



EASTERN AFRICA NETWORK FOR TRYPANOSOMOSIS

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# **Proceedings of the 7<sup>th</sup> Annual EANETT Conference**

**Whitesands Beach Hotel,  
Mombasa, Kenya**

**16<sup>th</sup> – 18<sup>th</sup> November, 2005**

**Trypanosomiasis Research Institute (TRC/KARI)**



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### Introduction

The seventh Annual Conference of the Eastern Africa Network for Trypanosomosis (EANETT) was held at the Whitesands Beach Hotel in Mombasa. The conference is an annual event to review scientific activities undertaken by the Network members over the past year, and to draw up strategies and workplans for the coming year. It was a great pleasure for EANETT to welcome Malawi as the fifth African member country.

The three-day workshop, which provided an excellent opportunity for interaction by scientists from different countries, was well attended, with participants from all member countries and institutions, including Sudan, Kenya, Tanzania, Uganda, Malawi, Swiss Tropical Institute, and co-opted member P. Büscher (Antwerp, Belgium) and C. Gichuki (Kenyatta University, Kenya). EANETT was also happy to welcome the WHO representatives Deborah Kioy and Faustino Maiso and also the invited scientist Prof. C. Sugimoto (Obihiro University, Hokkaido, Japan), Dr. J. Watanabe (Minatoku University, Japan) and Dr. K. Schmidt (University Innsbruck, Austria). The invited scientist presented papers on their current projects, and expressed their interest in collaborating with EANETT.

The first two days were reserved for the presentations, which were divided into seven sessions. The 29 presented papers, covered public health, surveillance, animal reservoir, tsetse control, *T. b. gambiense* animal model, diagnosis, molecular characterization of trypanosomes and drug sensitivity of trypanosomes.

On the third day, group discussions took place covering the following topics: characterization of trypanosomes, epidemiology of HAT, tsetse control & research, public health & socioeconomics and human genetics & HAT. Each group made recommendations, which were discussed and adopted in the plenary.

The presentations and discussions, as indicated in the proceedings, revealed key areas of future research under the network, and formed the basis for developments of workplans for research and training during the coming year. The importance of the network in strengthening collaboration in research, training, prevention and control of sleeping sickness was highlighted. With the invited scientists possibilities for collaborations were discussed which will be pursued in bilateral contacts.



**Scientific Programme (overview)**

|                                     |                   |  |                           |
|-------------------------------------|-------------------|--|---------------------------|
| Wednesday,<br>16 <sup>th</sup> Nov. | 09.00 – 10.00     | Opening and Welcome                            | I. ElRayah,<br>G. Murilla |
|                                     | 10.00 – 10.30     | Coffee break                                   |                           |
|                                     | 10.30 – 12.15     | Session 1 “Tsetse”                             | L. Okedi                  |
|                                     | 12.15 – 13.45     | Lunch  |                           |
|                                     | 13.45 – 15.45     | Session 2 “Epidemiology”                       | I. El Rayah               |
|                                     | 15.45 – 16.15     | Tea break                                      |                           |
|                                     | 16.15 – 17.45     | Session 3 “Public Health & Socio-economics”    | G. Murilla                |
|                                     | 19.00             | Cocktail                                       |                           |
| Thursday,<br>17 <sup>th</sup> Nov.  | 09.00 – 10.45     | Session 4 “Diagnosis”                          | P. Büscher                |
|                                     | 10.45 – 11.15     | Coffee break                                   |                           |
|                                     | 11.15 – 12.15     | Session 5 “Drug Sensitivity”                   | R. Brun                   |
|                                     | 12.15 – 13.45     | Lunch  |                           |
|                                     | 13.45 – 15.15     | Session 6 “ Characterization”                  | J. Enyaru                 |
|                                     | 15.15 – 15.45     | Tea break                                      |                           |
|                                     | 15.45 – 16.45     | Session 7 “Animal Model & Isolation”           | C. P. Otim                |
|                                     | 16.45 – 17.45     | Feedback Workshop A & B                        | R. Brun,<br>G. Murilla    |
| 19.00                               | Conference dinner |  |                           |
| Friday,<br>18 <sup>th</sup> Nov.    | 09.00 – 11.00     | Group discussions                              | I. El Rayah               |
|                                     | 11.00 – 12.30     | Reports & recommendations of discussion groups |                           |
|                                     | 12.30 – 13.00     | Closing  | I. ElRayah,<br>G. Murilla |
|                                     |                   |  |                           |

Chairman of EANETT:

Dr. Intisar ElRayah (TMRI, Sudan)

Secretary of EANETT:

Mr. Marcel Kaiser (STI, Switzerland)

Organisers of the 7<sup>th</sup> annual EANETT conference

Dr. David Mwangangi (TRC, Kenya)

Mr. Marcel Kaiser (STI, Switzerland)

Conference venue: Whitesands Beach Hotel, Mombasa



## Scientific Programme (detailed)

Wednesday, 16<sup>th</sup> November, 2005

### Opening and Welcome (09.00-10.00)

**Chair: G. Murilla & I. El Rayah**

Dr. Grace Murilla, TRC, local organizer

Dr. Intisar El Rayah, EANETT chairperson

### Coffee break (10.00 – 10.30 )

### Session 1 “Tsetse“ (10.30 – 12.15)

**Chair: L. Okedi**

**Okedi, L. M. A.,** Matovu E., Abila P. P., and Enyaru J. C. K.

A COMPARISON OF VECTOR DYNAMICS AND DISTRIBUTION PATTERNS OF *GLOSSINA F. FUSCIPES* AND ASSOCIATED RISK FACTOR FOR SLEEPING SICKNESS TRANSMISSION ACROSS SELECTED FOCI IN SOUTH –EAST AND NORTH WESTERN UGANDA

**Florence Wamwiri<sup>1</sup>,** Gamba Nkwengulila<sup>2</sup> and Peter-Henning Clausen<sup>3</sup>  
TSETSE HOST PREFERENCES IN SLEEPING SICKNESS ENDEMIC AREAS OF WESTERN KENYA

**R.E. Changasi,** G.M. Tinega, J.M. Kiragu and G.A. Murilla  
TSETSE MASS REARING AT KARI-TRC

**Okoth<sup>1</sup>, S. O,** Kokwaro<sup>2</sup>, E. D., Kiragu<sup>1</sup> J. M, Murila<sup>1</sup>, G. A.  
SUSCEPTIBILITY AND TRANSMISSION CAPACITY OF ALLOPATRIC POPULATIONS OF *GLOSSINA PALLIDIPE* TO *TRYPANOSOMA BRUCEI RHODESIENSE*

**Patrick Abila**

PRELIMINARY RESULTS OF POPULATION GENETICS OF *GLOSSINA FUSCIPES FUSCIPES* FROM SLEEPING SICKNESS FOCI IN UGANDA

### Lunch (12.15 – 13.45)

### Session 2 “Epidemiology” (13.45 – 15.45)

**Chair: I. El Rayah**

**Malele, I. I.<sup>1</sup>,** Kinughi<sup>2</sup>, J. S., Kibona, S<sup>3</sup>., L. Matemba<sup>3</sup> & H.S Nyingilili<sup>1</sup>  
TRYPANOSOME INFECTION RATES IN TSETSE FLIES AND THE RISKS OF SLEEPING SICKNESS TRANSMISSION IN AND AROUND SERENGETI NATIONAL PARK

**Matovu E.,** Nerima B., Ogweng N., Enyaru J., Akol M., Sebikali C. & Otim C.  
PRELIMINARY FOLLOW-UP RESULTS OF MELARSOPROL TREATED HAT PATIENTS AT MOYO HOSPITAL, NORTHWEST UGANDA



**Sindato, C<sup>1</sup>.**, Malele, I. I<sup>2</sup>, Mwalimu, C<sup>3</sup>, H.S Nyingilili<sup>2</sup>, S. Kaboya<sup>1</sup>. & E. Kombe<sup>4</sup>

PREVALENCE OF SLEEPING SICKNESS IN BABATI DISTRICT

**J. Swilla and G. Nkya**

SURVEILLANCE OF HUMAN AFRICAN TRYPANOSOMOSIS IN MPANDA DISTRICT, TANZANIA

**Magona, J.W<sup>1</sup>.** & Walubengo, J.<sup>1</sup>

THE ROLE OF VETERINARY MEASURES IN THE CONTROL OF RHODESIAN SLEEPING SICKNESS

**Samuel Jemu**

MALAWI REPORT

**Tea break (15.45– 16.15)**

**Session 3 “Public Health & Socio-economics” (16.15 – 17.45)**

**Chair:** G. Murilla

**Karuga JW<sup>1</sup>.**, Wangula C.<sup>1</sup> and Bukachi SA.<sup>2</sup>

FAMILIAL CLUSTERING OF SLEEPING SICKNESS PATIENTS IN KENYA

**Hosham M. Osman<sup>a</sup>;** Mubarak M. Abdel Rahman<sup>b</sup>; Yasir O Mohammed.; Intisar E. Elrayah<sup>b</sup>

THE SOCIO-ECONOMIC CHARACTERISTICS OF POPULATION UNDER SLEEPING SICKNESS RISK AND THEIR KNOWLEDGE ABOUT THE DISEASE IN BAHER EL JABEL STATE- SOUTHERN SUDAN

**Bukachi SA.<sup>1</sup>,** Wandibba S.<sup>2</sup> and Nyamongo I.<sup>2</sup>

THE BURDEN OF HAT AND ITS ECONOMIC IMPACT IN KENYA

**Junichi Watanabe,** Yutaka Suzuki, Sumio Sugano and Chihiro Sugimoto

FULL-MALARIA: FULL-LENGTH CDNA LIBRARY OF MALARIA PARASITES AND A PLAN TO APPLICATION TO TSETSE FLY FOR ITS GENOME PROJECT

**Thursday, 17<sup>th</sup> November, 2005**

**Session 4 “Diagnosis” ( 09.00 – 10.45)**

**Chair:** P. Büscher

**Enyaru<sup>1,2</sup>,** J. C. K., Balyeidusha<sup>2</sup>, A., Matovu<sup>1</sup>, E., Nerima<sup>1</sup>, B., Akol<sup>1</sup>, M. and Sebikali<sup>1</sup>, C

MOLECULAR DIFFERENTIAL DIAGNOSIS OF AFRICAN ANIMAL TRYPANOSOMOSIS IN UGANDA



<sup>1</sup>Mohammed, Y.O.; <sup>2</sup>Karamalla, L.T.; <sup>3</sup>Mohamed-Ahmed, M.M.; <sup>2</sup>El Rayah, I. E.  
EVALUATION OF *TRYPANOSOMA BRUCEI* SPECIES INFECTING  
*GLOSSINA FUSCIPES FUSCIPES* AND STOMOXYS FLIES IN THE  
SOUTHERN SUDAN USING PCR TECHNIQUE

**Mohamed T. Azrag**<sup>1</sup>; Lubna T. Karamalla <sup>4</sup>; Yassir O. Mohamed <sup>3</sup>; Mubarak  
Mustafa<sup>3</sup> ; Intisar E. Elrayah<sup>5</sup>  
IDENTIFICATION OF *TRYPANOSOMA SPP* INFECTING CATTLE BY PCR  
TECHNIQUE IN BAHR EL JABEL STATE, SOUTHERN SUDAN.

