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Standardised tests in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle

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Abstract

Resistance to the drugs used to control African animal trypanosomosis is increasingly recognised as a constraint to livestock production in sub-Saharan Africa. The most commonly used tests for detection of trypanocidal drug resistance are tests using mice or ruminants, but these suffer from lack of standardisation and hence it may be difficult to compare the results of different investigators. Tests in mice are less expensive than tests in ruminants, but while tests in mice they may be useful

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as a general guide to resistance in a geographic area they should not be extrapolated to cattle on an individual trypanosome level. Moreover, the commonly used protocols are too laborious for their application to large number of trypanosome isolates on an area-wide basis. This paper presents guidelines for standardised testing of trypanocidal drugs in vivo, and introduces a simplified single-dose test for use in mice, which is convenient for use in areas with limited laboratory facilities. The single-dose test is appropriate for characterisation of geographic areas in terms of trypanocidal drug resistance using large numbers of trypanosome isolates, for making comparisons between areas, and for monitoring changes in trypanocidal drug resistance over time. Multiple-dose tests may be used to determine the degree of resistance of individual stabilates to be determined precisely in mice are also described, but for logistical reasons these will rarely be conducted on more than a few stabilates, and testing of a larger number of stabilates in the single-dose test will generally provide more useful information. Finally, we describe tests in cattle that may be used to determine the efficacy of recommended curative doses of trypanocidal drugs for the treatment of infection with individual trypanosome isolates, including *Trypanosoma vivax*, which is rarely infective for mice.

Keywords: Cattle–protozoa; Drug resistance; Trypanocides *Trypanosoma* spp.

1. Introduction

Control of African bovine trypanosomosis continues to rely in most endemic areas on chemotherapy and chemoprophylaxis using the salts of just three trypanocidal compounds, isometamidium, homidium and diminazene. All of these drugs have been in widespread use for over 35 years, and resistance has been reported in at least 13 African countries (Peregrine, 1994; Geerts and Holmes, 1998). Recent surveys in Eastern and Southern Africa (Ndung'u et al., 1999) and in West Africa (McDermott et al., 1999, 2000) have shown that the prevalence of trypanocidal drug resistance might even be higher than hitherto suspected. However, for most trypanosomosis areas, the extent and impact of drug resistance is not known (Geerts and Holmes, 1998). This information is crucial for technical staff and policy makers in making and implementing recommendations on trypanocidal drug usage and supportive tsetse and trypanosomosis control (ICPTV, 2000).

Several tests have been described for the detection of drug resistance in trypanosomes pathogenic for domestic ruminants (Geerts and Holmes, 1998). The most commonly used are tests in mice and ruminants, and these will be described in detail in this paper. Unfortunately, until now, there has often been considerable variation in the experimental protocols used to test for drug resistance and in the criteria for interpretation of the results, which are therefore not readily comparable between investigators. Hence, there is an urgent need to standardise these tests in order to allow comparison of drug resistance data on a temporal and spatial basis.

In the past, trypanocidal drug sensitivity testing in vivo has given emphasis to examination of individual trypanosome isolates in detail, for example by determination of the doses required to cure 50% (CD_{50}) or 80% (CD_{80}) of experimentally-infected animals. Where possible (i.e. for *Trypanosoma brucei* and *Trypanosoma congolense*, but not for *Trypanosoma vivax*), this has generally been done in mice because of the prohibitive cost of such detailed investigations in ruminants. Several authors have demonstrated a consistent

relationship between the results of tests in mice and those of tests in ruminants (Hawking, 1963; Sones et al., 1988; Peregrine et al., 1991; Ainanshe et al., 1992; Codjia et al., 1993; Ndoutamia et al., 1993). However, Sones et al. (1988) showed that the exact curative dose in cattle for an individual isolate could not be directly extrapolated from the results in mice because of differences in metabolic size. Kone (1999) later confirmed this observation.

Given the difficulties in using groups of cattle or large numbers of mice in Africa to assess the distribution and prevalence of drug resistance, here we introduce a new test in mice that is suited to characterisation of geographic areas in terms of the extent and probable impact of drug resistance by examination of large numbers of isolates using a simplified, single-dose protocol.

