gests that the group of trypanocidal drugs share some common, but not identical, pathways in their modes of action. It is too early to tell the precise number of DPs involved in isometamidium resistance. Work is in progress to analyse the DPs, and to use them in the extended analyses of trypanosome populations with defined drug resistance phenotypes.

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Area-wide appraisal of drug resistance in trypanosomes infecting cattle in East and Southern Africa

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Recent and ongoing international programmes addressing the problem of tsetse-transmitted animal trypanosomiasis, such as the DG-VIII-funded initiatives in East Africa and Southern Africa Farming in tsetse Controlled Areas (FITCA) and the Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) place great emphasis on vector control. However such programmes face major scientific, technical and socio-political challenges, and achievements in terms land area cleared or controlled have been modest. In the vast majority of countries in the affected area, control of animal trypanosomiasis relies heavily upon the use of chemotherapy and chemoprophylaxis; with the exception of Botswana and Zimbabwe, the use of trypanocidal drugs is the main method of controlling trypanosomiasis in all those countries in which the disease occurs. It can be argued that cattle only occur in many parts of Africa because of the availability of these drugs (Jordan, 1992). Evidence for the development of drug-resistant populations of trypanosomes in the field has been obtained in a number of African countries (Kupper & Wolters, 1983; Pinder & Authié, 1984; Ainanshe *et al.*, 1992; Clausen *et al.*, 1992; Codjia *et al.*, 1993, Peregrine *et al.*, this issue), and is potentially a major constraint to animal productivity and mixed crop-livestock agricultural systems over a vast area of the continent.

The extent and significance of trypanocidal drug resistance was investigated in priority areas of three East and Southern African Countries under DG-XII INCO-DC Project Number ER-BIC18CT95-006, Novel Approaches to the Epidemiology of Resistance to Drugs Used in the Control of Bovine Trypanosomiasis in East Africa. The project built on the results of two previous projects supported by DG-XII (no. TS3-CT93-240, Trypanocidal drugs: laboratory and field evaluation of novel controlled release systems, and TS2-CT880031, Animal trypanosomiasis: field and laboratory studies of drug-resistant African trypanosomes). Existing methods currently available for the demonstration of drug-resistance, namely therapeutic tests in domestic livestock and rodents, and tests on trypanosome isolates *in vitro*, have particular drawbacks (Sutherland and Holmes, 1991; Clausen *et al.*, this issue). In this project, these were supplemented with a novel approach, the use of ELISAs able to quantify the concentrations of trypanocidal drugs circulating in treated cattle (Eisler *et al.*, 1994, 1996, 1997; Eisler, 1996).

Study areas were selected in Kenya, Tanzania and Zambia on the basis of existing information on prevalence and impact of bovine trypanosomiasis. These were: Coast, Rift Valley and Western Provinces, Kenya; Coast, Tanga and Dar-es-Salaam Regions, Tanzania; and Eastern Province, Zambia. Sampling sites (total = 111) were selected at random within a designated area of each Province (Kenya and Zambia) or Region (Tanzania). Six thousand four hundred and thirty-six cattle (usually 50 per site) were examined for trypanosome infections between October 1996 and December 1998, using the haematocrit centrifugation buffy-coat technique and thick and thin Giemsa-stained blood films. Sera were collected for trypanocidal drug determination, and trypanosome stabilates were collected by mouse inoculation and in liquid nitrogen. Information on age, sex, breed, bodyweight, body condition score and history of trypanocidal drug treatment were also collected.

Trypanosome infections were detected in 823 cattle (overall infection rate 12.8%), with *Trypanosoma brucei*, *T. congolense* and *T. vivax* infection rates of 0.7%, 9.3% and 3.5% respectively. One hundred and eighty-six *T. congolense* stabilates (Kenya 52, Tanzania 63, Zambia 71) were tested for sensitivity to isometamidium chloride and dimi-

