African bovine trypanosomiasis: the problem of drug resistance

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The three trypanocides used to control tsetse-transmitted trypanosomiasis in domestic animals in Africa have been in use for over 40 years and, not surprisingly, resistance of trypanosomes to these drugs has emerged. Because of the relatively limited market in Africa and the high costs of developing and licensing new drugs, international pharmaceutical companies have shown little interest in the development of new trypanocides for use in either animals or humans. Therefore, the current challenge is to achieve optimal use of the relatively old existing drugs, and it is in this context that the problem of drug resistance has to be quantified – as discussed here by Stanny Geerts, Peter Holmes, Oumar Diall and Mark Eisler.

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In the 37 African countries with endemic animal trypanosomiasis, trypanocides play a key role in the control of the disease. There are currently only three trypanocides available for controlling tsetse-transmitted trypanosomiasis in domestic ruminants (caused by *Trypanosoma congolense, Trypanosoma vivax* and, to a lesser extent, *Trypanosoma brucei – Trypanosoma evansi* is not included in this review because it is not transmitted by tsetse). These are isometamidium and homidium, which have both prophylactic and therapeutic effects, and diminazene, which has only therapeutic properties. It is estimated that 35 million doses of these drugs are used in Africa each year, with about 50–70 million animals at risk from trypanosomiasis[1]. All three drugs have been on the market for >40 years and, for much of this time, they have been provided by a few European manufacturers. However, generic forms of these compounds from a variety of sources have recently become available.

In the past, the availability of trypanocides was strictly controlled in most African countries by Government Veterinary Departments. However, in recent times, with the privatization of veterinary services and a general trend towards deregulation of markets, trypanocides, along with many other pharmaceutical products, have become more freely available through local pharmacists, agroveterinary suppliers and the informal sector, and many are now purchased directly by farmers. In this way, availability has increased, but so have the risks of misuse. Trypanocidal drugs are probably the most commonly used veterinary products in sub-Saharan Africa (SSA), with the possible exception of anthelmintics and traditional remedies. They are often the first drugs tried by farmers in SSA when their cattle develop (any) symptoms of disease because they are affordable, at approximately US$1 per treatment. As a result, they are frequently used without an accurate diagnosis in tsetse-infested areas. It has been estimated from studies in Zambia that half the animals treated with trypanocides are not in fact infected with trypanosomes, and use of trypanocidal drugs by farmers may be unrelated to the prevailing tsetse challenge (Ref. 2; R.E. Mdachi, PhD Thesis, University of Glasgow, 1999; J. McDermott *et al.*, Abstract)*. There is also an understandable trend for farmers to restrict treatments to the more valuable animals in the herd, such as work oxen and milking cows. Unfortunately, farmers rarely have access to weighing scales and estimates have to be made of body weight in the calculation of the dose of drug to be administered. Interestingly, studies in Zambia[2] and Kenya (R.E. Mdachi, *op. cit.*) have indicated that under-dosing is less prevalent than might have been expected when farmers administer the drug, and that, where under-dosing does occur, it is more likely to be when the drug is administered by an animal health assistant or pharmacist. Finally, trypanocidal drugs might remain popular with farmers following effective control of tsetse, as shown in Eastern Province of Zambia, where the frequency of trypanocidal drug use remained as high as in an area where no tsetse control was taking place[2].

Extent of drug resistance

The development of resistance to therapeutic agents has been well documented for antibiotics, anthelmintics and insecticides. Thus, it is not surprising that drug resistance has also emerged to the three commonly used trypanocides, given their long use[1]. Trypanocide resistance has been demonstrated conclusively under laboratory conditions by inoculation of trypanosome isolates into bovines and treating with correct drug dosage regimens, or by administering prophylactic drug dosages and then challenging with tsetse infected with well-characterized trypanosome populations at regular intervals[3,4]. An ELISA test for isometamidium has demonstrated the presence of high drug levels in the blood of cattle harbouring trypanosomes, whereas the same drug concentrations were shown to be prophylactic for drug-sensitive isolates[5]. Furthermore, characterization of numerous trypanosome isolates in rodents has revealed

*McDermott, J. *et al.* (2000) Field studies on the development and impact of drug resistant animal trypanosomes in market oriented production systems in the southern Guinean Zone of West Africa. *Newsletter on Integrated Control of Pathogenic Trypanosomes and their Vectors (ICPTV Newsletter)* 2, 18–21.