**Lubna T. Karamalla**; Mubarak M. A/Rahaman; Khalil M. Khalil; Philippe  
Buscher; Intisar E. Elrayah  
PROSPECTS OF ELISA AS A DIAGNOSTIC TOOL FOR SLEEPING  
SICKNESS IN SOUTHERN SUDAN.

Noritaka Kuboki<sup>1</sup>, Oriol M. M. Thekisoe<sup>1</sup>, Noboru Inoue<sup>1</sup>, Darunee Tutasuvan<sup>2</sup>,  
and **Chihiro Sugimoto**<sup>1,3</sup>  
LOOP-MEDIATED ISOTHERMAL AMPLIFICATION METHOD FOR THE  
DIAGNOSIS OF AFRICAN TRYPANOSOMIASIS

**Coffee break (10.45 – 11.15 )**

**Session 5 “Drug Sensitivity ” (11.15 – 12.15)**

**Chair: R. Brun**

**Naomi Maina**<sup>1</sup>, Pascal Maser<sup>2</sup>, Joseph Mathu Ndungu<sup>1</sup> and Reto Brun<sup>3</sup>  
ROLE OF DRUG RESISTANT TRYPANOSOMES IN THE HIGH  
TREATMENT FAILURE RATES IN SOUTHERN SUDAN

**J.E. Auma**, Charles Otieno and G.A. Murilla.  
SENSITIVITY OF CLINICAL ISOLATES TO ARSENICALS (MEL B AND  
MEL CY)

**Hamisi M. Malebo**<sup>1,2,3</sup>, Sauda M. Swaleh<sup>1</sup>, Ahmed Hassanali<sup>4</sup>, Isaiah O.  
Ndiege<sup>1,4</sup>, Urs Sequin<sup>3</sup> and Reto Brun<sup>2</sup>  
TRYPANOCIDAL ACTIVITY OF RARE TANZANIAN MEDICINAL  
PLANTS *ANNICKIA KUMMERIAE* AND *ACRIDOCARPUS CHLOROPTERUS*

**Lunch (12.15 – 13.45)**

**Session 6 “Characterization” (13.45 – 15.15)**

**Chair: J. Enyaru**

<sup>1</sup>**Johnson K. Kinyua**, <sup>2</sup>Edward K. Nguu, <sup>2</sup>Francis Mulaa, F and <sup>1</sup>Joseph M.  
Ndung'u  
IDENTIFICATION OF VACCINE CANDIDATES AGAINST HUMAN  
TRYPANOSOMIASIS



**Konrad Schmidt**

BRINGING HUMAN GENETICS INTO HAT RESEARCH - GENERAL RATIONALE AND A PRELIMINARY STUDY DESIGN

**Oliver Balmer**

MICROSATELLITES AND FLUORESCENCE GENES FOR TRYPANOSOME STRAIN DISTINCTION IN FIELD AND LABORATORY STUDIES

**Tea break (15.15– 15.45)**

**Session 7 “Animal Model & Isolation” (15.45 – 16.45)**

**Chair: C. P. Otim**

**J.K. Thuita<sup>1</sup>**, D. Masiga<sup>2</sup>, J.M Kagira<sup>1</sup>, D. Mwangangi<sup>1</sup>, J.M Ngotho<sup>1</sup>, E Matovu<sup>4</sup>  
J.M. and M. Turner<sup>3</sup>

DIFFERENTIAL PATHOGENICITY OF CYCLICALLY TRANSMITTED HUMAN INFECTIVE TRYPANOSOMES IN ANIMAL MODELS

**<sup>1\*</sup>Kagira J.M.**, <sup>1,3</sup>Ngotho Maina, <sup>1</sup>Maina N., <sup>2</sup>Farah I.O. and <sup>3</sup>Han J.

INFLUENCE OF TRYPANOCIDAL THERAPY ON THE HAEMATOLOGY OF VERVET MONKEYS INFECTED WITH *T.B. RHODESIENSE*

**Mwangangi D.M<sup>1</sup>**, Thuita JK<sup>1</sup>, Ngotho J.M<sup>1</sup> and Ndung’u J.M<sup>2</sup>

OPTIMIZATION OF CHEMICAL IMMUNO-SUPPRESSION PROTOCOLS IN *MASTOMYS NATALENSIS* USING CYCLOPHOSPHAMIDE (CP) FOLLOWED BY A *T.B GAMBIENSE* INFECTION

**Pati Pyana<sup>1°</sup>**, Dieudonné Mumba<sup>1°</sup>, Stomy Karhemere<sup>1°</sup>, Philippe Büscher<sup>2°</sup>

ISOLATION OF *T.B. GAMBIENSE* FROM TREATMENT REFRACTORY PATIENTS IN R.D. CONGO

**Feedback Workshop A & B (16.45 – 17.45)**

**Reto Brun**

**Grace Murilla**

**Friday, 18<sup>th</sup> November, 2005**

**Chair: I. EL Rayah**

**Group discussions (09.00 – 11.00)**

**Group discussions, finalizing (11.00 – 12.30)**

**Reports & recommendations of discussion groups**

**Final discussion and closure (12.30 – 13.00)**

**G. Murilla & I. EL Rayah**



**A COMPARISON OF VECTOR DYNAMICS AND DISTRIBUTION PATTERNS OF *GLOSSINA F. FUSCIPES* AND ASSOCIATED RISK FACTORS FOR SLEEPING SICKNESS TRANSMISSION ACROSS SELECTED FOCI IN SOUTH-EASTERN AND NORTH-WESTERN UGANDA.**

**Okedi, L. M. A.,** Matovu E., Abila P. P., and Enyaru J. C. K.  
National Livestock Research Institute, Tororo, Uganda

The gambiense belt in Uganda extends from Lake Edward-Semiliki River-Lake Albert to the West Nile region into Sudan and Congo, while the rhodesiense belt extends from Lake Victoria-River Nile-Lake Kyoga. The disease has been endemic in Uganda since before 1896 from reports that gland palpation was a method of screening migrants to curb the spread of the disease (Leak, 1998; Lester, 1939). The vector species in both foci is predominantly *Glossina f. fuscipes*, a tsetse species found on the lake and riverine shorelines and streams/swamps draining into Lakes Victoria, Kyoga and the River Nile. *G. pallidipes* and *G. m. morsitans* co-exists with *G. f. fuscipes* in SE and NW Uganda in the rhodesiense and gambiense belts of the country respectively.

Tsetse vector dynamics data were estimated from odor-baited trap catches and the associated human activities that place humans at a continuous risk of contracting infections were observed. Surveys were conducted to compare the North-western and North-eastern districts where sleeping sickness outbreak foci are presently being reported for both forms of sleeping sickness in Uganda. Reports now show that *T. b. rhodesiense* form of sleeping sickness is in areas some 200km away from the traditional gambiense belt, the closest Uganda has reported of the two foci getting close. The fly per trap per day values ranged below at 5 for Kaberamaido, Soroti, Lira, while above 6.9 for Arua, and 2.0 for Moyo. Dissections of 79 flies caught at Omugo along River Enyau caught showed infection rates of 5% with 1.26% midgut and salivary gland infections, while dissections on the 57 tsetse samples showed overall infection rates in the two sub counties of Otuboi and Bululu was 36.84% midgut, of fly also harboured a salivary gland infection, i.e. 13 midgut, 12 proboscis and one salivary gland infection were detected in just 57 flies with overall FTDs below 5. Follow-up collections of fly parts and materials for parasitological and molecular characterization across both dry and wet seasons will yield data on the question of merger of the two forms of the disease, if it is already occurring.

*G. f. fuscipes* distribution in NW in Moyo and Arua districts tended to be in the proximity of rivers or streams with low densities recorded in dry seasons. In Arua district, it was observed that midgut and salivary gland trypanosome positive infections were consistently found in tsetse flies from one trap over eight-day survey. The grid is just one kilometer away from the Omugo sleeping sickness center, where massive disease control activities are emphasized. Generally, tsetse habitats being close to water shores allows for water-related human activities for livelihoods such as water-harvesting, building and farming activities that are water-dependent, placing rural folk into direct exposure with tsetse in their refugia (resting and breeding) habitats, as in North-western Uganda. In North-Eastern Uganda, habitats that stayed bushy along swamps and Lake Kyoga shoreline that were abandoned due to insurgency now with renewed cattle movements to and from markets, and the communal herding of livestock re-entering habitats that have stayed bushy for sometime along with domestic-related activities such as farming, water harvesting, and collecting firewood, elevated fly-man contact. Awareness is being raised among affected communities and health workers in the sleeping sickness affected areas.



## APPLICATION OF AVIAN ANTIBODIES FOR DETERMINATION OF FEEDING PREFERENCES OF *GLOSSINA* IN SELECTED HUMAN AFRICAN TRYPANOSOMIASIS (HAT) ENDEMIC AREAS OF KENYA

Florence Wamwiri<sup>1</sup>, Gamba Nkwengulila<sup>2</sup> and Peter-Henning Clausen<sup>3</sup>

<sup>1</sup>Kenya Agricultural Research Centre – Trypanosomiasis Research Centre, Alupe, PO Box 399 Busia.

<sup>2</sup>Department of Zoology and Marine Biology, University of Dar-es-Salaam, Po Box 35091, Dar-es-Salaam, Tanzania.

<sup>3</sup>Institute of Parasitology and International Animal Health, Free University Berlin, Koenigschweg 67, 14163 Berlin, Germany

Knowledge of the preferred hosts of *Glossina* in any particular area is an important contributing factor in assessment of disease risk in susceptible populations. A chicken egg-yolk enzyme-linked immunosorbent assay (ELISA) was developed to investigate the host range of tsetse flies in three historical HAT foci in western Kenya, namely Ruma National Park, Mageta Island and Busia. Antibodies against eight vertebrate host groups were raised in chicken and extracted from the egg-yolk. The specificity of the antisera was increased by cross-absorption to enable distinction of blood meals obtained from different families. Regarding sensitivity, the resulting antisera was capable of identifying all blood meals from laboratory-fed flies tested at 24 and 48 hours after feeding, but only 12% of those tested after 96h post-feeding. Three hundred and seventy-one blood meals from field-collected *G. pallidipes* and *G. fuscipes* were tested, of which 55% were identified to Family level. The preferred host group for *G. fuscipes* in Mageta Island and Busia were reptiles (82%, n=33) and unspecified bovids (70%, n=81) respectively. Out of 62 *G. pallidipes* blood meals from Ruma National Park tested, 74.2% were from Bovidae, while 11.3% were of Suidae origin. Of the 28 *G. pallidipes* blood meals collected from Busia, 57.1% were from Bovidae and 21.4% from reptiles. Eight blood meals, comprising of 3 from Busia and 5 from Ruma were from non-specified primates. The results infer that, in the presence of alternative hosts especially bovids, human hosts are not an attractive host for tsetse in this region. This may account for the very low incidence of reported HAT cases here.