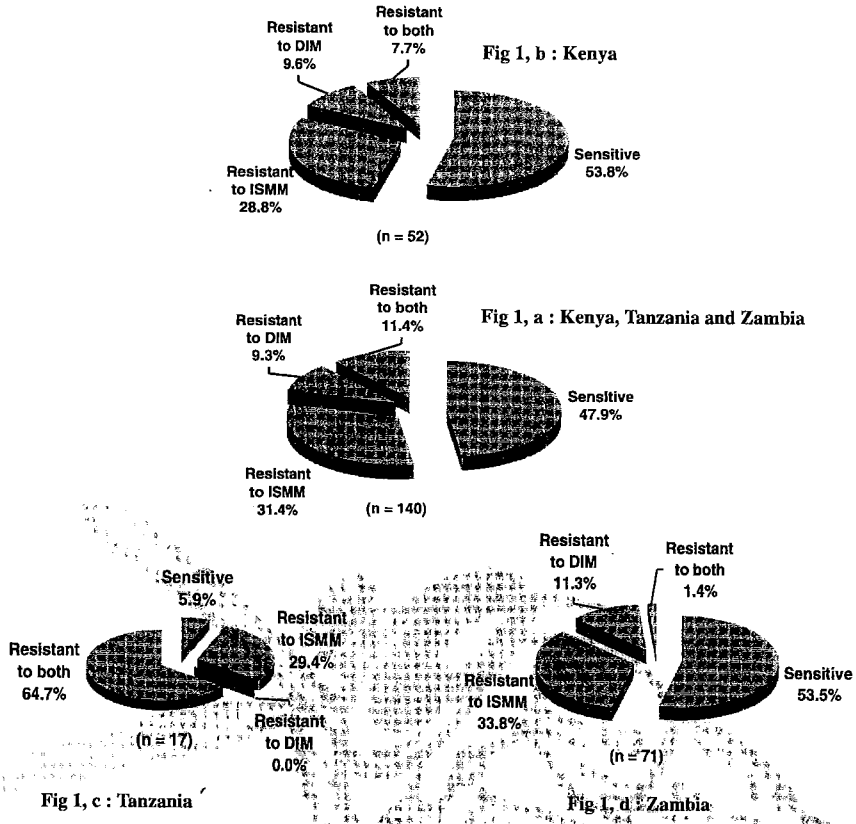

Figure 1. *Percentages of* Trypanosoma congolense *stabilates sensitive or resistant to trypanocidal drugs on the basis of tests in mice using discriminatory doses of 1.0 mg/kg b.w isometamidium chloride (ISMM) and 20 mg/kg b.w. diminazene aceturate (DIM). (a) Kenya, Tanzania and Zambia (b) Kenya, (c) Tanzania (pools of up to 5 stabilates; all stabilates comprising a pool were from a single site) and (d) Zambia.*

nazene aceturate in mice, using discriminatory doses of 1.0 mg/kg bodyweight (bw) isometamidium chloride and 20 mg/kg bw diminazene aceturate (Geerts *et al.*, this issue). Stabilates from Tanzania were pooled prior to testing; seventeen pools of up to 5 stabilates of *T. congolense* from six sites (5 sites in Tanga Region and 1 site in Coast Region) were tested for sensitivity to isometamidium chloride and diminazene aceturate in mice, using the same discriminatory doses. All stabilates comprising a pool were from a single site. Hence a total of 140 individual stabilates or pools were tested, of which 47.9% were sensitive to both drugs, 31.4% were resistant to only isometamidium, 9.3% were resistant to only diminazene, and 11.4% were resistant to both drugs (Fig 1, a). In total, 42.9% were resistant to isometamidium and 20.7% were resistant to diminazene.

In both Kenya and Zambia, about 46% of *T. congolense* stabilates were resistant to one or other of the two trypanocides tested, and in both countries over 35% of stabilates were resistant to isometamidium. In Kenya there was slightly more diminazene resistance (17.3% of *T. congolense* stabilates) than in Zambia (12.7%), and considerably more evidence of multiple resistance, with 7.7% and 1.4% of stabilates resistant to both drugs in the two countries respectively (Fig. 1, b and c). Multiple resistance appeared to increase across Kenya from West to East, and was observed in 0% of stabilates from Western Province, in 8.7% of stabilates from Rift Valley Province, and in 14.3% from Coast Province. Longitudinal studies conducted in Kwale District, Coast Province, Kenya revealed multiple resistance in 85% of an additional 34 *T. congolense* stabilates (Mdachi, 1999).

Of the seventeen pools of up to 5 stabilates of *T. congolense* isolated in Tanzania and tested for sensitivity to isometamidium chloride and diminazene aceturate in mice, only 1 pool (5.9%) was sensitive to both drugs, 5 pools (29.4%) were resistant to isometamidium only, none was resistant to diminazene only, and 11 pools (64.7%) were resistant to both drugs (Fig 1, d). These results indicated the presence of resistance to isometamidium at five sites, and resistance to both isometamidium and diminazene at three of the sites.