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significant differences in drug sensitivity that correlate well with the drug resistance patterns observed for these populations in cattle[6]; M.C. Eisler *et al.*, Abstract)†.

Although drug resistance has now been reported in at least 13 African countries[1,4], the reports have usually been of isolated cases, and there has been little attempt to undertake spatial or temporal assessments of the scale of the problem. An important prerequisite to undertaking such studies is the availability of reliable and simple tests for drug resistance, as the failure of drug treatment might have causes other than drug resistance. These might include significant under-dosing, treatment with bogus drugs, or rapid re-infection after treatment with a short-acting therapeutic drug. Although a variety of tests for trypanocide resistance have been developed over the years, using animals (ruminants and laboratory animals) or *in vitro* approaches[1,4], there have been few attempts to compare the reliability and acceptability of these different techniques. Unfortunately, in most African laboratories, *in vitro* cultivation of trypanosomes is not yet readily available and one has to turn to less sophisticated tests using animals.

The optimal animal tests are probably those that are conducted in the definitive hosts (i.e. cattle, sheep or goats), but these are expensive to undertake. Instead, tests on laboratory mice have become the preferred method, at least for testing isolates of *T. congolense*, which generally grows well in mice. Recently, progress has been made in developing a standardized test through the Concerted Action Programme entitled 'Integrated Control of Pathogenic Trypanosomes and their Vectors' (ICPTV: http://www.dis.strath.ac/vie/icptv), which is an integral part of the research and development module of the Programme Against African Trypanosomiasis (PAAT). As a result, there is now a standardized protocol for a single-dose mouse test, which has been assessed in a variety of countries and is gaining general support (S. Geerts *et al.*, Abstract)‡. This single-dose mouse test is intended to compare and characterize areas in terms of the extent of drug resistance in *T. congolense* or *T. brucei* (very few *T. vivax* grow in mice) by examination of as many isolates as possible, rather than to characterize individual stabilates. The greater the proportion of trypanosome populations in an area that express resistance in naturally infected cattle, the greater the proportion that will show resistance in a test in mice using an appropriate single drug dose. The test uses discriminatory dosages of 1.0 mg kg^{-1} isometamidium chloride and 20 mg kg^{-1} diminazene aceturate and allows considerable reduction in the number of animals, and also the labour needed, compared with the CD_{50} test, in which the dose required to cure completely 50% of the mice is determined by infecting and treating at least five groups at several different dosages. Although there is a good correlation overall between the results obtained using the test in mice and those obtained using the test in ruminants, the curative dose to be used in cattle cannot be extrapolated from the results in mice[6].

The simplified test will allow more reliable comparisons to be made and more accurate assessments of the spatial and temporal importance of drug resistance in *T. congolense* and *T. brucei* across Africa. For example, a recent study on 140 *T. congolense* isolates randomly collected in Kenya, Tanzania and Zambia using single-dose mouse testing showed trypanocidal drug resistance to be widespread but variable across eastern and southern Africa (M.C. Eisler *et al.*, *op cit.*)†. The problem was shown to be particularly severe in coastal regions of Kenya and Tanzania, where multiple drug resistance was common and where there was a history of heavy use of trypanocides. Elsewhere, for example western Kenya and eastern Zambia, there was some evidence of drug resistance but no evidence of multiple drug resistance, and use of the sanative pair[7] (i.e. the alternate use of isometamidium–homidium and diminazene) could be expected to be effective in controlling resistant infections (Fig. 1). The results were broadly consistent with those obtained using an ELISA for isometamidium[8] as an indicator of levels of drug usage, and also of resistance (where effective concentrations were found in the sera of cattle that were nevertheless infected). The methodologies employed were shown to be useful in the investigation of trypanocidal drug resistance on an area-wide basis, and a similar approach could be used in other regions of SSA.

