populations requires further examination. It is also important to determine the extent to which control of tsetse can be used to limit the rate of development and spread of drug-resistant trypanosomes in the future.

References

Boyt, W.P. (1986) *A Field Guide for Diagnosis, Treatment and Prevention of African Animal Trypanosomiasis.* FAO, Rome.

Clausen, P.-H., Leendertz, F.H., Blankenburg, A., Tietjen, U., Mehlitz, D., Sidibe, I. and Bauer, B. (1999) A drug incubation glossina infectivity test (DIGIT) to assess the susceptibility of *Trypanosoma congolense* bloodstream forms to trypanocidal drugs (xenodiagnosis). *Acta Tropica* 72, 111–117.

Eisler, M.C., Elliott, C.T. and Holmes, P.H. (1996) A simple competitive enzyme immunoassay for the detection of the trypanocidal drug isometamidium. *Therapeutic Drug Monitoring* 18, 73–79.

Eisler, M.C., Brandt, J., Bauer, B., Clausen, P.-H., Delespaux, V., Holmes, P.H., Ilemobade, A., Machila, N., Mbwambo, H., McDermott, J., Mehlitz, D., Murilla, G., Ndung'u, J.M., Peregrine, A.S., Sidibé, I., Sinyangwe, L. and Geerts, S. (2001) Standardised tests in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle. *Veterinary Parasitology* 97, 171–183.

Fox, R.G.R., Mbando, S.O., Fox, M.S. and Wilson, A. (1993) Effect on herd health and productivity of controlling tsetse and trypanosomiasis by applying deltamethrin to cattle. *Tropical Animal Health and Production* 25, 203–214.

Geerts, S. and Holmes, P.H. (1998) *Drug Management and Parasite Resistance in Bovine Trypanosomiasis in Africa*. PAAT Technical and Scientific Series No. 1. FAO, Rome.

Itty, P., Swallow, B.M., Rowlands, G.J., Mulatu, W. and d'Ieteren, G.D.M. (1995) The economics of village cattle production in a tsetse-infected area of southwest Ethiopia. *Preventive Veterinary Medicine* 22, 183–196.

Leach, T.M. and Roberts, C.J. (1981) Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the Eastern hemisphere. *Pharmacology and Therapeutics* 13, 91–147.

Mulligan, H.W. (1970) *The African Trypanosomiases*. George Allen & Unwin, London.

Ndoutamia, G., Moloo, S.K., Murphy, N.B. and Peregrine, A.S. (1993) Derivation and characterization of a quinapyramine-resistant clone of *Trypanosoma congolense*. *Antimicrobial Agents and Chemotherapy* 37, 1163–1166.

Peregrine, A.S. (1994) Chemotherapy and delivery systems: haemoparasites. *Veterinary Parasitology* 54, 223–248.

Peregrine, A.S., Mulatu, W., Leak, S.G.A. and Rowlands, G.J. (1994) Epidemiology of bovine trypanosomiasis in the Ghibe valley, Ethiopia: multiple drug resistance and its effective control. In: Proceedings of the 7th International Symposium on Veterinary Epidemiology and Economics, 15–19th August, 1994, Nairobi, Kenya. *The Kenya Veterinarian* 18, 369–371.

Scott, J.M. and Pegram, R.G. (1974) A high incidence of *Trypanosoma congolense* strains resistant to homidium bromide in Ethiopia. *Tropical Animal Health and Production* 6, 215–221.

Trail, J.C.M., Murray, M., Sones, K., Jibbo, J.M.C., Durkin, J. and Light, D. (1985) Boran cattle maintained by chemoprophylaxis under trypanosomiasis risk. *Journal of Agricultural Science* 105, 147–166.

Van den Bossche, P., Doran, M. and Connor, R.J. (2000) An analysis of trypanocidal drug use in the Eastern Province of Zambia. *Acta Tropica* 75, 247–258.

Whiteside, E.F. (1962) Interactions between drugs trypanosomes and cattle in the field. In: Goodwin, L.G. and Nimmo-Smith, R.H. (eds) *Drugs, Parasites and Hosts*. J. and A. Churchill, London, pp. 116–141.