## TSETSE MASS REARING ACTIVITIES AT KARI-TRC

RE Changasi, GM Tinega, JM Kiragu and GA Murilla

Kenya Agricultural Research Institute-Trypanosomosis Research Centre (KARI-TRC), P.O. Box 362, Kikuyu, Kenya.

### Introduction

The Lambwe Valley of western Kenya has long been known as a source of animal and human Trypanosomosis, the sole vector of which is *Glossina pallidipes*. Repeated attempts to eradicate the vector using conventional techniques (bush clearing, traps, targets and aerial spraying with insecticides) have been unsuccessful. Joint FAO/IAEA expert mission to Lambwe Valley in 1984 recommended the use of sterile insect technique (SIT) for *G. pallidipes* eradication. In collaboration with the Kenya Government, the International Atomic Energy Agency (IAEA) is funding an SIT programme to eradicate *G. pallidipes* from Lambwe Valley. Mass rearing of tsetse is a prerequisite for SIT. So far, this collaboration has facilitated the setting up of functional and well-equipped insectaries for tsetse mass rearing at KARI-TRC. If successful, this will be the first inland SIT programme against tsetse in Africa. The initial breeding stock of *G. pallidipes* was a Ugandan strain obtained from Vienna.



#### Materials and method

*Feeding:* The colony is maintained on *in vitro* silicone membrane with bovine blood collected aseptically from TRC's herd. The flies are offered a blood meal three alternate days a week.

*Temperature and relative humidity:* Maintained at 24 °C and 70-80% relative humidity.

*Pupae collection:* The pupae is collected on daily basis into petri dishes and stored in normal insectary conditions for 25 days and then transferred into emergence cages where they emerge after 28-31 days.

*Sexing:* The flies are sexed immediately after emergence and transferred into holding cages (48 females/cage and 60 males/cage).

*Mating:* Zero day female flies are mated with ten-day-old male flies at a ratio of 4:1.

*Growth parameters:* A check for mortality rate is done once a week (Wednesday) while for fecundity and emergence rate is five days a week (Monday-Friday).

#### Results and discussion

A self-sustaining colony of *G. pallidipes* has been established. The current tsetse fly population stands at 20,000 breeding females. This population is expected to reach 40,000 by the end of this year. The overall fly population for successful SIT project in Lambwe Valley is 200,000 breeding females and is expected to be achieved by November 2007. Currently, the fecundity of the colony is 0.5-0.6, mortality rate is 1.2% and the emergence rate is 90%. Male flies for experimental/research purposes are available. An irradiation facility for blood and fly sterilisation has been established. The constraints faced include an initial high mortality due to unstable environmental conditions has been addressed

### **SUSCEPTIBILITY AND TRANSMISSION CAPACITY OF SUBPOPULATIONS OF *GLOSSINA PALLIDIPES* TO HUMAN INFECTIVE *TRYPANOSOMA BRUCEI RHODESIENSE***

**Okoth<sup>1</sup>, S. O., Kokwaro<sup>2</sup>, E. D., Kiragu<sup>1</sup> J. M, Murila<sup>1</sup>, G. A**

<sup>1</sup>Trypanosomiasis Research Centre-Kenya Agricultural Research Institute, Box 362, Kikuyu

<sup>2</sup>Department of biological Sciences, Kenyatta University, Box 43844 Nairobi

#### Introduction

Dynamics of vector-transmitted parasitic diseases depend on the vectorial capacity, which is a function of intrinsic biological characteristics of the vector (Welburn and Maudlin, 1999). Key among them is refractoriness to trypanosome establishment and fly longevity. Some tsetse subpopulations harbour intracellular endosymbiont that are thought to provide nutritional supplements to the fly and thus enhance transmission competence (Scott *et al.*, 1993; Occurrence of the endosymbionts, agglutinins and lectins vary from one tsetse population to another, and may influence vectorial capacity. In this study, entomological factors associated with transmission of the human infective *T. b. rhodesiense* by *G. pallidipes* subpopulation from a sleeping sickness focus of Busia, and a non sleeping sickness focus of Nguruman was evaluated.

#### Materials and methods

This study was carried out using *Glossina pallidipes* from Busia and Nguruman subpopulations.

Busia lies between latitude 0° 136' South and 0° North and longitudes 33° 54' east and 34° 25' 24'' East, while Nguruman lies at latitude 1° 55' S and longitude 35° 25' E on the floor of the rift valley

in southern Kenya. Flies from each area were maintained at relative humidity  $70 \pm 2\%$  and temperature  $25 \pm 1^{\circ}\text{C}$  by *in vivo* feeding from rabbit ears. The F1 pupae were collected, incubated and the emerging teneral used in the experiment. Flies were infected with a *T. b. rhodesiense* clone, KETRI 3537 *in vivo* from infectious mice. Day 25 post infection, flies were allowed to transmit infection to fresh mice then dissected. Infection status was confirmed by microscopy and PCR analysis.

#### Results and discussion

Daily survival rates of flies from the two regions did not differ significantly ( $t=0.420$ ,  $df=7$ ,  $P=0.687$ ). Mean percent survival to day 25 was also not significantly different ( $t=0.989$ ,  $df=8$ ,  $P=0.395$ ). Although the infection rates in flies were higher in the Nguruman subpopulation (58.9%;  $N=63$ ) than in Busia (47.6%;  $N=39$ ), these differences were not significant ( $\chi^2=0.387$   $df=1$ ,  $P=0.534$ ). Higher infection was detected by microscopy among the Busia subpopulation (19%) than the Nguruman subpopulation (17.9%) flies. Contrastingly, PCR detected higher proportion of trypanosome infections among the Nguruman (41.0%) than the Busia (28.6%) subpopulation flies. There was significant difference in infection load among the three parts dissected ( $F=26.167$ ;  $df=2$ ;  $P<0.001$ ). Comparison of mean infection loads between the Busia and Nguruman subpopulation groups showed no significant difference ( $\chi^2=0.646$ ,  $DF=2$ ,  $P=0.439$ ). There was no significant difference in mean PCV Busia ( $t=1.35$ ,  $df=31$ ,  $P=0.187$ ) between treated and control mice. Busia flies showed higher competence in transmission of *T. b. rhodesiense*.

### **PRELIMINARY WORK ON POPULATION GENETICS OF *GLOSSINA FUSCIPES* *FUSCIPES* IN UGANDA**

**Abila P.P.**, Enyaru J.C.K., Muwanika V., and Okedi L.M.A  
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Tsetse flies are the vectors of Human African Trypanosomiasis (HAT) and animal trypanosomiasis in Africa. Laboratory and field studies on tsetse have demonstrated; differences in behaviour and susceptibility to infection due to genetic variation. In order to understand the epidemiology of tsetse borne trypanosomiasis, vector species should be characterised accurately. The variation within and among populations can be obtained by population genetics studies which exploit methods that reveal detectable genetic variation. The DNA-PCR based approaches for population genetic analysis are available; RAPD provide a quick method of generating comparative genetic data, microsatellite DNA markers estimate genetic variation within and among populations. The present work aims at: evaluating the genetic variability within and between different *G. f. fuscipes* subpopulations

Four sleeping sickness foci were selected basing on existing foci characteristics *viz*: Epidemiological (active transmission), ecological terms (peri-domestic, riverine). Samples were drawn from disease endemic districts: Soroti, Kamuli, Iganga and Tororo. Control samples were drawn from Lira where active transmission has been reported only recently. *G. f. fuscipes* were sampled using biconical traps. The legs of the *G. f. fuscipes* samples were excised and placed in dry 1500 $\mu\text{l}$  vials and transported to the laboratory. Samples were dissected and examined for trypanosome infection. Samples collected ( $N=250$ ): Iganga ( $n=52$ ), Kamuli ( $n=62$ ), Lira ( $n=50$ ), Soroti ( $n=24$ ), and Tororo ( $n=62$ ). Flies from Iganga and Kamuli were peri-domestic, while those



from Tororo, Soroti and Lira were riparian. Only one salivary gland infection was detected from Tororo, mouthparts (Iganga [4], Lira [5], and Tororo [2]), mid-gut (Iganga [7], Kamuli [4], Lira [3], and Tororo [6]).

Genomic DNA was extracted from three legs per sample using the salt-extraction procedure. PCR to amplify DNA fragments using Pgp13 loci specific microsatellite were used for characterization of samples from Soroti. A total of 10 microsatellite primers described by Luna et al. (2001) have been screened for the analysis of population genetics. The PCR was performed in 25 $\mu$ l reaction solutions as described by Gariou-Papalexidou et al. (2000). PCR products were subjected to electrophoresis on 12% polyacrylamide gels. Ethidium bromide staining was done in order to visualise the bands. Gel image and documentation was achieved using a digital camera. Alleles were detected using AlphaDigiDocTM AD-1200 computer software. The microsatellite data generated was coded and analyzed using MSTools Excel Ad in program for: Heterozygosity and Allele frequency. Observed Heterozygosity was high (0.84615  $\pm$  0.1). Seven (7) alleles were detected. Allelic frequency for Pgp13 locus for *G. f. fuscipes* samples from Soroti district were as follows: 1(19.23%), 2(23.08%), 3 (11.54%), 4(3.85%), 5(3.85%), 6(30.77%), 7(7.69%)

The preliminary results confirm that the primer sets described from *G. palpalis gambiense* amplify *G. f. fuscipes* DNA. For more genetic analysis, PCR will be performed with dye labelled primers. ABI prism will be used for band detection and fragment size analysis. Knowledge of levels of gene flow, population isolation and variability will provide a sound basis for rational: prioritized decisions when and where to control tsetse in study areas

## **TRYPANOSOME INFECTION RATES IN TSETSE FLIES AND THE RISKS OF SLEEPING SICKNESS TRANSMISSION IN AND AROUND SERENGETI NATIONAL PARK**

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A study was carried out during the dry season in and around Serengeti National Park to assess the rate of trypanosome infection in tsetse and therefore the trypanosomiasis infection risks. Villages covered included Robanda and Makau, whereas study sites within the park were Retima Hippo Pool, Kilimafedha, Simiyu, Death Valley and Sopa. Flies were trapped using pyramidal traps and a mobile trap. A total number of 2508 tsetse flies were caught with an average of 95 tsetse flies per trap per hour. The main species of tsetse trapped included *Glossina swynnertoni*, *G. pallidipes*, *G. morsitans* and *G. brevipalpis* in the order of decreasing abundance. *G. swynnertoni* was found to be more infected than *G. pallidipes* such that it appears to play a big role in the transmission of African trypanosomiasis. 678 flies were dissected and the overall infection rate recorded was 5.9%. Trypanosomes identified by using a light microscope were as follows: *vivax* - type (5.9%), *brucei* type (2.5%) and *congolense* type (0.9%), in the order of decreasing prevalence. Only tsetse caught from Robanda, Kilimafedha, Death Valley and Makau had the *brucei*- type of infection. The prevalence of *brucei* – type was 1.7% within the park and this was recorded from flies trapped at Kilimafedha and Death Valley whereas outside the park (Robanda and Makau) the prevalence of *brucei* - type was 1.6% and 5.9% respectively. Although the two settlements are outside the park, they share the same ecosystem with Serengeti National park. The relatively high density of vectors and the infection rates in trapped flies, is an indication that the transmission risks (both outside and within SNP) of both animal and human trypanosomiasis can not be ignored

Similar survey will be conducted during the wet season, followed by analysis of FTA collected samples to distinguish between human and non human infective trypanosomes of *brucei* - type of infection. Continued deployment of insecticide-impregnated targets was recommended in order to reduce the fly population, with an ultimate aim of minimizing the risk of sleeping sickness transmission in and around the park.