Mechanisms and genetics of drug resistance

Although the development of simplified and standardized tests to detect drug resistance is undoubtedly of great importance, there is also a need to investigate at a more fundamental level the mechanisms associated with drug resistance in trypanosomes. It is rather surprising, given the length of time these drugs have been available and the widespread interest in drug resistance, that relatively little work has been done on how these drugs are taken up by trypanosomes and the processes that are changed when drug resistance emerges. Unfortunately, this is an area that has not been funded by pharmaceutical companies, and other funding agencies have been reluctant to support such work. It is hoped that this situation is slowly improving. Progress is being made in elucidating the role of nucleoside transporters in resistance to trypanocidal drugs[9]. Furthermore, changes in the mitochondrial electrical potential (MEP) have

†Eisler, M.C. *et al.* (2000) Area-wide appraisal of drug resistance in trypanosomes infecting cattle in East and Southern Africa. *ICPTV Newsletter* 2, 16–18.
‡Geerts, S. *et al.* (2000) *In vivo* tests for the detection of resistance to trypanocidal drugs: tests in mice and in ruminants. *ICPTV Newsletter* 2, 6–7.

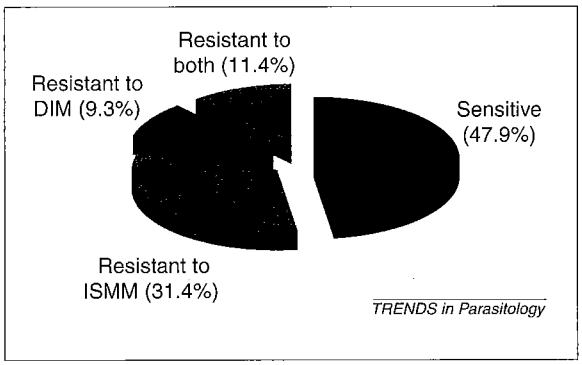

Fig. 1. Percentages of *Trypanosoma congolense* isolated from cattle in tsetse-infested areas of Kenya ($n = 52$), Tanzania ($n = 17$) and Zambia ($n = 71$) that were sensitive or resistant to trypanocidal drugs on the basis of tests in mice using discriminatory doses of 1.0 mg kg^{-1} body weight isometamidium chloride (ISMM) and 20 mg kg^{-1} body weight diminazene aceturate (DIM)[23].

been demonstrated in isometamidium-resistant trypanosomes[10]. As the MEP is closely linked with the rate of isometamidium uptake, which seems to be a good indicator of the degree of drug resistance, an *in vitro* test measuring the MEP might provide a rapid indication of the degree of drug resistance if it could be carried out using a small number of trypanosomes directly isolated from the blood of infected animals.

Interesting work is also going on to identify genetic markers for isometamidium resistance, which might be developed later on into reagents for the identification of resistant trypanosomes using PCR (P. Majiwa *et al.*, Abstract)§. Recent data suggest that isometamidium resistance in *T. congolense* involves several genes (V. Konde and P. Majiwa, Abstract)¶. There is an urgent need to examine the genetic background of drug resistance in trypanosomes in greater detail. The mono- or polygenic nature and dominance or recessiveness of the genes involved could have far-reaching implications on the spread and eventual control of resistance.

Impact of drug resistance

It is essential to assess not only the distribution of drug resistance, but also the constraints it imposes on effective control. Whether or not drug-resistant trypanosomes are less pathogenic than are susceptible ones remains a controversial issue. However, recent studies at the International Livestock Research Institute (ILRI, Nairobi, Kenya) did not find any differences in virulence among four populations of *T. congolense* ranging from extremely sensitive to strongly resistant to isometamidium (ILRI, Abstract)**.

§Majiwa, P. *et al.* (2000) Molecular approaches to the identification of DNA markers for drug resistance in *Trypanosoma congolense*. *ICPTV Newsletter* 2, 14–15.
¶Konde, V. and Majiwa, P. (2000) Alterations in gene transcription patterns associated with the development of isometamidium resistance in *Trypanosoma congolense*. *ICPTV Newsletter* 2, 15–16.
**ILRI (1996) Divining the mechanisms of drug resistance in trypanosomes. *Newsletter of the International Livestock Research Institute* 2, 7–10.

Although drug resistance reduces the efficiency of trypanocides, there is significant evidence from the field that, even where drug resistance has been clearly demonstrated, for example in the Ghibe valley of Ethiopia, continuing use of trypanocides (to a limited extent and in combination with other control measures) might still provide beneficial effects to cattle[11,12]. Although to be thoroughly effective trypanocides should lead to the death of trypanosomes, it is more probable that, in most situations, they cause restrictions in replication, which then allow the trypanosomes to be cleared by the immune system. Evidence to support this comes from studies using immunosuppressed mice: doses of trypanocide that were effective in immunocompetent mice were ineffective in immunosuppressed animals, and rapidly led to the development of substantial drug resistance in trypanosomes passaged through such animals[13]. Although immunosuppression is more pronounced in trypanosome-infected mice, it is also present in infected cattle[14].

