Combating sleeping sickness in cattle and people

RIU

Validated RNRRS Output.

A safe, accurate and easy-to-use test is now available to screen for trypanosomes. Spread by tsetse fly, these tiny parasitic organisms cause serious diseases like nagana in cattle and sleeping sickness in people. Previously, screening to prevent the spread of these diseases was slow and inaccurate. Now, just one drop of blood is enough to provide the DNA needed for analysis, and this can easily be attached to a sample-collection card and posted to a laboratory for testing. The system could have a major impact on livestock and on poor producers' health and livelihoods, and is already being used in parts of Uganda, Zambia, Tanzania, Nigeria, Malawi, and Zimbabwe. But, because most people aren't aware of its benefits, this ready-to-use technique urgently needs to be promoted.

Project Ref: **AHP01:**
Topic: **2. Better Lives for Livestock Keepers: Improved Livestock & Fodder**
Lead Organisation: **Centre for Infectious Diseases, University of Edinburgh, UK**
Source: **Animal Health Programme**

Document Contents:

Description

Research into Use
NR International
Park House
Bradbourne Lane
Aylesford
Kent
ME20 6SN
UK

Geographical regions included:

Malawi, Nigeria, Tanzania, Uganda, Zambia, Zimbabwe,

Target Audiences for this content:

Livestock farmers,

AHP01

A. Description of the research output(s)

1. Working title of output or cluster of outputs.
In addition, you are free to suggest a shorter more imaginative working title/acronym of 20 words or less.

Diagnostics for identification and differentiation of African trypanosomes

2. Name of relevant RNRRS Programme(s) commissioning supporting research and also indicate other funding sources, if applicable.

DFID Animal Health Programme, Cunningham Trust, World Health Organisation, The Leverhulme Trust.

3. Provide relevant R numbers (and/or programme development/dissemination reference numbers covering supporting research) along with the institutional partners (with individual contact persons (if appropriate)) involved in the project activities. As with the question above, this is primarily to allow for the legacy of the RNRRS to be acknowledged during the RIUP activities.

Funding agency	***Project no.***	***Project title***
AHP	R7596	Decision support system for the control of trypanosomiasis in South-East Uganda: improving public health and livestock productivity through the cost-effective control of trypanosomiasis in livestock
AHP	R7360	Field methods and tools for resource poor farmers and extension workers to improve targeting and appropriate use of drugs for control of African bovine trypanosomiaisis
AHP	R7597	A low cost haemoglobinometer as a decision support tool for bovine disease diagnosis in sub Saharan Africa
AHP	R8318	Decision support for endemic disease control in sub-Saharan Africa – private sector drivers for technology adoption for resource poor farmers.
AHP/LPP	R6559	Preliminary study of the effects of host physiology on the efficacy of cattle as baits for tsetse control.
AHP	R7173	Cattle management practices in tsetse-affected areas.
AHP	R7364	Improving the control of tsetse: The use of DNA profiling to establish the feeding responses of tsetse to cattle.
LPP/AHP	R7539	Environmental risks of insecticide-treated cattle in SA livestock systems.
AHP	R8214	Integrated vector management: controlling malaria and trypanosomiasis with insecticide-treated cattle.

LPP	ZC0254	General model for predicting the effect of insecticide-treated cattle on tsetse populations.

Project Partners (contact person):