2. Methods for the detection of resistance to trypanocidal drugs

The protocols for these tests were developed at a workshop organised by the EU Concerted Action on Integrated Control of Pathogenic Trypanosomes and their Vectors held in Nairobi (June 1999) entitled "Drug Delivery and Resistance in the Context of Integrated Disease Management" (ICPTV, 2000). Efforts have been made to keep the materials and methods as simple and cheap as possible in order to permit their use under a wide variety of conditions and without access to well-equipped laboratory facilities.

This paper provides guidelines for

1. A new, simplified single-dose test that allows a large number of isolates of *T. congolense* or *T. brucei* to be tested in mice relatively easily and at low cost. This test should be used in studies in which a reasonably large number of cattle (e.g. 50) are sampled at each of several sites selected randomly from all possible sites in an area in order to evaluate the problem of drug resistance on an area-wide basis. Selection of sampling sites may be stratified on the basis of production systems, agro-ecological zones or other appropriate factors. The important features of this test are shown in Table 1.
2. A multi-dose test in mice, which allows the degree of resistance of individual *T. congolense* or *T. brucei* stabilates to be determined precisely. This test may be used

Table 1
Comparison of trypanocidal drug sensitivity testing in mice using multiple dosage rates with a new approach using a single dosage rate

	Multi-dose test	New single-dose test
Number of mice required to test		
A single drug	36	12
Two drugs	66	18
Three drugs	96	24
Number of trypanosome isolates typically examined	1–10	>50
Provides detailed information on individual isolates	Yes[a]	No
Allows comparisons between geographic areas	No	Yes

[a] Detailed information such as CD_{50} or CD_{80} will nevertheless be of only limited value in predicting curative drug doses required in cattle.

if detailed comparison of the level of drug resistance of different trypanosome isolates is required. Since this test requires a large number of experimental animals per isolate, it is unlikely to be conducted on more than a few isolates from any given area for logistical reasons. Hence, whenever possible, testing of a larger number of stabilates using the single-dose test is preferable to characterise the drug resistance situation in an area. However, the multi-dose test could be used to maximise the information obtained where only a limited number of stabilates can be obtained from an area of interest. The major differences between the single-dose and multi-dose tests in mice are shown in Table 1.

3. A test in cattle to determine the efficacy of recommended curative doses of trypanocidal drugs for the treatment of infection with individual isolates of *T. vivax*, *T. congolense* or *T. brucei*. This test is useful because of the difficulty in extrapolating the curative dose in cattle from the results of test in mice (Sones et al., 1988; Kone, 1999), and because of the poor infectivity of most *T. vivax* for mice.

2.1. Tests in mice

2.1.1. Isolation of trypanosomes

Blood of cattle infected with populations of *T. congolense* or *T. brucei* can be inoculated directly into outbred mice without the need for carrying liquid nitrogen to the field. Mice to be inoculated with trypanosomes preferably should be immunosuppressed 24 h beforehand either by intraperitoneal injection with 300 mg/kg cyclophosphamide (e.g. Endoxana®, Asta Medica, Cambridge, UK), or by gamma or X-irradiation with 6.5 Gy (650 rads). However, immunosuppression may reduce the viability of mice under field conditions and should be used with discretion.

The species of trypanosome present should be verified, since it may not be what was originally observed in the field if a mixed infection was present. *T. vivax*, which is rarely infective for mice, will usually be eliminated spontaneously after passage through mice. It may be necessary to expand the trypanosome population by additional passages in mice to produce sufficient inoculum (1×10^5 trypanosomes per mouse) for mice used in drug-sensitivity testing. However, the number of passages in mice should be limited to two or three in order to minimise selection of sub-populations from the original isolate.

2.1.2. Mice

For the single-dose test, a group of 6 mice is required for each drug to be tested, and an additional control group of 6 mice. For the multi-dose test, at least 6 groups of 6 mice (1 control and 5 treatment groups) are necessary for each drug to be tested.

For both tests, the strain, sex and age of the mice should be recorded. The mice should be weighed to an accuracy of 1 g and body weights should not vary by more than 10%.

2.1.3. Trypanocidal drugs

For the single-dose test, isometamidium chloride and diminazene aceturate should be used at dose rates of 1.0 and 20 mg/kg BW, respectively. There is no fixed recommendation for the homidium salts, and until further data becomes available the same dose (1.0 mg/kg BW) as for isometamidium should be used.

