R5955CB – Field testing ELISA to help control trypanosomiasis*
*Field evaluation and application of recently developed immunoassay techniques for improving chemoprophylactic and chemotherapeutic strategies in the control of African bovine trypanosomiasis

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Executive summary

- Bovine trypanosomiasis in sub-Saharan Africa is a major constraint to livestock productivity and poverty alleviation as some 40 million cattle are kept in tsetse infested areas.
- Trypanocidal drugs remain the primary means of trypanosomiasis control for farmers.
- Until recently, no accurate way to measure trypanocidal drug levels in animals existed to help optimise drug use. Improved drug use would save farmers scarce cash and minimise environmental damage.
- The project successfully developed and field-tested trypanocidal drug–ELISAs (enzyme-linked immunosorbent assays) in Kenya, Tanzania and Zambia. The technology was transferred and is now well established in these three countries. Local personnel were trained to conduct assays and completed over 10,000 determinations (iterations of assays).
- Targeted, user-friendly software was developed to conduct quality-assurance analyses and data collation. This information, along with data on parasitology and productivity, was put into a database.
- The technology – and the skills needed to use it – will enable local researchers to collect information to advise farmers on appropriate drug use.

Project dates: January 1994 – March 1997

Background

Increased livestock productivity plays a key role in poverty alleviation, economic growth and environmental stability in developing countries. It does this through enhanced food production, income generation and by assisting cropping systems. Bovine trypanosomiasis, on the other hand, is
the major animal disease constraint to agricultural production in sub-Saharan Africa. Over 40 million of Africa’s cattle population are kept in tsetse infested areas.

Trypanocidal drugs remain the mainstay of its control, either as a treatment (chemotherapy) or to prevent disease (chemoprophylaxis). This will probably remain the case as drugs are the only method of control readily available to individual farmers. Other strategies, such as vector control, tend to rely on government or community involvement which is difficult to sustain.

As no new drugs are available or likely to appear due to high development costs, ensuring the optimal use of existing trypanocidal drugs is important. This is not without difficulty. Until recently, it has been difficult to measure the levels of trypanocidal drugs in animals. Evaluation of factors influencing the duration of chemoprophylaxis is dependent upon knowledge of drug concentrations present in treated animals. Hence, chemotherapeutic or chemoprophylactic strategies have been based on experience rather than scientific fact and against fears of possible drug resistance.

Evidence of drug-resistant populations of trypanosomes has been obtained in the field in a number of countries. But, proving drug resistance is generally difficult in Africa. This project provided a novel approach for demonstrating drug-resistance, namely the use of ELISAs (enzyme-linked immunosorbent assays) able to quantify the concentrations of trypanocidal drugs circulating in treated cattle.

Objectives
The project aimed to transfer ELISA techniques developed in the University of Glasgow to African Institutes currently investigating and controlling tsetse-transmitted bovine trypanosomiasis. Specific aims included:

- Evaluating trypanocidal drug ELISAs under field conditions in selected African sites to provide a method of evaluating existing drug regimens and provide a basis for rationalising chemoprophylactic and chemotherapeutic regimens.
- Providing methods for measuring levels of trypanocidal drugs in cattle serum at certain African Veterinary Laboratories.
- Obtaining measurements of trypanocidal drugs levels in the serum of treated cattle under various conditions of field tsetse challenge.
- Obtaining data on the prevalence of drug resistant trypanosomes in the presence of levels of drug that would normally be protective.
• Investigating the correlation between chemotherapeutic or chemoprophylactic strategy, blood levels of drug, and susceptibility to infection.
• Provide recommendations for the improvement of trypanosomiasis control strategies based on the drug assay results.
• Develop computer software for data management and analysis of trypanocidal drug ELISA results in African laboratories.
• Liaise with the joint United Nations Food and Agriculture Organisation (FAO)/IAEA Agriculture Laboratory for the standardisation of trypanocidal drug ELISA reagents as well as their incorporation into kits to ensure future availability of this technology.
• Training counterpart staff in the use of drug ELISAs and the calculation and analysis of results.