## **PRELIMINARY FOLLOW-UP RESULTS OF MELARSOPROL TREATED HAT PATIENTS AT MOYO HOSPITAL, NORTHWEST UGANDA**

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This work is a follow-up of previous reports that a non-functional (mutant) *Trypanosoma brucei* adenosine transporter (TbAT1) could be involved in melarsoprol treatment failure at the Omugo focus, Northwest Uganda. We have carried out surveys to investigate the occurrence of trypanosome isolates harbouring TbAT1 mutations in Moyo District of the same endemic area. Melarsoprol treatment failure in Moyo is hitherto undocumented, but is believed not to be as alarming as that reported for Omugo. The aim of this study therefore, is to compare presence of mutations in TbAT1 of isolates from Moyo with treatment outcome as well as in vitro drug

sensitivity of the parasites to melarsoprol and DFMO. Using PCR and RFLP analysis, the prevalence of TbAT1 mutants in Moyo district was shown to be 14% as compared to 30% previously reported from Omugo in Arua district. Patient follow-up will confirm if these mutants are of the clinical drug resistance phenotype, thereby generating more information on TbAT1 contribution to melarsoprol treatment outcome. Already, 10 of the 20 patients with mutant TbAT1 have confirmed as relapse cases. One of them has been successfully treated with DFMO. This points to the potential of TbAT1 as a risk factor for melarsoprol treatment failure.

## PREVALENCE OF SLEEPING SICKNESS IN BABATI DISTRICT, TANZANIA

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Human African Trypanosomiasis (HAT) or sleeping sickness is one of the most important but equally most neglected tropical infections. It is caused by a protozoan, *Trypanosoma brucei*, which is transmitted to humans through the bite of a tsetse fly (*Glossina* spp).

Babati is one of the Districts of Manyara (formerly part of Arusha region) region where HAT is endemic. The study was carried out to ascertain the presence of HAT in Tarangire National Park (located in Babati district) and the villages surrounding the park, following the quetches that, there are some USA tourists who contracted the disease when they visited the park in June 2005. The study area was Tarangire National Park and the surrounding villages. Concentration and Field's stain techniques were employed to examine the presence of trypanosomes in human blood samples. Tsetse flies were trapped, sorted out into species and dissected under light microscope to examine the presence of trypanosomes in the midguts and salivary glands. An interview was administered using interview schedule and retrospective data on HAT were sought from the In-charges of health facilities within and outside the park. Of all the blood sampled individuals (n=306), 161 (52.6%) and 145 (47.4%) were females and males respectively. The overall mean age was 24.9 years old (range=1- 67 and standard deviation (S.D) =14.8). The mean age by sex was 22.6 (range=1-60 and S.D= 12.7) and 27.4 (range = 1-67 and S.D = 16.5) in females and males respectively. All (176 from within the park and 130 from outside the park) blood sampled individuals were found negative when screened for HAT. In the entomological survey, a total number of 857 flies were trapped and 287 dissected. The overall infection rate in the salivary glands was found to be 1.4%. The following species were recorded in the order of decreasing abundance *Glossina swynnertoni* (91.6%), *G. pallidipes* (4.6%) and *G. morsitans* (3.9%). From the questionnaires it was revealed that the In-charges of the health facilities were aware of HAT disease. This study concludes that, there is no cause for alarm about HAT in the area, however vector control programs need to be scaled up and insecticide impregnated targets need to be deployed permanently around camping sites, Rangers posts and Tarangire HQ, and in the villages surrounding the park; Also deliberate efforts are needed to strengthen diagnosis capability in the health facilities in the villages surrounding the park.

Similar survey need to be extended to cover the wet season in order to ascertain the seasonal epidemiological variation of the disease. The assessment of the status of animal reservoirs is also recommended.

**Key words:** Human African Trypanosomiasis (HAT), disease, trypanosomes, tse tse flies (vector), Tarangire National Park and the villages surrounding the park.

## SURVEILLANCE OF HUMAN TRYPANOSOMIASIS IN MPANDA DISTRICT

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Human African Trypanosomiasis (HAT) is caused by flagellate protozoan parasites (*Trypanosoma brucei rhodesiense* and *T. brucei gambiense*), that are transmitted by tsetse flies (*Glossina*). In Tanzania sleeping sickness is endemic in 6 regions namely Kigoma, Arusha, Tabora, Kagera, Mbeya and Rukwa. Currently active foci exist in Tabora and Kigoma regions. The trend of HAT in Western Tanzania regions shows that it is a re-emerging public health problem. Of about 2479 people at risk in Mpanda district (Rukwa Region), 369 (15%) were actively screened for trypanosomes and malaria parasites. The active screening detected only 2 (0.6%) cases of trypanosomiasis and 71(19.7%) cases of malaria. The passive survey detected 12 cases at the two poorly equipped and staffed health facilities. Most if not all suspected HAT cases are either referred to Kaliua H/C (Tabora) or Kasulu Hospital (Kigoma) where treatment is being provided for free. These findings indicate that the capacity building in the health facilities within the tsetse zone is crucial to reverse the current compromised diagnosis and referral system which exerts a heavy burden (time and money) to the patients/caretakers. Moreover the results indicate that, in areas where the disease was considered to have been arrested the disease has erupted again and the active foci and tsetse zones are expanding; Mpanda, Urambo, Kasulu and Kibondo have been good examples of these areas. Regular medical surveillance which involves case detection and easy treatment, and vector control, is the backbone of the strategy for the active control of sleeping sickness. The government has to give more attention to sleeping sickness than what is doing at the moment. Since there are no proper regular surveys on the extent of transmission, the records of people affected are not realistic. Moreover, few skilled personnel and health centers equipped for treating the disease are the major drawbacks.

Key words: trypanosome, surveillance, sleeping sickness, foci, parasite

## THE ROLE OF VETERINARY MEASURES IN THE CONTROL OF RHODESIAN SLEEPING SICKNESS

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The latest epidemics of sleeping sickness due to *Trypanosoma brucei rhodesiense* occurring in Southeast Uganda have been going on since 1976. These epidemics are often focalised and are propagated by domestic animal reservoirs such as cattle, pigs, sheep and goats that harbour *T. brucei rhodesiense*. The most important vector for sleeping sickness in Southeast Uganda is *Glossina f. fuscipes*. Conventionally, control relies on tsetse trapping to control the vector and screening of people in the affected areas or foci to detect and treat cases. While the role of animal reservoirs has been highlighted and documented as important in the epidemiology of Rhodesian sleeping sickness, it has not been very much exploited in the control of sleeping sickness epidemics. However as examples, 1791 cattle in Buteba focus, 939 cattle and 224 pigs in Kisoko focus and 1771 cattle and 18 pigs in Osukuru focus were simultaneously treated with diminazene aceturate at a dosage of 7 mg/Kg body weight and dressed with deltamethrin pour-on (Spoton, Coopers, Harare, Zimbabwe) along the backline at 1 ml per 10 Kg body weight. Up to 14 years for Buteba focus, 12



years for Kisoko focus and 4 years for Osukuru focus have elapsed without resurgence of sleeping sickness since mass treatment of livestock to eliminate reservoir *T. b. rhodesiense* infection accompanied with simultaneously suppressing of tsetse populations with deltamethrin pour-on were carried. This has proved to be a useful emergency measure in the control of Rhodesian sleeping sickness, especially where communities in a mixed crop-livestock farming system are affected.

## FAMILIAL CLUSTERING OF SLEEPING SICKNESS PATIENTS IN KENYA

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### Introduction

In Kenya, sleeping sickness endemic foci is limited to western Kenya, characterized by sporadic outbreaks, and recrudesces. Familial clustering of the disease has been reported in history for many years. Its importance as an epidemiological property has been observed in West Africa. The foregoing has also been confirmed in Uganda but the extent to which this has been occurring in Kenya is largely unknown. This study attempted to establish the percentages of past patients who were related and the nature of such relationships. This was based on the assumption that any attempts to understand socio-cultural and economic impact of sleeping sickness needs to comprehend familial relationship which are likely to magnify the impact of the disease in the affected families. The duration between the cases was also evaluated. The objectives of this study were achieved through a review of the available sleeping sickness patients' medical records at the referral hospital and interview with key-informants.

### Materials and methods

Three hundred and twenty three medical records of SS cases were reviewed with the view of establishing the histories recorded by the medical personnel at admission and in the course of treatment. The key-informants were recruited from people likely to have more knowledge on patients such included, Medical personnel from TRC Alupe, former patients and people coming from patients' localities.

### Results

The results showed that 29% of the past patients were related. Fifty two percent were male while the rest 48% were female. Twenty five percent of those patients had more than one relative. Apatit village had the highest (40%) number of relatives, which also constituted 84% of the patients from the village. Members of nuclear family affected by the disease formed more than half (54%) of the patients and this mainly occurred during the outbreaks. The duration in between the diagnosis of one family member to another was also analyzed. The results show that most (63%) of the relatives were diagnosed within one month of infection.

### Conclusion

The study confirms the familial nature of sleeping sickness in Kenya. The disease affects families' livelihoods and more so when more than one member gets infected. There is therefore a need to compare the socio-economic impact of the disease on families with relatives and those with none. The study also confirms the importance of spot check on family members whenever a new case of sleeping sickness is reported.



## **THE SOCIO-ECONOMIC CHARACTERISTICS OF POPULATIONS UNDER SLEEPING SICKNESS RISK AND THEIR KNOWLEDGE ABOUT THE DISEASE IN BAHER EL JABEL STATE- SOUTHERN SUDAN**

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The study was conducted in Juba area (Baher Eljabel State) to explain the socio-economic characteristics of the respondents and their knowledge about Sleeping Sickness. The study involved four areas, Rejaf, Jebel Kujur, Lologo and Terkaka.

A serological surveillance was conducted in each of the selected areas. The results were tabulated according to the age group, gender, and place of residence. The internally displaced group (IDG) showed a statistically ( $P < 0.05$ ) higher sero-prevalence rate when compared with the permanent resident groups (PRG) where either disease had been or not been recorded.