Table 2
Standardised protocols for testing trypanocidal drug resistance in mice and cattle[a]

	Single-dose test in mice[b]	Multi-dose test in mice[b]	Test in calves[c]
Number of groups of animals			
Treatment groups per drug	1	5	1
Control groups	1	1	Not necessary
Number of animals/group	6	6	3–6
Inoculum			
Number of trypanosomes	10^5	10^5	10^5
Route of administration	IP	IP	IV
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	N.D.	N.D.	1
Drug administration			
Time	24 h post-inoculation	24 h post-inoculation	First peak parasitaemia
Route	IP	IP	IM
Parasitological examination			
Method	Tail blood wet smear	Tail blood wet smear	Buffy-coat
Frequency	Two times per week[d]	Two times per week[d]	Three times per week
Duration of follow-up (days)	60	60	100
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[f]	Less than 5/6 cured		Less than 3/3 cured

[a] N.D.: not yet determined; IM: intramuscular; IP: intraperitoneal; IV: intravenous.

[b] Mice should be weighed to an accuracy of 1 g and body weights should not vary by more than 10%; strain, sex and age of mice should be recorded.

[c] Calves must be kept in a fly-proof stable or other environment non-endemic for trypanosomosis.

[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 5 out of 6 control mice must become parasitaemic; if not, the test must be repeated. If one test or control mouse dies prior to detection of parasitaemia, for interpretation see text.

[f] Resistance of individual stabilates in mice should not be extrapolated to cattle.

In the multi-dose test, the following dose ranges should be used: 0.01–20 mg/kg BW for isometamidium chloride and for homidium chloride or bromide and 1–60 mg/kg BW for diminazene aceturate. An approximate geometric progression (as presented in Table 2) of at least 5 different doses should be used.

The drugs should be dissolved in an appropriate quantity of sterile distilled water such that the required dose is contained in 0.2 ml, which is injected intraperitoneally (IP). The controls should receive the same amount of water without drug by the IP route. For each drug the source, the manufacturer and the batch number should be recorded.

2.1.4. Test protocol

The main features of the protocols are summarised in Table 2.

1. Groups of 6 mice should be inoculated IP with 1×10^5 of the trypanosomes to be tested.
2. About 24 h after inoculation of the trypanosomes, trypanocidal drug treatment should be administered by the IP route.
3. Following drug treatment, parasitological examination of the mice should be conducted twice a week using phase-contrast or dark-ground microscopy (magnification ×250) of a wet smear of tail blood. When *T. brucei* (which may be highly pathogenic in mice) are being tested, the frequency of parasitological monitoring should be increased to three times a week for the first 2 weeks.
4. When parasitaemias are observed in the control mice, the number of days to first detection of parasitaemia should be recorded and the mice euthanased.
5. The treated groups should be followed until a relapse occurs or until 60 days post-treatment.

2.1.5. Interpretation

In the case of the single-dose test, parasitaemias must be observed in at least 5 out of the 6 control mice; otherwise the test must be repeated. If one control mouse dies prior to detection of parasitaemia, the test is valid provided that parasitaemia is detected in all of the remaining 5 mice. If more than one control mouse dies prior to detection of parasitaemia, the test must be repeated.

A trypanosome isolate is considered as drug-sensitive if at least 5 out of the 6 treated mice are cured, i.e. they remain aparasitaemic until the end of the 60-day observation period. If fewer than 5 mice are cured, the isolate is considered resistant to the dosage used. If one test mouse dies prior to detection of parasitaemia, the isolate is considered as drug-sensitive if at least 4 out of the remaining 5 treated mice are cured; if fewer than 4 of the remaining 5 mice are cured, the isolate is considered resistant to the dosage used. If more than one test mouse dies prior to detection of parasitaemia, the test must be repeated.

For the multi-dose test, CD_{50} CD_{80} or CD_{95} values may be calculated using standard logit or probit analyses (Peregrine et al., 1991).

If resistance to two or more drugs (multiple resistance) is demonstrated simultaneously in the same isolate using either test, it is important to determine if this multiple resistance is present at the level of the individual trypanosome. For this purpose clones should be derived from the original isolate and tested in mice for resistance to the same drugs (Codjia et al., 1993; Mulugeta et al., 1997; Peregrine et al., 2000).