1. Centre for Infectious Diseases, The University of Edinburgh, UK (Professor Sue Welburn; sue.welburn@ed.ac.uk; Dr Kim Picozzi; kim.picozzi@ed.ac.uk)
2. Faculty of Veterinary Medicine, Makerere University, Kampala Uganda (Professor John David Kabasa; kabasajd@vetmed.mak.ac.ug; Dr Charles Waiswa cwaiswa@vetmed.mak.ac.ug)
4. Department of Biochemistry, University of Cambridge (Dr Mark Carrington; mc115@mole.bio.cam.ac.uk)
5. London School of Hygiene and Tropical Medicine, London, UK (Dr. P Coleman; paul.coleman@lshtm.ac.uk
6. BioSciences Research Institute, University of Salford, UK (Professor Geoff Hide; G.Hide@salford.ac.uk)
7. Coordinating Office for the Control of Trypanosomiasis in Uganda (COCTU)
(Dr Laurence Semakula; Coctu.maaif@yahoo.com)
(Freddie Kansiime; freddiekansiime@yahoo.com)
8. Ministry of Agriculture, Animal Industries and Fisheries Commissioner for Animal Health; (Dr Nicholas Kauta; nicholaskauta@yahoo.co.uk)
9. Farming in Tsetse Controlled Areas (FITCA); (Dr Simon Gould and Dr Ambrose Gidudu; fitca@utlonline.co.ug)
10. Ceva Sante Animale, Libourne, France (CEVA) (Martin Mitchell martin.mitchell@ceva.com)

4. Describe the RNRRS output or cluster of outputs being proposed and when was it produced? This requires a clear and concise description of the output(s) and the problem the output(s) aimed to address. Please incorporate and highlight (in bold) key words that would/could be used to select your output when held in a database.

The output is a sensitive, accurate, safe and convenient new **diagnostic** system enabling **identification** and **differentiation** of all pathogenic trypanosomes – which cause acute and chronic sleeping sickness, in people, **zoonotic sleeping sickness** and **nagana** in cattle.

The system was developed during 2000-2005 and involved two stages: adaptation of a **commercially** available **sample collection** and storage media (Whatman FTA® cards) and development of a suite of bespoke molecular tools based on specific **DNA-probes**[1,2,3,4,5] It can be used for routine diagnosis of sleeping sickness cases in individuals[1,5] as well as for **epidemiological** investigations[6,7], large-scale cattle screening operations[8,9,10]and **monitoring** and **evaluation** of **control programmes**[11].

It represents a major improvement over previously available methods. Cattle and **wildlife** can act as asymptomatic carriers of *T. brucei rhodesiense,* which can be transmitted via the bite of a **tsetse** fly to people, who then develop sleeping sickness[12,13,14]. The **human-infective** parasites are morphologically indistinguishable from non-human infective *T. brucei brucei*, which also infects cattle and wildlife[1,15,16].

Microscopic examination of blood smears, the classical approach, is of low sensitivity, labour and infrastructure heavy, and cannot differentiate between human-infective and non-infective parasites[4,1]. The new system is 50-times more sensitive. Injection of fresh blood samples into mice can amplify the number of parasites in a sample,

making it easier to detect parasites by microscopic examination, but this is inconvenient, costly, takes several days to yield a result and raises animal welfare issues – and many samples fail to develop in mice.

The new system involves collection of a single drop of blood (or tissue) on an FTA® card, which captures and stabilises any DNA (including that from parasites) in the sample and inactivates potentially dangerous pathogens. The cards can thereafter be safely handled, stored at room temperature, and can even be sent through the post. In the laboratory, a small punch is used to obtain a disc from the card containing the sample. This is then tested using DNA-probes identify the trypanosome species present in the sample, highly accurate and very sensitive. Multiple testing of a single sample is possible because only a tiny amount of material is required for each test.

The cards can also be used to detect a wide range of other diseases using the DNA captured on the card, either at the same time or up to several years later: this allows, for example, retrospective baselines to be generated at little cost using these **archived bio-bank** materials.

5. What is the type of output(s) being described here?
Please tick one or more of the following options.

Product	***Technology***	***Service***	***Process or Methodology***	***Policy***	***Other Please specify***
	x		*x*		

6. What is the main commodity (ies) upon which the output(s) focussed? Could this output be applied to other commodities, if so, please comment

The output is primarily focused on livestock and human public health.

The diagnostic system was developed to (a) detect human-infective sleeping sickness parasites in samples obtained from cattle, other livestock and wildlife (to differentiate human-infective *T.b.rhodesiense* from non human-infective *T.b.brucei*) and to (b) identify the burden of pathogenic trypanosomiasis (nagana) carried by local livestock (*T. vivax, T.congolenese*).