The percentage of males was 64.6 % and that of females 35.4% in all areas. The educational level of the respondents in all areas was 24.95%, this indicated that approximately three out of four respondents were uneducated. The study showed that the majority of respondents in all areas were agro-pastoralists (48.2 %), housewives (20.9 %), employee (13.6 %), and the rest of respondents were unemployed, students or others. The study showed that the family size in all areas was the same, it equals 6.6 persons per family. Concerning the type of residence, the place of residence includes town, village and camps. The types of residence vary even within one locality according to the population groups and the individual occupations.

The study indicated that the knowledge about Sleeping Sickness in the four areas collectively were 62.2 %. Knowledge about the way of transmission was 26.8 %, which indicated the lack of knowledge about the disease in these areas. The study also indicated that in all areas the knowledge of Sleeping Sickness symptoms was weak (22.1 %).

**Key words:** Baher Eljabel State- Sleeping Sickness- serological surveillance- Socio economics- knowledge on HAT.

## **THE BURDEN OF HUMAN AFRICAN TRYPANOSOMIASIS AND ITS ECONOMIC IMPACT IN HOUSHOLDS IN KENYA**

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### **Introduction**

Human African Trypanosomiasis (HAT) affects the most economically productive age group of individuals; hence the economic cost to the nation as a whole is high. The disease also has considerable impact on patients' households: children, health, education and nutrition, particularly, if the patient is a wage earner. The percentage of "HAT treatment cost" financed from household savings, a key variable in national investment strategies, has a significant impact on the economic growth.



## Methods

Patients' records of former HAT patients in Kenya from 1990-2002 were reviewed and the patients followed up to establish the economic impact of the disease on their households.

## Results

The total number of patients during this period were 207 and 58% of them were in the 15-45 age group bracket. Female patients were the majority in the 0-34 age group while males were the majority (64%) in the 45 and above age group. Most (80.8%) of the patients were farmers and 75% of them reported earning an average of one to forty-nine US\$ per month. Many (90%) of the respondents used more than one method of treatment before being properly diagnosed as suffering from HAT. About 38% of the HAT respondents took more than four months before getting appropriate treatment and in the process, many (85.6%) used up to US\$48 in seeking treatment. The HAT patients took an average of five weeks in hospital while undergoing treatment. This had varied impact on the individuals and households: school drop-outs, loss of jobs, loss of property, less or no house/farm work done, inability/inadequate performance of normal duties, a lot of time taken caring for the patient, inability to provide food, lack of money for school fees, emotional effects.

## **PRODUCTION AND ANALYSES OF FULL-LENGTH cDNA LIBRARY OF TSETSE FLY**

**Junichi Watanabe**, Yutaka Suzuki, Sumio Sugano, Noboru Inoue, Masahira Hattori, Chihiro Sugimoto

As the consequence of genome sequencing, analyses of transcriptome become of great importance. It is also true that the study of expressed genes is invaluable in the organism which has a large genome size or repetitive sequences. It is the case with Tsetse fly.

We have developed a method to produce a full-length cDNA library using an oligo-capping method. It essentially replaces the cap structure of the intact mRNAs with a synthetic oligo-RNA primer and selectively clone the full-length cDNAs. We have produced libraries from various human tissues and malaria parasites. In addition, we have recently started to make libraries from *Drosophila* fly. The results have been published as DBTSS (<http://dbtss.ims.u-tokyo.ac.jp>) and Full-malaria (<http://fullmal.ims.u-tokyo.ac.jp>).

On the bases of these achievements, we plan to produce full-length cDNA libraries from various tissues of Tsetse fly, including the mid gut, the salivary glands, etc.

Once the libraries are produced, large scale 5' and 3' end-one-pass sequencing will be performed. Assemble of these sequences will produce a large number of exact gene structures, which are based on the evidence. These will provide valuable data for prediction of gene structures from the genome sequences. Comparisons with the genome sequence of Tsetse fly will reveal the exact transcription start sites of expressed genes and identify their promoter regions. Comparative biological study with *Drosophila* fly is of particular interest to elucidate the unique features of Tsetse fly, including parasitism of trypanosomes and oviparity. Furthermore, the library clones will be valuable resources for the study of this fly, including drug designing for Tsetse control, development of anti-vector vaccines and transmission blocking measures of trypanosomes. Our efforts will contribute to improve human and animal health in endemic areas of trypanosomiasis.

## MOLECULAR DIFFERENTIAL DIAGNOSIS OF AFRICAN TRYPANOSOMOSIS IN UGANDA

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The infection rates of animal trypanosomosis in Arua and Moyo districts varied from 0 in goats, to 39.6% in pigs. In each district pigs appeared to be more susceptible to trypanosome infection with infection rates of 39.6% in Arua-Omugo, 25.0% Arua-Koboko and 13.0% in Moyo-Metu. A total of 106 trypanosomal DNAs from infected domestic animals were analysed by PCR using primers based on ribosomal Internal transcribed spacer-1 region (ITS-PCR) and *Trypanosoma brucei* primers (TBR-PCR) techniques. ITS-PCR resolved only 77 (72.6%) of the 106 trypanosomal DNA samples into different trypanosome species and the 29 (27.4%) were negative by ITS-PCR, which could be due to low amounts of DNA in the extracts. However, TBR-PCR resolved the 106 trypanosome isolates in 88 *Trypanozoon*, 12 *T.vivax* and 6 *T.congolense*. In addition, 58 (89.2%) of the 65 (53 from cows and 12 from pigs) trypanosome isolates from domestic animals in *T.b.gambiense* endemic areas, north west Uganda, were positive by TBR-PCR, indicating that they were *Trypanozoon* trypanosomes while 7 (10.8%) of the trypanosome isolates were TBR-PCR negatives. The negative trypanosome isolates were later confirmed to be 2 *T.congolense* and 5 *T.vivax* using species-specific primers. Furthermore, the 31 of the 58 TBR-PCR positive trypanosomal DNA samples, so far analysed, were TgsGP-PCR negative and SRA negative, indicating that they were neither *T.b.gambiense* nor *T.b.rhodesiense*. There is probably no mixed infection of the two diseases, *T.b.gambiense* and *T.b.rhodesiense*, in domestic animals in North West Uganda. Analysis of trypanosomes derived from domestic animals in *T.b.rhodesiense* areas showed that, 79 (90.8%) of the 87 trypanosomes isolated from cattle were positive by TBR-PCR, indicating that they are *Trypanozoon* while 8 (9.2%) were negative, suggesting that they could be other trypanosome species. When subjected to SRA-PCR, 10 (11.5%) of the 87 trypanosomes isolates derived from cattle were positive, indicating that they could be *T.b.rhodesiense* circulating in cattle which is similar to the percentage of *T.b.rhodesiense* previously obtained in cattle in Serere, Soroti district. Some of the SRA-PCR negatives could be *T.b.rhodesiense* since this technique appears to miss some of the parasitologically confirmed cases of *T.b.rhodesiense* sleeping sickness which could be due to modified SRA genes or loss of the SRA genes from the expression sites in which they reside during the gene rearrangements associated with antigenic variation.

## EVALUATION OF *TRYPANOSOMA BRUCEI* SPP INFECTING *GLOSSINA FUSCIPES FUSCIPES* AND *STOMOXYS* FLIES IN THE SOUTHERN SUDAN USING PCR TECHNIQUE.

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Ethanol-fixed samples of *Glossina fuscipes fuscipes* and *Stomoxys* species were tested for trypanosome infection of *T. brucei* subspecies using PCR technique. The crude target DNA

sequences were extracted by incubating the flies in Nonidet PCR template buffer containing proteinase-K. The DNA amplification sets of conditions (optimization of PCR standard reaction conditions) were adjusted (Lubna *et al* 2005) for each pair of primers used. The primers used included TBR<sub>1-2</sub>, SRA<sub>A-E</sub> and TgsGP<sub>FOR-REV</sub>. The results of the PCR analysis showed that 46.55% of the *G. f. fuscipes* and 39.36% of the *Stomoxys* individuals were infected with *Trypanosoma brucei* trypanosomes. However, out of the infected individuals 66.67% and 29.62% of *G. f. fuscipes* and 0.00% and 100% of the *Stomoxys* flies harboring *T. b. gambiense* and *T. b. brucei*, respectively. *T. b. rhodesiense* infection was only detected in one sample of *G. f. fuscipes*. The results are discussed in relation to epidemiology and control of the current epidemics of Human African Trypanosomosis (HAT) in the Southern Sudan.

### **IDENTIFICATION OF *TRYPANOSOMA BRUCEI* SPP INFECTING CATTLE BY PCR TECHNIQUE IN BAHR EL JABEL STATE, SOUTHERN SUDAN.**

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Polymerase Chain Reaction (PCR) technique was used to identify *T. brucei* spp. trypanosomes infecting bovine to subspecies level in Juba area, Southern Sudan. The blood samples collected on filter paper from cattle were found positive for trypanosomes by using the Microhaematocrit Centrifugation Technique (HCT). Out of 43 parasitologically positive cattle only 37.5% were confirmed to be infected with *T. brucei* trypanosomes using the TBR<sub>1-2</sub> primers. However, none of these samples reacted positive for *T. b. gambiense* nor to *T. b. rhodesiense* using SRA<sub>A-B</sub> and TgsGP<sub>FOR-REV</sub> primers, respectively. The result obtained certainly reveals that cattle are infected with the Nagana causative trypanosome *T. brucei brucei* that is mainly transmitted by tsetse flies, and the cattle found in the area are prone to tsetse bites, consequently might act as reservoirs for sleeping sickness.

Key words: PCR; Trypanosomes spp., identification, cattle, Bahr El Jabel state, Southern Sudan

### **ELISA PROSPECTS AS A DIAGNOSTIC TOOL FOR SLEEPING SICKNESS IN SOUTHERN SUDAN**

**Lubna T. Karamalla**; Mubarak M. A/Rahaman; Khalil M. Khalil; Philippe Buscher; Intisar E. Elrayah

Southern Sudan has suffered a series of epidemics of Human African Trypanosomosis (HAT) since the last century. Although the Sudan lies in the interface of the geographical distribution of the sleeping sickness two types Gambian and Rhodesian, the disease epidemics have been mainly attributed to the Gambian type. Efforts aiming at controlling the disease depend on case-detection and treatment, but attainment of the people in remote areas is the main obstruction. Consequently there is need to identify additional approaches to overcome this obstacle. Considerable attention has been given to use devices that detect trypanosomal-specific antibodies. Hence in the present study

CATT and ELISA techniques were tested for specificity and sensitivity to choose the best in terms of applicability and cost-effectiveness one to survey remote areas for sleeping sickness.