2.2. Test in calves

Neither the single-dose nor the multiple-dose tests in mice are able to predict accurately the curative doses of trypanocidal drugs for cattle infected with a particular trypanosome isolate. Hence, the test in calves should be used to determine whether or not drugs are efficacious at recommended curative doses in cattle infected with a particular trypanosome isolate. The test in calves may also be used for the investigation of drug resistance in *T. vivax*, which is not usually infective for mice.

2.2.1. Animals

About 3- to 6-month-old calves should be used, preferably of a genotype similar to that of cattle in the area under study (e.g. Zebu) and without prior exposure to tsetse or trypanosomosis. They should also be negative for anti-trypanosomal antibodies by the indirect fluorescent antibody test or ELISA (Luckins and Mehlitz, 1978) if these tests are available.

Due to individual variation in the response to trypanocidal drug treatment among ruminants inoculated with the same *T. congolense* isolate (Hawking, 1963; Peregrine et al., 1991; Ndoutamia et al., 1993; Kone, 1999), it is advisable to use a minimum of three and preferably six animals. However, economic considerations may preclude the use of more than a single animal per stabilate for drug-sensitivity testing. The results of studies using only one animal should be interpreted with caution.

2.2.2. Test protocol

1. About 1 month prior to experimental work the animals are moved to a fly-proof stable. In order to remove existing parasite burdens, treat sub-cutaneously with a broad-spectrum anthelmintic (e.g. ivermectin, 200 μg/kg BW), with diminazene aceturate at 3.5 mg/kg BW, with long-acting oxytetracycline at 20 mg/kg BW, and spray or wash with acaricide.
2. Beginning 2 weeks prior to inoculation of trypanosome isolate, and continuing until the end of the experiment, monitor PCV and parasitaemia at least three times a week by the examination of peripheral blood from a marginal ear vein using the phase-contrast buffy-coat technique (Murray et al., 1977). Cattle should be clinically examined throughout the study on a regular basis, preferably daily.
3. After confirming the viability of a trypanosome isolate microscopically, inoculate the stabilate into a jugular vein of a calf, and continue the clinical and parasitological monitoring as described above. If a significant deterioration in clinical condition is observed, PCV and parasitaemia status should be monitored the same day.
4. At the first peak of parasitaemia, weigh the animal and treat intramuscularly, on the same day, with one of the following: a 2% w/v solution of isometamidium chloride at a dose of 0.5 mg/kg BW; a 7% w/v solution of diminazene aceturate at 3.5 mg/kg BW; or a 2.5% w/v solution homidium chloride/bromide at a dose of 1.0 mg/kg BW. Choose whichever drug is used most commonly in the area under investigation. When injecting isometamidium, which has irritant properties, it is advisable to divide the drug between two injection sites and to ensure no leakage of the drug along the track of the needle when it is withdrawn.
5. If cattle relapse (i.e. trypanosomes reappear after treatment with the first trypanocidal drug), they should be monitored (parasitaemia, PCV and body weight) at least three times per week (and daily if possible) for a maximum of 50 days from the date of relapse, to obtain information on the pathogenicity of drug-resistant trypanosomes. The time between treatment and relapse should also be recorded.
6. Relapsing cattle should be re-treated if the PCV falls by one-fifth of the value measured at the time relapse is first detected, or if the PCV falls below 15%, or if considered necessary on the basis of clinical examination. If re-treatment is necessary, weigh the animal and treat intramuscularly, on the same day, with the second most commonly used drug, using the dosage regimens given above. NB: the second trypanocide should

not be administered less than 30 days following treatment with the first trypanocide in order to avoid possible side effects (Eisler et al., 1997). Also, isometamidium and homidium should ideally not be administered within 3 months of each other to avoid the complication of additive therapeutic effects in view of the long residence times of these drugs (Eisler, 1996; Murilla et al., 1999).

7. If cattle relapse again (i.e. trypanosomes reappear after treatment with the second drug), they should be monitored (parasitaemia, PCV and body weight) at least three times per week (and daily if possible) for a maximum of 50 days from the date of relapse, to obtain information on the pathogenicity of drug-resistant trypanosomes.
8. Relapsing cattle should be re-treated if the PCV falls by one-fifth of its value at the time of relapse, or if the PCV falls below 15%, or if considered necessary on the basis of clinical examination, in which case weigh the animal and treat intramuscularly, on the same day, with the third most commonly used drug, using the dosage regimens given above. NB: the third trypanocide should not be administered less than 30 days following treatment with the second trypanocide in order to avoid possible side effects.
9. If no relapse is detected after 100 days following administration of the first, second or third trypanocidal drug, the experiment should be terminated.