A similar approach could be used to detect a wide range of pathogens in samples obtained from livestock: archived materials applied to the platform technology can further be examined for other **babesia**, **theileria**, **anaplasma**, etc.

It could also be used to detect pathogens in other sectors, such as fisheries, and is also applicable in the human health sector.

7. What production system(s) does/could the output(s) focus upon?
Please tick one or more of the following options. Leave blank if not applicable

Semi-Arid	***High potential***	***Hillsides***	***Forest-Agriculture***	***Peri-urban***	***Land water***	***Tropical moist forest***	***Cross-cutting***
x	*x*	*x*	*x*	*x*			*x*

8. What farming system(s) does the output(s) focus upon?

Smallholder rainfed humid	***Irrigated***	***Wetland rice based***	***Smallholder rainfed highland***	***Smallholder rainfed dry/cold***	***Dualistic***	***Coastal artisanal fishing***
x				*x*	*x*	

9. How could value be added to the output or additional constraints faced by poor people addressed by clustering this output with research outputs from other sources (RNRRS and non RNRRS)?

The diagnostic approach developed for sleeping sickness and nagana, i.e. sample collection using FTA® cards and subsequent testing using specific DNA-probes, could be applied to the diagnosis, monitoring and surveillance of a wide range of diseases of livestock and people, including the following from the Animal Health Programme for which RiUP proformas are being prepared: rabies, TB/brucellosis, integrated tsetse control, East Coast fever, nematodes.

The approach might also be applicable to:
Livestock Production Programme: tannins to reduce parasitic infections.
Post Harvest Fisheries Research Programme: development of a polymerase chain reaction method for the rapid and highly sensitive detection of aquatic vibrios.

Validation

B. Validation of the research output(s)

*10**. How** were the output(s) validated and **who** validated them?*

Please provide brief description of method(s) used and consider application, replication, adaptation and/or adoption in the context of any partner organisation and user groups involved. In addressing the "who" component detail which group(s) did the validation e.g. end users, intermediary organisation, government department, aid organisation, private company etc. This section should also be used to detail, if applicable, to which social group, gender, income category the validation was applied and any increases in productivity observed during..

The diagnostic system has been rigorously tested under field and laboratory conditions. These tests have been described in peer-reviewed papers published in appropriate and respected journals.

The diagnostic tools were initially validated in the laboratory at the University of Edinburgh against a panel of well characterised stocks of human-infective and non human-infective trypanosomes species. This demonstrated that the tools were species specific and highly sensitive.

Validation trails showed that microscopy consistently underestimates point prevalence of *T. brucei* infections in

cattle by between 10- and 80-fold. In a trial in Iganga, Uganda, microscopy gave a prevalence rate of 0.2% for T. brucei: the new systems gave a prevalence rate of 8.1%. Similarly in Kamuli, Uganda, microscopy underestimated prevalence rates by a factor of 10.

Using the new sensitive diagnostic for *Tb,rhodesiense*, it was shown that the animal reservoir was underestimated by a factor of 18.

The system was validated in the field in Uganda in collaboration with the Ugandan Ministry of Agriculture, Animal Industries and Fisheries (MAIFF), Makerere University, Livestock Health Research Institute (LIRI), Farming in Tsetse Controlled Areas (EU-FITCA) and the Coordinating Office for Control of Trypanosomiasis in Uganda (COCTU).

FITCA carried out block treatment operations in 2002/3 in which 65,400 cattle in specific sub-counties were treated with trypanocidal drugs in Tororo, Iganga, Kamulu and Soroti districts (15% coverage per district). The project used the molecular tools to monitor the operation's impact.

*11. **Where and when** have the output(s) been validated?*

Please indicate the places(s) and country(ies), any particular social group targeted and also indicate in which production system and farming system, using the options provided in questions 7 and 8 respectively, above..

The diagnostic tools have been validated in laboratory trials conducted at the University of Edinburgh and in field trials conducted in Uganda, between the years 2000-2006. All validation published in peer refereed journals.