An active surveillance of sleeping sickness using CATT/*T.b.gambiense* with diluted blood (CATT/DB) protocol was conducted in Juba low endemic focus, Bahr El Jebel State, and in Khartoum, Khartoum State, as non-endemic control area. The results obtained showed that only 55 out of 803 and one out of 203 individuals screened were sero-positives in Juba and Khartoum area, respectively, in contrast 6 people were confirmed parasitologically infected with the disease only in Juba area. Samples of serum and blood onto filter paper were collected during these surveys to evaluate the specificity and sensitivity of CATT/*T.b.gambiense* on diluted plasma (CATT/PL) and on eluates of dried blood on filter paper (CATT/FP); and ELISA/*T.b.gambiense* on diluted plasma (ELISA/PL) and on eluates of dried blood on filter paper (ELISA/FP).

203 samples of plasma and blood on filter paper were collected from the participants in Khartoum (non endemic area). CATT showed negative reaction with all eluates and it reacted positively only with one plasma sample. The specificity of CATT/FP and CATT/PL was estimated to be 100% and 99.5%, respectively. When ELISA test was performed on the plasma and the filter paper eluates, three different samples reacted positively. The specificity was estimated to be 98.5% for both ELISA/FP and ELISA/PL. The specificity of the tested serological techniques was not significantly ( $P>0.5$ ) different.

In the low-endemic area, 803 persons participated in the survey. Only 55 plasma and 803 blood filter paper samples were collected. 14/55 and 19/55 were considered sero-positive cases when ELISA and CATT tests were performed on the plasma. Out of these sero-positives only 6 individuals were confirmed parasitologically positive. In contrast 37/803 and 10/803 samples reacted positively when ELISA and CATT test were carried out on the eluates but only 4 persons were confirmed parasitologically positive. The specificity of ELISA/FP (95.87%) and CATT/FP (99.25%) was significantly higher ( $p<0.05$ ) than that of ELISA/PL (83.67%) and CATT/PL (73.46%). Although the highest sensitivity proportions were scored when CATT and ELISA tests were performed on the plasma (100%) rather than on the eluate (67%) samples. The use of plasma was eliminated for the difficulties faced while collecting and preserving the samples.

The cost of the serological techniques tested with the subsequent parasitological techniques used to confirm infection was calculated based on the consumables and reagents price. In the given circumstances it appeared that ELISA/FP is the most applicable. Hence the authors propose to replace CATT/FP by ELISA/FP for screening population at remote areas for *T.b. gambiense* infection.



## LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP): A NEW DNA AMPLIFICATION TECHNIQUE FOR DIAGNOSIS OF AFRICAN TRYPANOSOMOSIS

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While PCR is a method of choice for the detection of both human and animal trypanosomosis, the expense of this method negates its use as a diagnostic method for the detection of African trypanosomosis in endemic African countries. Loop-mediated isothermal amplification (LAMP) reaction is a method that amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions using only simple incubators. Here, we present our conditions for a highly sensitive, specific and easy diagnostic assay based on LAMP technology for detection of trypanosome parasites including *Trypanosoma brucei* subspecies (*Trypanosoma brucei brucei*, *T. b. gambiense* and *T. b. rhodesiense*); *T. congolense* and *T. evansi*. Our series of experiments have developed LAMP for detection of trypanosome infections in experimentally infected mice and pigs. Furthermore LAMP has been applied in epidemiological studies for animal trypanosomosis. With advantages of rapidity (amplification in 1 hour), simplicity (requiring only a water bath/heatblock), amplification at a constant temperature, ability to produce large amounts of DNA which can be visualized by naked eye due to white turbidity indicating positive amplification and amplifying blood DNA from filter papers, LAMP has the potential to replace PCR and to be utilized even by moderately trained technicians in resource poor countries.

## ROLE OF DRUG RESISTANT TRYPANOSOMES IN THE HIGH TREATMENT FAILURE RATES IN SOUTH SUDAN

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Melarsoprol, an arsenical, is the first line drug for treatment of the late stage disease of Human African Trypanosomiasis (HAT). Increased failure rates have been reported from various *T. b. gambiense* endemic areas and are thought to be due to resistant trypanosomes. Resistance of trypanosomes to melarsoprol is ascribed to reduced uptake of the drug via the P2 nucleoside transporters. In this study, the role of resistant trypanosomes in the high melarsoprol treatment failure in sleeping sickness patients in Ibba, South Sudan was investigated. Eighteen *T. b. gambiense* stocks isolated from patients admitted at Ibba MSF-F hospital were used. *In vitro*, the isolates were sensitive to melarsoprol and diamidines. The gene that codes for the P2 transporters - TbATI was amplified and all the isolates contained a full-size TbATI open reading frame. The PCR products of 10 of the isolates were directly sequenced and compared to the published sequences: *TbATI*<sup>Sensitive</sup> and *TbATI*<sup>Resistant</sup>. The TbATI sequences of the 10 isolates were more similar to *TbATI*<sup>Sensitive</sup> than to *TbATI*<sup>Resistant</sup>. All sequenced (10) isolates, however had a new coding mutation and a few new mutations were also noted in one isolate. The findings of this study imply that *T. b. gambiense* isolated from South Sudan were sensitive to melarsoprol and therefore the treatment failures reported might not be related to melarsoprol resistance. They further implies that a change over from melarsoprol to eflornithine as the first line drug for the second stage disease may not have been necessary.

Key words: *T. b. gambiense*, Sudan, Melarsoprol, *TbATI* gene

## SENSITIVITY OF *TRYPANOSOMA RHODESIENSE* ISOLATES TO ARSENICALS (MEL B AND MEL CY)

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### Introduction

The current therapies for HAT are unsatisfactory for various reasons, unacceptable toxicity, poor efficacy, and undesirable route administration. Melarsoprol, which is the main chemotherapeutic agent for late stage HAT, has limitations in that it is insoluble in water and must be given intravenously dissolved in propylene glycol, a solvent that is highly irritating to tissues.

Melarsomine -an arsenical patented under the name Cymelarsan (Mel cy) for veterinary use has been shown to be active against *T. brucei* stocks both *in vitro* and *in vivo* (Zweygarth & Kaminsky 1989). Mel Cy is a white powder that is highly water-soluble.

### Objective

To determine sensitivity of *Trypanosoma rhodesiense* isolates to Cymelarsan both *in vitro* and in mice.



#### Materials and methods

Six *T. rhodesiense* (KETRI 265, 2487, 2562, 3189, 3200 & EATRO 1152), 1 *T. brucei* (KETRI 2362) were used *in vitro* with Mel B and Mel Cy and two *T. rhodesiense* (KETRI 3189 & 3200) were tested in mice against Mel cy.

*In vitro experiments:* Bloodstream forms were seeded into 96 well plates in serial dilutions of drug ranging from 2.5µg/ml to 50ng/ml. The cultures were incubated at 37C in a humid atmosphere containing 5% CO<sub>2</sub>. After 48 hours the MIC was determined microscopically.

*In vivo experiments:* Swiss white mice weighing 20-30g were inoculated i.p. with 1 x 10<sup>5</sup> tryps/ml. Groups of 6mice were treated 24 hours after infection with a single dose of 0.3, 0.6, and 1.2-mg/kg bwt Cymelarsan. Mice were monitored every other day by wet film for 60 days. Mice were considered cured when there were no trypanosomes detected for the 60 days duration

#### Results

Mel Cy MIC values ranged between 31.6- 83.4ng/ml, whereas those of Mel B ranged between 25.2-62.7ng/ml. When tested against *T. brucei* MIC values were 60.6 and 63.4 ng/ml for Mel Cy & Mel B respectively. When compared with Mel B, Mel Cy was 0.7-1.8 more active *in vitro*. •*In vivo* results showed Mel Cy CD<sub>100</sub> for *T. rhodesiense* KETRI 3189 to be 0.3mg/kg and 1.2 mg/kg for KETRI 3200. Mice infected with either isolate were cured but dose required for isolate KETRI 3200 (0.3mg/kg) was 4 times higher that for isolate KETRI 3189 (1.2mg/kg).

#### Conclusion

Mel Cy was as effective as Mel B. The efficacy of Cymelarsan needs to be further evaluated, as this would offer an alternative to melarsoprol.

## CHARACTERIZATION OF ANTI-TSETSE AND TRYPANOSOME TRANSMISSION BLOCKING VACCINE CANDIDATES ISOLATED FROM THE MIDGUT OF *GLOSSINA PALLIDIPES*

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### Introduction

Vaccination has had limitations due to antigenic variation of the Variant Surface Glycoproteins (VSGs). As a result, efforts towards development of a vaccine based on the trypanosome antigens has not been successful. Currently, the possibility to use flagellar pocket proteins, microtubule associated proteins and congopain has been documented with positive results. However, the results are still preliminary and other approaches in vaccine development would be invaluable in search of better trypanosome and tsetse fly control. A novel approach to trypanosomiasis control is the use of anti-tsetse or transmission blocking vaccines. A recent study (Kinyua et al., 2005) indicated the potential of midgut proteins as anti-tsetse and transmission blocking vaccine candidates. Deaths and reduced larviposition of flies fed on serum from immunized rabbits was observed. The present study reports characterization of the protective midgut protein fractions as reported by Kinyua et al., 2005.

### Methods and results

Biochemical and Immunological characterization was done on fractions of midgut proteins (DET and AQ) from the tsetse fly *Glossina pallidipes* previously ascertained to have anti-tsetse and transmission blocking properties. Purified IgGs were used in an affinity chromatography for the purification of antigenic proteins. The SDS-PAGE profile of the affinity purified antigens revealed antigens of  $M_r$  12,500- 100,000 for the DET fraction while antigens of  $M_r$  14,000-85,000 were enriched for AQ fraction. Similar results were observed with western blot analysis. Some of the antigens isolated by immunoaffinity chromatography had glycoprotein moieties. An SDS-PAGE of immunoaffinity purified glycoproteins revealed glycoprotein antigens of  $M_r$  28,200, 14,100 and 12,500 for the DET fraction while a glycoprotein antigen of  $M_r$  85,000 was revealed for the AQ fraction.

Two-DET of the immunoaffinity purified antigens gave a more defined immunogenicity picture of the protein sub-units in both the DET and AQ fractions. Polypeptide spots of  $M_r$  40,000, 38,000, 32,000 and 30,000 were predominant in the DET fraction while polypeptides of  $M_r$  6,000-83,000 were enriched for the AQ fraction. Some relatively low molecular weight proteins were observed for the AQ fraction. The polypeptides could be subunits of a native protein.

An SDS-PAGE of the native DET and AQ immunoaffinity proteins revealed proteins of high molecular weight. Proteins of  $M_r$  150,000, 66,000 and 53,000 were predominant for the DET fraction while proteins of  $M_r$  160,000, 97,000 and 55,000 were predominant for the AQ fraction. The fact that numerous antigens were identified on denaturing SDS-PAGE suggests that the native proteins are composed of antigenic subunits. However, the relative contribution of the various subunits in protection could not be quantified in this study. The results of this study indicate that several polypeptides may have protective efficacy against trypanosomiasis and hence the need to carry out a study with the purified antigens either singly or in combination.