2.2.3. Interpretation

If no relapse is detected after 100 days following administration of the first trypanocidal drug, the trypanosome isolate is defined as sensitive to the dose of drug used. If no relapse is detected after 100 days following administration of the second or third trypanocidal drug, the trypanosome isolate may be sensitive to this drug dose, although the effects of possible selection bias must be considered (see Section 3). Relapse infections detected within 100 days of administration of a trypanocidal drug are indicative of resistance.

The results for individual cattle should be recorded. If relapse occurs in more than 20% or more of the cattle tested (i.e. for a total of between one and four cattle, at least 1 relapse; for a total of 5 or 6 cattle at least 2 relapses), the isolate may be said to exhibit resistance to the dose of drug used.

If breakthrough trypanosome infections are detected following administration of the first drug, there may be some selection bias in the sensitivity tests for the second and third drug (see Section 3). Sensitivity to a second or third drug should therefore be confirmed in a repeat test, using the drug in question first.

3. Discussion

This paper describes three methods for the investigation of trypanocidal drug resistance using laboratory animals. The first is a novel, simplified approach to the use of mice for investigation of drug resistance on an area-wide basis, whereas the other two are standardised protocols for the use of mice and cattle to obtain more detailed information on individual trypanosome isolates. All three methods are applicable to laboratories with relatively limited resources, as is frequently the case in trypanosomosis-endemic areas.

These methods were developed in response to the requirement for standardised methods for assessment of drug resistance in trypanosomes pathogenic for domestic animals,

particularly cattle (ICPTV, 2000). This requirement stems from the need to be able to assess levels of trypanocidal drug resistance in an area, to monitor its development over time, and to make comparisons among areas. This information is of crucial importance to those involved in the control of African bovine trypanosomosis, from the level of policy makers and advisors to the level of individual animal health workers and farmers responsible for treatment of livestock, in order to better manage the use of the few available trypanocides with a view to minimising the impact of trypanocidal drug resistance. Standardised methods should also assist in the detailed investigation of factors predisposing to the development of drug resistance, and in the formulation of strategies to prevent or delay such development and to limit the prevalence of resistant strains.

Characterisation of an area in terms of trypanocidal drug resistance requires investigation of a large number of trypanosome stabilates collected during the course of an unbiased and extensive survey. The details of such a survey are outwith the scope of this paper, but it is recommended that the study design, including sample size, should be carefully considered. The survey should include isolates from a wide area using appropriate epidemiological sampling techniques, for example random and stratified sampling (ICPTV, 2000). The single-dose test in mice lends itself to the rapid screening of a large number of isolates collected during such a survey. For example, in a recent multi-centre study conducted at approximately 130 sampling locations in Kenya, Zambia and Tanzania, 140 trypanosome isolates were investigated using this method over a 3 year period (Geerts et al., 1999).

It is important that a standardised inoculum of 10^5 trypanosomes be used for each mouse since it has been shown that the outcome of the test may be dependant on the number of trypanosomes inoculated (Sones and Holmes, 1992; Mamman et al., 1995). It is also important that intraperitoneal drug treatment is administered approximately 24 h after inoculation of the trypanosomes, since trypanosomes (especially *T. brucei*) might invade sites inaccessible to the drug if treatment is carried out later than this, e.g. at the time of development of patent parasitaemia (Jennings et al., 1977a,b). Delaying the administration of drug by 24 h is a reasonable precaution against trypanosomes succumbing to overwhelming local drug concentrations in the peritoneal cavity prior to the dissemination of the parasites in the host circulation. Otherwise some drug-resistant isolates might be misclassified as sensitive.

The doses recommended for the single-dose test (1.0 mg isometamidium chloride per kg BW; 20 mg diminazene aceturate per kg BW) were selected on the basis of the multi-centre study referred above (Geerts et al., 1999). In that study, large numbers of trypanosome stabilates from cattle in tsetse-infested areas in Coast, Rift Valley and Western Provinces of Kenya, Coast, Dar-es-Salaam, and Tanga Regions of Tanzania and the Eastern Province of Zambia were tested in mice using three different dose rates (0.1, 1.0 and 10 mg isometamidium chloride per kg BW; 1.0, 20 and 40 mg diminazene aceturate per kg BW) for each drug. The dose rates recommended here are those that discriminated best between these areas in terms of the proportion of trypanosome isolates resistant to each drug. They are also the doses most likely to reveal changes over time in the prevalence of drug resistance in areas investigated on more than one occasion using the single-dose test. Further research is still necessary to identify the best discriminatory dose rate for the homidium salts.