Current Situation

C. Current situation

*12. **How and by whom** are the outputs currently being used? Please give a brief description.*

The diagnostic system has been used to provide answers and supply information that was previously difficult or impossible to obtain:

It has been used by researcher from Uganda and the University of Edinburgh to demonstrate that movement of *T. b rhodesiense* infected cattle from sleeping sickness endemic areas of Uganda, undertaken as part of a major restocking programme, was responsible for introducing Rhodesian sleeping sickness to six districts which were previously disease-free.

The diagnostic system is currently being used to guide the planning and implementation, and will shortly be used to monitor and evaluate, a large-scale campaign – Stamp Out Sleeping Sickness (SOS) - to control sleeping sickness through the treatment of cattle in eastern Uganda. Its use enabled samples to be collected in the field

which were then rapidly analysed in the laboratory to produce up-to date maps that clearly showed the geographical spread of the parasite in cattle populations as well as its prevalence. Following the intervention phase of the campaign the system will be used to monitor and evaluate its effectiveness.

*13. **Where** are the outputs currently being used? As with Question 11 please indicate place(s) and countries where the outputs are being used.*

The diagnostic system is currently being used in the Ugandan districts of Kabaramido, Dokolo, Amolitar, Lira, Apac and Soroti; in Eastern Province Zambia; in Serengeti ecosystem and sleeping sickness foci Tanzania; Jos plateau, Nigeria, Malawi, Zimbabwe.

14. What is the scale of current use? Indicating how quickly use was established and whether usage is still spreading.

The diagnostic system is currently being most intensively used in six districts of Uganda to underpin the large-scale SOS campaign. Several thousand samples will be collected from cattle on to FTA cards, which will subsequently be tested in the laboratory using species-specific diagnostic probes as part of the campaigns monitoring and evaluation programme.

15. In your experience what programmes, platforms, policy, institutional structures exist that have assisted with the promotion and/or adoption of the output(s) proposed here and in terms of capacity strengthening what do you see as the key facts of success?

The diagnostic system has been rapidly adopted in Uganda because it represents a significant improvement on alternative approaches and methods, is simple and convenient to use to collect samples in the field and, uniquely, enables accurate and sensitive detection of *T.b.rhodesiense* parasites in samples. Recognition that sleeping sickness is a priority disease in the country, and the on-going intervention campaign to prevent and reverse the spread of *T.b.rhodesiense* sleeping sickness in Uganda, have both enhanced the speed of uptake.

Capacity building is required to increase awareness of the system amongst those with responsibility for screening for *T.b.rhodesiense* sleeping sickness and to demonstrate its advantages over alternative approaches. At the level of sample collection, this will involve simple training programmes for field workers and their line managers. To enable national and regional laboratories to analyse the samples using DNA-probes will require training in the method. Where the necessary equipment, resources and facilities do not exist, the laboratories will also need to supplied, including hardware, reagents and training.

Current Promotion

D. Current promotion/uptake pathways

*16**. Where** is promotion currently taking place? Please indicate for each country specified detail what promotion is taking place, by whom and indicate the scale of current promotion.*

The diagnostic system is currently being promoted for sample analysis in Uganda, Tanzania and Zambia.

17. What are the current barriers preventing or slowing the adoption of the output(s)? Cover here institutional issues, those relating to policy, marketing, infrastructure, social exclusion etc.

Uptake of the diagnostic system for *T.b.rhodesiense* sleeping sickness is constrained by lack of awareness of the benefits the system offers, lack of experience of the laboratory methods and lack of the necessary equipment, resources and facilities. To operate the system will require access to reagents for which adequate funds will be needed. Technology needs to be transferred to suitable centres in Uganda, Tanzania, Malawi, S.Sudan, Zambia for research and surveillance activities and operations.

18. What changes are needed to remove/reduce these barriers to adoption? This section could be used to identify perceived capacity related issues.

Awareness of the benefits of the diagnostic system need to be raised in countries where sleeping sickness, zoonotic trypansomiasis and nagana occur. This is necessary at all levels including policy makers, those responsible for disease control, surveillance and monitoring, front-line animal and human health staff and laboratory staff. This will entail demonstrating that the new approach offers significant benefits over the traditional approach, which depended on visual identification of parasites in blood or other samples using microscopy.