A cDNA library has been constructed for the midgut protective antigens and functional analysis is in progress.

## BRINGING HUMAN GENETICS INTO HAT RESEARCH – GENERAL RATIONALE AND A PRELIMINARY STUDY DESIGN

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The risk of becoming infected with *Trypanosoma brucei gambiense* or *rhodesiense* is subjected to various environmental risk factors (e.g. factors influencing vector densities, animal reservoirs for parasites). Nevertheless, an influence of human genetic variations on susceptibility to infection or course of disease has been shown for other vector-borne infectious diseases like malaria. Malaria is a well-known example of the impact of selective pressure from infectious diseases on the human genome, e.g. the single point mutation in the gene encoding the  $\beta$  chain of haemoglobin leading to haemoglobin S (HbS), which significantly lowers the risk for cerebral malaria in carriers of one copy of this variant. HbS has only occurred in autochthonous populations from areas with endemic malaria, raising to allele frequencies of  $> 10\%$ . Understanding of such host genetic factors associated with a disease gives insight into pathophysiologically important pathways, and consequently might help to define new treatment options, to find drug targets, or even to develop a vaccine (e.g. DARC and *Plasmodium vivax*). It might also help to identify populations at higher risks for epidemic outbreaks of an infectious disease, and allows to correct for host genetic factors when investigating other parameters associated with the disease (e.g. clinical complications, environmental risks).

In case of human African trypanosomiasis (HAT), hardly anything is known about the influence of human genetic factors on resistance or susceptibility to the disease. However, the magnitude and the lethal character of the disease and examples from animal trypanosomiasis imply, and observations of familial clustering of cases, ethnic differences in infection rates, and first studies on genes with immunomodulating functions indicate that host genetic variants are associated with HAT. Furthermore, an evolutionary link exists between the dependence of *T. brucei spp.* on receptor-mediated uptake of human host lipids, a trypanolytic factor in human host lipids (the HDL associated apoL-I), and the adaptation to the human infective strains of *T. brucei* to this deadly host environment (e.g. expressed SRA in *T. b. rhodesiense*).

To investigate for any association of human genetic factors with HAT, a cross-sectional case control study is planned. The influence of environmental factors on the rate of infections (entomological, parasitological, and socio-economic factors) has to be controlled for by separate parts of the study, thus allowing to better match cases (detected infection with *T. b. spp.*) and controls (no infection with *T. b. spp.*) for these factors prior to the genetic analyses. For the case of *T. b. gambiense*, a separate group for analysis should be formed by individuals with positive CATT, but undetectable parasite load. In the human genetic studies, frequencies of genetic variations between cases and controls will be compared, first in a candidate gene approach (e.g. IL10, TNF, apoL-I), also including host plasma lipids, and later on in a marker based genome wide association scan using state-of-the-art technology (e.g. Affymetrix 500K GeneChip®) in order to detect other genes with a strong and significant association with HAT for further analyses. Sample taking is scheduled for an area with *T. b. rhodesiense* (Western Tanzania) and with *T. b. gambiense* (Southern Sudan) during active field surveillance. For later confirmation and comparison of any results, timely proper sampling in other areas with HAT will be beneficial. Entomological, parasitological, and socio-economic analyses should be conducted in an expert network from various EANETT members. Capacity building in the field of (human) genetic analyses in a North-South but also South-South collaboration is intended. All study parts will be subjected to ethical clearance by the appropriate authorities.



## MICROSATELLITES AND FLUORESCENCE GENES FOR TRYPANOSOME STRAIN DISTINCTION IN THE FIELD AND LABORATORY STUDIES

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It has abundantly been shown that different *Trypanosoma brucei* strains (genotypes) can have very different life-histories (e.g. growth rates, virulence, drug resistance). It is also increasingly being recognized that co-infections with multiple genotypes of the same parasite species is relatively frequent and may have important consequences for the course of infection, parasite evolution and transmission dynamics. Strain distinction is therefore crucial to address many questions of importance in *T. brucei*. Methods to distinguish *T. brucei* genotypes thus far were unpractical in many situations. Specifically, genotyping of field samples was not feasible easily, because parasites needed to be cultured first. And distinction of multiple genotypes in co-infections was extremely cumbersome in experimental studies, because parasites needed to be cloned before genotyping. We have therefore developed two methods to solve these problems.

We have characterized a set of 14 microsatellite markers (Balmer, O. & Palma, C., MacLeod, A., Caccone, A. (in press): Characterization of di-, tri-, and tetranucleotide microsatellite markers with perfect repeats for *Trypanosoma brucei* and related species. *Molecular Ecology Notes*.). The great advantage of these markers is that they can be applied to small amounts of parasite DNA and hence can be applied to field samples such as single tsetse flies, without having to culture the parasites. The markers are variable in samples from all over Africa. They also amplify *T. evansi* and *T. equiperdum*. Some of these markers further amplify *T. congolense* and *T. vivax* and should therefore not be used when simultaneous infection with these trypanosomes is possible.

We have furthermore developed live fluorescence markers to allow an easy distinction of different strains in experimental studies involving multiple *T. brucei* genotypes (Balmer, O. & Tostado, C. (2006): New fluorescence markers to distinguish co-infecting *Trypanosoma brucei* strains in experimental multiple infections. *Acta Tropica* 97: 94-101.). Different strains can be transfected with fluorescence gene constructs of differing colour, thus making the strains easily distinguishable by eye or by FACS (fluorescence activated cell sorter). The constructs are expressed in all life-stages. We have shown that these transfections are stable over time in culture, in the tsetse fly and in the mouse. They allow us to simultaneously and accurately track the population growths of two *T. brucei* strains infecting a single mouse individual.



## **THE PATHOGENESIS OF FLY-TRANSMITTED *TRYPANOSOMA BRUCEI* *RHODESIENSE* INFECTIONS IN A VERVET MONKEY MODEL OF HAT**

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### Introduction

Infections in the KETRI vervet monkey model of HAT are initiated through syringe inoculation of bloodstream trypanosomes. Tsetse transmitted infections would, however, mimic the natural mode of infection of humans and expectedly produce a better disease model

### Objective

To evaluate the pathogenesis of tsetse transmitted *T.b. rhodesiense* clones in vervet monkeys.

### Materials and methods

We carried out pathogenesis studies of fly (*Glossina pallidipes*) transmitted *T.b. rhodesiense* clones: KETRI 3741, 3801, 3804, and 3928. Mature tsetse infections were confirmed by xenodiagnosis, after which two to three monkeys were infected with each clone via the bite of a single infected fly.

### Results

Five of the nine (62.5%) individuals developed chancres four to eight days after infection (DAI). The pre-patent period was 4 (4-10) days (median and range), indicating that trypanosomes migrated from the site of fly bite to the systemic circulation almost immediately and independently of the development of chancre. The period to detection of parasites in cerebrospinal fluid (CSF) was 16 (8-40) days (median and range), marking the onset of late stage disease. Interestingly, CSF trypanosome and white cell trends showed a positive linear association ( $r=0.3244$ ,  $p=0.001$ ), progressing in severity through lag (transition), intermediate and terminal phases. Haematology changes included anaemia of a microcytic hypochromic type and severe progressive thrombocytopaenia. White blood cells decreased, with maximum leucopenia at 28-44 DAI followed subsequently by a leukocytosis. The course of infection ranged 22-120 days, strain 3801 producing a more acute disease than strains 3741, 3804, and 3928.

### Conclusion

These data closely mimic the human disease showing that the fly transmitted model is well suited for evaluation of haemathical changes in HAT.



## INFLUENCE OF TRYPANOCIDAL THERAPY ON THE HAEMATOLOGY OF MONKEYS INFECTED WITH *TRYPANOSOMA BRUCEI RHODESIENSE*

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### Introduction

Human African Trypanosomiasis is a re-emerging protozoal disease which is associated with complex haematological changes. Monitoring of the haematological parameters is important, not only for rapid appraisal of the disease management, but also in understanding the pathogenesis and diagnosis of relapsed infections.

### Objective

The aim of this study was to determine the sequential haematological changes in the vervet monkeys infected and treated with sub-curative and curative trypanocidal drugs.

### Methodology

Three vervet monkeys were infected intravenously with  $10^4$  trypanosomes of a stabilate *Trypanosoma brucei rhodesiense* KETRI 2537. They were treated with diminazene aceturate at 28 days post infection (dpi) and with melarsoprol (Mel B) following relapse of infection at 140 dpi.

### Results

All the monkeys developed a disease characteristic of trypanosomiasis with a pre-patent period of 3 days. Treatment with diminazene aceturate caused a clearance of trypanosomes from both the blood and cerebrospinal fluid (CSF). The parasites later relapsed in the CSF (63-98 dpi) and blood (135-140 dpi). There was a significant decline in red and white blood cell (WBC), and platelet counts (PLT) between 7 and 28 dpi. The treatment caused an improvement in the values with red blood cell (RBC), hematocrit (HCT), hemoglobin (HB) and red cell distribution width (RDW), achieving the pre-infection values between five and seven weeks after treatment. The PLT and WBC counts also recovered to pre-infection levels within one and two weeks, respectively. Most of the parameters were later characterized by fluctuations, and declined at one to two weeks before the trypanosomes relapsed. Following melarsoprol treatment at 140 dpi, most values recovered within two weeks and stabilized at pre-infection levels, during the 223 days post treatment monitoring period.

### Conclusions

In conclusion, *T.b. rhodesiense* infection causes a complex haematological response in vervet monkeys. Treatment leads to a rapid recovery of platelets and WBC counts while erythrocytes take a longer time to achieve normal levels. Serial monitoring of these haematological parameters, which are easy to obtain, can be used as an adjunct in the diagnosis and prognosis of the disease.

## OPTIMIZATION OF CHEMICAL IMMUNO-SUPPRESSION PROTOCOLS IN *MASTOMYS NATALENSIS* USING CYCLOPHOSPHAMIDE FOLLOWED BY A *T.B GAMBIESE* INFECTION

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### Introduction

Cyclophosphamide (CP) is an immunosuppressant drug often used in cancer treatment. It acts by reducing natural cell killer activity of the host by causing depletion of leucocytes /neutrophils. *Trypanosoma brucei gambiense* infection is usually characterized by low parasitaemia in both lab rodents and higher animals (man, monkey). Earlier work in *Mastomys natalensis* had shown multiplication of *T.b gambiense* could be improved by using CP at rate of 200mg/Kg bwt.

### Objective

To determine the range of CP dosages in *Mastomys natalensis* for expansion of *T.b gambiense*

### Specific Objectives

Determine the success of development of parasitaemia in *Mastomys natalensis*

Determine clinical picture in the immuno-suppressed host animals

### Activities

60 *Mastomys natalensis* weighing between 70-80g divided into 9 groups, comprising of 6 animals each, and a control group of six were used

Treatment was given at dosage rate of 100, 150, and 250mg/Kg bwt intraperitoneally given once, twice or thrice for each dosage level prior to infection.