The use of a single dosage in mice means that the data obtained for any individual trypanosome isolate are of limited usefulness and should not be used to predict the response

to treatment of an infected bovine host, for the reasons stated earlier. Nevertheless, the doses recommended for this test are many-fold greater than the CD_{50} values (0.018 mg isometamidium chloride per kg BW; 2.3 mg diminazene aceturate/kg BW (Peregrine et al., 1991)) for *T. congolense* IL1180, a typical, well-characterised, pathogenic, drug-sensitive population. Hence, isolates not cleared by these doses in mice may be said to be resistant with reference to *T. congolense* IL1180. If more detailed characterisation of the level of drug resistance of an individual trypanosome stabilate is required, the multi-dose test may be conducted.

Special consideration should be given to situations where resistance to two or more drugs is present in individual isolates. In these situations it is important to determine whether the multiple resistance is clonal, i.e. present at the level of the individual trypanosome, as was demonstrated at Ghibe, Ethiopia (Codjia et al., 1993; Mulugeta et al., 1997; Peregrine et al., 2000). The sequential use of two different members of a "sanative pair" (Whiteside, 1958), e.g. isometamidium and diminazene would be expected to eliminate an infection with a trypanosome population in which multiple resistance was not clonal, but not one in which it was.

The tests in mice are primarily applicable to those trypanosome species that are readily infective for mice, i.e. *T. brucei* and *T. congolense*. Directly inoculating mice in the field has the advantage that supplies of liquid nitrogen are unnecessary. Liquid nitrogen may be used for the direct stabilation of *T. vivax* from infected cattle under field conditions, but experiences of the authors in several countries suggest that the recovery rate of viable parasites following this procedure is often poor. Other methods such as the use of survival analysis of longitudinal block-treatment study data (Mdachi, 1999) and the isometamidium ELISA (Eisler et al., 1996, 2000; Mdachi, 1999) may be more useful for investigation of drug resistance in this trypanosome species.

Finally, given that there is only an approximate relationship between the drug sensitivity of a stabilate in mice and that in cattle, it may be necessary to ascertain whether treatment with a manufacturers recommended drug dosage is likely to be successful in cattle infected with this isolate. This test has the advantage that it is applicable to all trypanosome species infective for cattle, including *T. vivax*. Although, in the past tests have been frequently conducted in individual cattle, larger numbers of cattle (3–6) are necessary to achieve reliable results. As stated above, there may be some selection bias in the sensitivity tests for the second and third drug because the trypanosome populations under evaluation have already undergone selection by the first drug and might not be completely representative of the original population. For example, within a heterogeneous trypanosome population one sub-population might be resistant to the first drug and sensitive to the second, and another sub-population sensitive to the first drug and resistant to the second. By the time of testing against the second drug, the first drug would have eliminated the sub-population resistant to the second drug, and the conclusion would be that the isolate was resistant to the first drug and (incorrectly) that it was sensitive to the second.

An alternative approach to the investigation of trypanocidal drug resistance is the use of field observational methods, such as longitudinal block-treatment studies. These may be conducted without the need for isolation of trypanosomes, and are therefore more appropriate for areas without laboratory facilities. Such studies are outwith the scope of this paper, and are described elsewhere (Eisler et al., 2000).

In conclusion, the standardised in vivo tests described here are intended for use in investigating drug resistance in trypanosomes infective for domestic ruminants on an area-wide basis, with a view to formulation of official drug use policies and recommendations to veterinarians, extension workers and farmers. Where drug resistance is found to be a constraint to the control of trypanosomosis, application of other strategies may be recommended, such as tsetse suppression and use of trypanotolerant cattle, either as alternatives to drug use or in combination with drug use in an integrated control programme (ICPTV, 2000). It is hoped that in future investigators will follow these standardised protocols wherever possible, to facilitate comparisons of the drugs resistance situation among geographic areas and to allow changes within areas over time to be documented.

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