The traditional approach depended on human resource, including medical, veterinary, and entomological field teams; microscopes, generators, and vehicles; and institutional infrastructure (allowances, staffing). But it undoubtedly underestimated the risk posed to man from domestic livestock reservoirs of *T. b. rhodesiense*. Poor quality of data, derived from an inefficient and insensitive and diagnostic system would impact on the reliability of disease surveillance programmes.

Following-up on awareness raising, training will be required to equip field and laboratory workers with the necessary skills to implement the system.

Setting up national centres or regional hubs where the testing of samples can be undertaken would facilitate adoption in country and be advantageous. The stability and safety of the sample collection system, using the FTA® cards, and the ability to transport them by post would makes regional laboratories an attractive prospect offering cost-savings and enhancing regional and inter-country cooperation and capacity building.

19. What lessons have you learnt about the best ways to get the outputs used by the largest number of poor people?

The diagnostic system is not used directly by poor people, rather it is instrumental in demonstrating the burden of disease in animals and in highlighting the public health implications of zoontoic disease spread among public and private animal health professionals, paraprofessionals and ancillary workers, such as community-based animal health workers, for the benefit of poor people in sleeping sickness endemic areas of Africa. Because the system is a huge improvement over previously available system and was able to answer previously unanswerable questions, the system was rapidly taken up in Uganda. The lesson learnt is once people are aware of the benefits of the system and resources and facilities are available, uptake rapidly followed.

Impacts on Poverty

E. Impacts on poverty to date

20. Where have impact studies on poverty in relation to this output or cluster of outputs taken place? This should include any formal poverty impact studies (and it is appreciated that these will not be commonplace) and any less formal studies including any poverty mapping-type or monitoring work which allow for some analysis on impact on poverty to be made. Details of any cost-benefit analyses may also be detailed at this point. Please list studies here.

It is too soon to demonstrate impact on poverty. However a system that enables effective control of an important zoonotic disease – sleeping sickness – will have a major impact on poverty and reduce the vulnerability of poor livestock dependent households.

21. Based on the evidence in the studies listed above, for each country detail how the poor have benefited from the application and/or adoption of the output(s)

It is too soon to demonstrate such impacts.

Environmental Impact

H. Environmental impact

24. What are the direct and indirect environmental benefits related to the output(s) and their outcome(s)?

The diagnostic system will have no direct environmental impacts. The system can be used to underpin and support effective control of Rhodesian sleeping sickness, which will remove a major constraint to improving the health and wellbeing of people living in endemic areas.

25. Are there any adverse environmental impacts related to the output(s) and their outcome(s)?

The diagnostic system is not expected to be associated with any adverse environmental impacts, but would facilitate the effective control of an important, unpleasant and, if untreated, invariably fatal, zoonotic disease.

26. Do the outputs increase the capacity of poor people to cope with the effects of climate change, reduce the risks of natural disasters and increase their resilience?

By facilitating the effective control of an important zoonotic disease, the overall vulnerability of people living in Rhodesian sleeping sickness endemic areas of Africa will be reduced.