Analysis of sera (n = 6247) of cattle from these three countries using isometamidium-ELISA showed isometamidium usage to be highly variable within regions. Overall the highest usage rates were observed in Tanzania, where the drug was detectable in 15% of cattle, and the lowest in Zambia (4.1% of cattle). In Kenya (overall detection rate 5.9%), higher isometamidium usage was observed in Coast (6.8%) and Rift Valley Provinces (8.9%) than in Western Province (3.6%). These patterns broadly corresponded with levels of isometamidium resistance found in *T. congolense*.

Attempts to characterize drug sensitivity of *T. vivax* in calves met with limited success. This was mainly due to poor viability of stabilates prepared directly in the field. However, the isometamidium-ELISA provided evidence of resistance to this trypanocide in *T. vivax*. When 222 sera from *T. vivax*-infected cattle were tested in the ELISA, isometamidium was detected in 28 (12.6%). These 28 were either from Tanzania, from where 20.7% of 82 sera from *T. vivax*-infected cattle were positive for isometamidium, or from Kenya from where the equivalent percentage was 8.1% of 136 sera from infected cattle. *Trypanosoma vivax* was rare among Zambian cattle, and was found in only 4 of the 1597 cattle examined. Isometamidium was detected in none of the sera of these 4 cattle.

In conclusion, the work described here has shown trypanocidal drug resistance to be widespread but variable in the area investigated. The problem was shown to be particularly severe in coastal regions of Kenya and Tanzania, where multiple drug resistance was common. Elsewhere, for example Western Kenya, and Eastern Zambia, while there was some evidence of dug resistance, there was no evidence of multiple drug resistance, and use of the principal of the sanative pair (Whiteside, 1958) could be expected to be effective in controlling resistant infections. Finally, 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 sub-Saharan Africa where reliance is placed on drugs to control tsetse transmitted bovine trypanosomiasis.

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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

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Historical Background

Livestock production is of great importance in the southern Guinean zone of West Africa, particularly in northern Côte dIvoire, southern Mali and southern Burkina Faso. Cattle contribute meat, milk and traction power. Livestock trading is also an important economic activity, as the region is a source and transit area for cattle moving to the humid coastal areas.

Kénédougou province of Burkina Faso borders Mali and is in close proximity to Côte dIvoire. The government of Burkina Faso has prioritised increasing livestock production in the province. In the late 1970s and early 1980s, group ranches were established in the central Samorogouan zone of the province. Trypanosomosis proved to be a major constraint to cattle production in this zone. Prevalences of trypanosome infections in cattle of 20% and greater were usual and trypanocides were widely used (representing approximately 70% of all veterinary drug expenditures). Drug resistance, both single and multiple, became an increasing problem (Authié, 1984; Pinder and Authié, 1984, Clausen *et al.*, 1992), such that trypanosomosis in cattle in Samorogouan was often unaffected by some trypanocides administered at maximum dose rates. In these areas, alternative control measures, particularly pour-ons, have been used with success (Bauer *et al.*, 1995). In other zones of Kénédougou province, resistance to trypanocidal drugs appears to have been less marked and variable, both spatially and temporarily. These variations in drug resistance levels provided an opportunity to investigate factors that influence the development of drug resistance and its impact in the field.

Field studies

To investigate the importance of trypanosomosis, resistance to trypanocidal drugs and the potential impact of integrated control measures on these factors, a 3-phase study was initiated. The first phase was a cross-sectional study in which tsetse apparent density, cattle trypanosome prevalence, trypanocidal drug use and livestock husbandry data were collected from 45 (of 166) randomly-sampled villages in all 4 livestock production systems in Kénédougou province. This study was conducted from mid-June to mid-August 1998. The second phase studied the occurrence of trypanocidal drug resistance in villages with high trypanosomosis risk (10% trypanosome prevalence). In 10 such villages, cattle were block treated with isometamidium at 1 mg/kg bodyweight (bw) and followed at 2-weekly intervals for trypanosome infections by the buffy-coat phase-contrast technique. Trypanosomes isolated from cattle both before and after the block treatment were assessed for resistance to isometamidium and diminazene in mice. This study was conducted from mid-November 1998 to mid-February 1999. A third phase will investigate variations in livestock production parameters associated with different drug-resistance levels. This paper reports on the results from the first two phases.

Tsetse challenge and trypanosomosis risk

The four livestock production system zones on which the sampling was stratified (see Figure 1) had varied tsetse distribution and density. The northern zone (NDorola) is driest. Cotton is the main agricultural activity and livestock production is of mixed pastoral / agro-