Tsetse challenge

Another important aspect that influences the efficacy of trypanocides in the field is the force of infection. There is now a considerable body of evidence to suggest that there is a close correlation between the degree of tsetse challenge and the prevalence of drug resistance[1,15] (R.E. Mdachi, *op. cit.*). Where tsetse numbers are very high, drug resistance is much more likely to develop. In part, this is probably because there is increased usage of these drugs in such situations and this increases drug pressure, but there is also an indication that increased challenge *per se* increases the likelihood of animals becoming infected with drug-resistant trypanosomes. The drug pressure then encourages such strains to spread rapidly. This can be expressed in a quantitative way. If, for instance, 10% of the trypanosome strains in an area are resistant and an animal is challenged by one trypanosome strain drawn at random from the resistant and sensitive subpopulations combined, there will be a one-in-ten chance of the resulting infection being resistant. However, if in the same area, an animal is challenged with ten trypanosome strains, there will be a probability of 0.651 that the animal is challenged with at least one resistant strain, based on a binomial distribution of p sensitive and q resistant.

There is certainly evidence from the field that, where drug resistance emerges as a problem, the control of tsetse and a reduction in the level of challenge can be a very effective measure in reducing the incidence of drug resistance and enhancing the efficacy of these drugs so they can continue to be used in such circumstances[16,17].

Minimizing the development of drug resistance

Identifying tactical measures to reduce the incidence of drug resistance and to minimize its impact and spread is of great importance. As a result of the

ICPTV workshop in Nairobi, a number of useful measures were identified (Anon, Abstract)[††]. The most important one is the reduction of the number of treatments, which can be achieved by integrating drug usage with other control measures[18]. Such an integrated approach includes: the use of sanative pairs of drugs[7] wherever possible; limiting treatment to clinical cases (a balance has to be found between economic benefit through the treatment of subclinical cases and delaying resistance development through the reduction of drug pressure); and the introduction of vector control, including the use of insecticidal pour-ons or other measures to reduce tsetse challenge, such as zero-grazing of dairy cattle in netted enclosures, as practiced by some farmers on the Kenyan coast[1,18]. The use of trypanotolerant livestock is also an important option in West Africa, and possibly elsewhere[19].

Quality assurance of trypanocidal drugs

In recent years, a further issue has arisen associated with the liberalization of veterinary drug supply and marketing: the growing problem of poor-quality drugs finding their way onto the market. In some cases, products with no trypanocidal activity have been identified and in other situations compounds with reduced activity have been marketed. Such products are not only less effective when used by farmers, but also greatly increase the risk of drug resistance developing (especially when under-dosing also allows the survival of the heterozygote-resistant trypanosomes). Unfortunately, quality controls on pharmaceutical products used in the developing world are frequently inadequate and there is already considerable evidence that the problem is widespread for a variety of pharmaceutical products[20–22]. To ensure that drugs of sufficient quality are available, the PAAT committee has proposed that, in collaboration with pharmaceutical companies, regional testing laboratories should be established in Africa supported by specialist laboratories in Europe. The development of such centres would allow a random testing of products purchased from local pharmacies as well as the testing of sample batches produced by different pharmaceutical companies. The results of such tests by independent laboratories and the publishing of the results through the PAAT-L discussion forum (http://www.fao.org/paat/html/paat_1) would provide a very useful service to veterinary authorities across Africa. It is hoped to establish such a service within the coming year.

Conclusion

It is clear that trypanocides remain a vital part of the armoury used by farmers across Africa to control trypanosomiasis and it is in everyone's interest that these drugs remain as effective as possible for as long as possible. In order to achieve this, it is important to monitor, on a regular basis drug, usage patterns by farmers, and the prevalence and incidence of drug resistance in different countries, and to put in place measures to ensure that only drugs of the highest quality find their way onto the markets in Africa. Finally, where drug resistance does emerge as a serious problem, clear guidelines on the course of actions to be followed should be made available to livestock owners and veterinarians.

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