Annex

References

1. Welburn, S.C, Picozzi, K., Fevre, E.M., Coleman, P.G., Odiit, M., Carrington, M. and I. Maudlin (2001). Identification of human infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-associated (*SRA*) gene. *Lancet* 358: 2017-19.
2. Cox, A., Tilley, A., McOdimba, F., Fyfe, J., Eisler, M.C., Hide, G and S.C. Welburn (2005). A PCR based assay for detection and differentiation of African trypanosome species in blood. *Experimental Parasitology* 111: 24-29.
3. Tilley, A., Welburn, S.C., Fevre, E.M., Feil, E.J., and G. Hide (2003) *Trypanosoma brucei:* Trypanosome strain typing using PCR analysis of mobile genetic elements (MGE-PCR). *Experimental Parasitology* 104 (1-2): 26-32.
4. Picozzi, K., Tilley, A., Fèvre, EM., Coleman, PG., Odiit, M., Magona, J, Eisler, M.C and S.C. Welburn (2003) The diagnosis of trypanosome infections: applications of novel technology for reducing disease risk. *African Journal of Biotechnology*. 1 (2): 39-45.
5. Picozzi, K., Fevre, E.M., Odiit, M., Carrington, M., Eisler, M.C., Maudlin, I and S.C. Welburn (2005). Sleeping sickness in Uganda: a thin line between two fatal diseases. *British Medical Journal*. Nov 26;331 (7527):1238-41
6. Coleman, P.G. and S.C. Welburn (2004) Are fitness costs associated with resistance to human serum in *Trypanosoma brucei rhodesiense*? *Trends in Parasitology* 20: 311-315.
7. Fevre, E.M. Coleman, P.G., Odiit, M.D., Magona, J., Welburn, S.C., and M.E.J. Woolhouse (2001). The origins of a new sleeping sickness outbreak (caused by *Trypanosoma brucei* infection) in eastern Uganda. *Lancet* 358: 625-628.
8. Kaare, M.T., Picozzi, K., Mlengeya, T., Fèvre, E.M., Mtambo, M.M., Mellau, L.S., Cleaveland, S and Welburn, S.C. (2007) Sleeping sickness – a re-emerging disease in the Serengeti? *Travel Medicine and Infectious Disease. In press*
9. Magona J.W., Mayende, J.S., Olaho-Mukani, W., Coleman, P.G., Jonsson, N.N., Welburn, S.C and M. C. Eisler (2003). A comparative study on the clinical, parasitological and molecular diagnosis of bovine trypanosomosis in Uganda. *Onderstepoort Journal of Veterinary Research* 70(3): 213-8.
10. Fèvre, E.M., Tilley, A., Picozzi, K., Fyfe, J., Magona, J.W., Shaw., D.J., Eisler, M.C., and S.C. Welburn. (2006) Central point sampling from cattle in livestock markets in areas of human sleeping sickness. *Acta Tropica.* 97(2):229-32.
11. Fèvre, E.M., Picozzi, K., Fyfe, J., Waiswa, C., Odiit, M., Coleman, P.G. and S.C. Welburn (2005). A burgeoning epidemic of sleeping sickness in Uganda. *Lancet* 366: 747-747.
12. Welburn, S.C and I. Maudlin (1999) Tsetse Trypanosome Interactions: Rites of Passage. *Parasitology Today* 15:399-403
13. Welburn, S.C. Coleman, P.G., Fevre, E and I. Maudlin (2001) Sleeping sickness – a tale of two diseases. Trends in Parasitology 17: 19-24.
14. Hutchinson, C., Fevre, E., Carrington, M and S.C. Welburn (2003). Farmer went to market: Lessons learnt from the re-emergence of *T. brucei rhodesiense* sleeping sickness in Uganda. *Lancet Infectious Diseases* 3(1):42-5.

15. Welburn S.C., Fevre, E.M., Odiit, M., Maudlin, I and M.C. Eisler (2006) Crisis, what crisis? Control of Rhodesian sleeping sickness. Trends in Parasitology. 22 123-8.
16. Welburn, S.C., Picozzi, K., Kaare, M., Fèvre, E.M., Coleman, P.G and T. Mlengeya (2005). Control Options for Human Sleeping Sickness in Relation to the Animal Reservoir of Disease. In Conservation and Development Interventions at the Wildlife/Livestock Interface: Implications for Wildlife, Livestock and Human Health. Eds. Osofsky, S. A., Cleaveland, S., Karesh, W. B., Kock, M. D., Nyhus, P. J., Starr, L., and A. Yang. IUCN, Gland, Switzerland and Cambridge, UK. 220 pp.
17. Odiit, M *et a*l., (2005) Quantifying the level of under-reporting of sleeping sickness cases. *Tropical Medicine International Health* 10(9): 840-9.
18. Odiit, M *et al.,* (2004) Assessing the patterns of health-seeking behaviour and awareness among sleeping-sickness patients in eastern Uganda. *Annals of Tropical Medicine and Parasitology* 98(4): 339-48.