**Highlights**

The project achieved the greater part of its objectives using laboratory and field studies. Being quantitative, the trypanocidal drug ELISA technique was more technically demanding than similar assays used for animal disease diagnosis. A series of calibration standards and quality control samples, for example, are included on every ELISA plate. Satisfactory results must be obtained for these controls before results for field samples may be interpreted.

Despite these demands, trypanocidal drug ELISAs are now well established in Kenya, Tanzania and Zambia. Personnel have been trained to conduct assays with a high degree of proficiency. Over the project period, some 10,000 determinations were conducted. These achievements have been aided by the development of IBM compatible, user-friendly software to conduct quality-assurance analyses and data collation. Further, these data and data on parasitology and productivity are included in a database.

Activities were conducted in the three aforementioned countries. Studies were done on a commercial beef cattle ranch in a semi-arid zone of Kenya, and in the coastal zone of Tanzania. These suggested that blood levels of the prophylactic drug isometamidium (Samorin/Trypamidium) were relatively consistent and associated with relatively predictable periods of prophylaxis. However, climatic conditions and level of tsetse challenge appeared to cause variations in the prophylactic period from year to year. Drug resistance seemed to be disproportionately more severe during seasons of relatively heavy challenge.

Other studies were completed on small-holder dairy cattle of imported and mixed breeding in Kenya’s Coast Province, and Zebu cattle in Eastern Province, Zambia. These showed similarly consistent drug levels where treatments were administered by skilled and qualified personnel. However, where poorly qualified individuals administered treatments, much wider variations in blood levels and in resulting periods of prophylaxis were observed. This is increasingly commonplace due to the
privatisation of veterinary services. Clear evidence of under dosing was also obtained in certain situations.

There are many determinants of whether animals exposed to tsetse challenge become infected. It appears difficult to correlate the isometamidium concentration in the circulation of an individual animal with the probability of it becoming infected. However, drug levels are clearly related to the overall number of infections expected in a population. It is therefore advisable to conduct determinations on a herd basis, an application to which the ELISA technology used in this project lends itself admirably.

In addition, information was obtained on drug delivery mechanisms in different situations, particularly in Zambia where a Belgian Government Project (ASVEZA - Assistance to the Veterinary Services of Zambia) created a drug distribution cost-recovery system. Precise figures are available that suggest a marked reduction in the use of prophylactic rather than curative trypanocidal drugs. Finally, in one area, evidence was obtained of inappropriate administration of isometamidium treatments where the prevalence of infection may be overestimated.

Impact
The successful development and field testing of trypanocidal drug-ELISAs and its transfer to African Institutions represents a significant step forward in the fight against Africa's most serious animal disease. The development and provision of IBM compatible software to collate and analyse results reinforces the ELISA’s value as a tool for African researchers to investigate the epidemiology and importance of trypanocidal drug resistance. The sustainability of the research findings is ensured, by both the transfer of outputs to African laboratories and the continuation of research in an EU funded project.

The project also strengthened the research capacity of collaborating African Institutes by providing equipment and training for researchers and technicians. They now have the ability to use these specific ELISA techniques to diagnose and improve control of trypanosomiasis in Africa.

Collaborators
1. Department of Agriculture of Northern Ireland
2. Centre for Tropical Veterinary Medicine, University of Edinburgh
3. Veterinary Services of Zambia (ASVEZA) and Department of Veterinary and Tsetse Control Services, Lusaka
4. Zambia Regional Tsetse and Trypanosomiasis Control Programme, ODA/DFID Insect Pest Management Initiative Project, Lusaka, Zambia
5. Animal Disease Research Institute, Dar-es-Salam. Mkwaja Ranch, Tanga Tanzania
6. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna
7. Kenya Trypanosomiasis Research Institute (KETRI), Veterinary Investigation Laboratory, National Dairy Development Programme, Kenya
8. International Livestock Research Institute, Nairobi
9. Institute of Tropical Medicine, Antwerp, University of Gent, Belgium
10. Veterinary Department, Mali

**Selected publications**