Control group consisted of six untreated infected *Mastomys*

The mice were infected with a *T.b gambiense* strain, KETRI 2565, isolated from Southern Sudan 1982, and preserved in 10% glycerol. Parasitaemia was monitored from 3 days up to 30 days post infection.

All the treated groups were given a booster dosage of 200 mg/Kg bwt on weekly basis for three weeks

### Results

The group that had been subjected to 100 mg/Kg X 3, 150mg X 2 and 250 mg X 1 became parasite positive at 4 days post infection.

The parasitaemia was initially at antilog<sub>10</sub> 5.4 but increased to as high as antilog<sub>10</sub> 6.0 (Woo method) by 28 days post infection.

Given dosage of 250mg more than once and 150mg/Kg more than twice was fatal to the rodents

Dosage level of 150mg/Kg and 100mg/Kg once and less than X 3 respectively did not give different results from the control animals

### Discussion and Conclusion

The above results show that the 250mg/Kg bwt and 300mg/Kg bwt given once and as split dosage respectively prior infection are good dosage levels for the expansion of *T.b gambiense* isolates in *Mastomys natalensis*. However, the recommended dosages need to be tested for field isolates not adapted to laboratory conditions

It seems boosting the dosage on weekly basis after the third week of infection, had no advantage over control animals

Given the earlier and the present results on immuno-suppression, 200mg/Kg bwt given once and a boost of a similar dosage is sufficient to produce good suppression *M. natalensis* for *T.b gambiense* multiplication.

The above results give the scientists working on HAT more flexibility to choose immuno-suppression dosages ranging from a total of 200 to 300mg/Kg bwt .

Earlier work had shown 200mg/Kg bwt given once produced good suppression

## **ISOLATION OF *T.B. GAMBIENSE* FROM TREATMENT REFRACTORY PATIENTS IN R.D. CONGO**

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### Introduction

Isolation of *T.b. gambiense* from human patients is difficult. Success rates of inoculation into classical laboratory rodents such as Swiss mice and albino rats are low. Better recipients are *Mastomys natalensis* and *Grammomys surdaster*. In the latter species, respectively 89% and 50% success rates were obtained with inoculation of freshly collected blood and cerebrospinal fluid in Kinshasa (Büscher et al. 2005).

For remote collection of blood and CSF samples prior to inoculation in *Grammomys*, cryostabilisation in a bull semen cryomedium has been proposed. This technique is now being used in a clinical study on improved follow-up in Mbuji-Mayi, Kasai Province, R.D. Congo, where treatment failure rates are alarmingly high. This study is part of a larger study to isolate *T.b. gambiense* strains from relapsing patients and controls for further *in vitro* and *in vivo* drug profiling and molecular characterisation. We here describe the validation of Triladyl as a medium for cryopreservation of patients' samples and the comparative susceptibility of *Grammomys surdaster* and *Mastomys natalensis* for *T.b. gambiense*.

### Materials and methods

#### *Cryomedium*

Egg yolk is collected with a syringe through the punctured egg shell, cleaned with alcohol. One volume of egg yolk is mixed with 3 volumes of Triladyl and 3 volumes of Phosphate Buffered Saline Glucose (PSG, Lanham and Godfrey 1970 ). The mixture is divided over 2 ml aliquots and kept frozen at -20°C until use.

#### *Cryopreservation of venous blood on heparin*

Blood with confirmed presence of trypanosomes is divided over 3 Eppendorf tubes at 1.5 ml and centrifuged for 5 minutes at 3,000 rpm in a microcentrifuge. The plasma is discarded and 250 µl of the buffy coat in each tube is transferred to a cryotube and mixed with 250 µl of the cryomedium. The tubes are placed in a sock and frozen in the vapour phase of liquid nitrogen for 1 hour. After that they are taken out of the sock and transferred into the liquid nitrogen until further use.

*Cryopreservation of cerebrospinal fluid (CSF)*

CSF is taken by lumbar puncture and checked for the presence of trypanosomes. The CSF is divided over 3 Eppendorf tubes at 1.5 ml and centrifuged for 5 minutes at 3,000 rpm for 5 minutes. The supernatant is discarded leaving about 300 µl of sediment in each tube. The sediment is mixed with 300 µl of cryomedium where after the tubes are processed as for blood.

*Inoculation of rodents with cryostabilized blood or CSF*

*Grammomys surdaster* and *Mastomys natalensis* are bred at the Institute National de Recherche Biomédicales in Kinshasa. The cryopreserved CSF and blood samples are thawed quickly in a water bath at 37°C. Rodents are inoculated intraperitoneally with 0.3 ml. The parasitemia is checked regularly (every day during the first week) for at least 2 months. When first peak parasitemia is too low for cryostabilisation of the isolate, blood from the parasitemic animal is subinoculated into a naïve animal which is followed up as described above.

*Cryostabilisation of the T.b. gambiense isolate growing in rodents*

When parasitemia is about 10<sup>6,9</sup> according to the matching method of Herbert and Lumsden (1976), the animal is anesthetised with Nembutal (intraperitoneally at 0.2 ml/250 g body weight). Blood is taken by heart puncture on heparin (about 1 ml blood for *Grammomys* or *Mastomys*) and processed as for patients' blood taking care to prepare several cryotubes per animal and to store them over several cryocontainers. After one week, one cryovial of each isolate is checked for viability of the cryopreserved trypanosomes.

Results

In a first experiment, 8 CSF samples from patients who relapsed after melarsoprol treatment were inoculated at 0.3 ml each in one *Grammomys* rat. The animals were checked for up to 97 days. Six animals became positive within 6 to 97 days with parasitemia ranging from 1 to 4 trypanosomes per microscopic field (Table 1). Five isolates have been cryostabilized.

|                               |     |     |     |     |     |    |   |   |
|-------------------------------|-----|-----|-----|-----|-----|----|---|---|
| <i>Grammomys</i> nr.          | 1   | 2   | 3   | 4   | 5   | 6  | 7 | 8 |
| day of first peak parasitemia | 6   | 6   | 14  | 14  | 37  | 97 | n | n |
| tryps/field                   | 2-3 | 2-3 | 2-3 | 2-3 | 1-4 | 1  | n | n |

In a second experiment, 4 CSF samples from melarsoprol relapsing patients and 2 blood samples from pentamidine relapsing patients were inoculated at 0,3 ml of each sample in 1 *Grammomys* and 1 *Mastomys* rat. The animals were checked for 50 days. Five *Grammomys* and 1 *Mastomys* became positive (all CSF, 1 blood) within 15 to 34 days with parasitemia ranging from 1 to 10 trypanosomes per microscopic field (Tables 2 and 3). Five isolates have been cryostabilized.

|                               |   |    |     |     |     |     |
|-------------------------------|---|----|-----|-----|-----|-----|
| <b><i>Grammomys</i> nr.</b>   | 1 | 2  | 3   | 4   | 5   | 6   |
| sample type                   | B | B  | CSF | CSF | CSF | CSF |
| day of first peak parasitemia | n | 15 | 15  | 17  | 20  | 23  |
| trypanosomes/field            | n | 1  | 1   | 10  | 1   | 1   |

|                            |   |   |     |     |     |     |
|----------------------------|---|---|-----|-----|-----|-----|
| <b><i>Mastomys</i> nr.</b> | 1 | 2 | 3   | 4   | 5   | 6   |
| sample type                | B | B | CSF | CSF | CSF | CSF |



|                               |   |   |    |   |   |   |
|-------------------------------|---|---|----|---|---|---|
| day of first peak parasitemia | n | n | 34 | n | n | n |
| trypanosomes/field            | n | n | 5  | n | n | n |

#### Discussion

From the first experiment, it is concluded that Triladyl/egg yolk/PSG is an excellent cryomedium for patients' blood and CSF keeping the trypanosomes alive for later inoculation into susceptible rodents. Thus, it becomes possible to collect and preserve patients' samples in liquid nitrogen for later inoculation into susceptible rodents in a central laboratory with animal house facilities.

From both experiments, it is clear that the isolation success rate in *Grammomys surdaster* is high (75% and 83%) compared to *Mastomys natalensis* (17%). In both rodent species, first peak parasitaemia remains low (1-10 tryps/field) and subinoculation is sometimes needed before cryostabilisation of the isolates.

Since the reproduction rate of *Grammomys* is much lower than of *Mastomys*, the availability of sufficient *Grammomys* for inoculation is limited. Therefore, it is proposed to carry out a comparative isolation experiment with immunosuppressed *Mastomys* and *Grammomys* to investigate whether immunosuppression of *Mastomys* may render the species more susceptible for *T.b. gambiense* infection. Depending of the results, isolation of the trypanosomes from relapsing patients will continue in one of both species where after the drug sensitivity profile of the isolates will be investigated.

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## Summary of Group Discussions & Recommendations

### Group 1: Public health and socioeconomics

#### Actions taken

Studies on socio-economic impacts of SS, socio-economic factors influencing disease transmission and benefits accruing to communities once the disease is controlled. Studies and documentation on disease burden

- Uganda: Done in S. - Eastern Uganda. Burden of disease done in S. E. but more studies needed
- Tanzania: A proposal already submitted but not yet funded
- Sudan: Socio economic study done but more required
- Kenya: Studies done
- Malawi: No study done

#### Strengthen the socio-economic component in research centres.

- Uganda: One PhD undergoing training
- Tanzania: None
- Kenya: Two undergoing training
- Sudan: Collaboration with Universities
- Malawi: None

#### Creation awareness/education about sleeping sickness

- Uganda: TV, calendars
- Tanzania: Workshop with policy makers and currently working on national HAT control policy  
Disease awareness continues
- Kenya: Awareness through local leaders, and press
- Malawi: Little on going but need for more

#### Capacity building

##### Training personnel in the socio-economic methodologies.

- Uganda, Malawi, Sudan, Tanzania: None,
- Kenya: Done but need for standardization of methodologies

##### Development of training materials (IEC).

- Sudan, Tanzania: None
- Malawi: Developed with assistance from WHO
- Uganda: Developed and distributed
- Kenya: Developed pamphlets, booklets in local and national languages  
Two people currently on training of IECs

##### Training of medical personnel in counselling skills.

- Uganda, Kenya: Done
- Tanzania, Malawi, Sudan: Not done



**Equip selected health centres with diagnostic facilities.**

Uganda, Tanzania: Done

Kenya Malawi: Not done

**Recommendations**

1. Workshop on standardization of tools, terms methodologies, OVis should be done before the next conference
2. Short term training on methodologies
3. Joint proposal targeting WHO-SEB by EANETT countries. Deadline FEB, 16 2006
4. Inventory of Socio-economists in EANETT country members
5. Studies on KAP necessary in all EANETT countries.



## Summary of Group Discussions & Recommendations

### Group 2: Characterisation of trypanosomes

Many points of last year's recommendation of group 2 have been started to be addressed. Last year's recommendations still apply.

#### A) Isolation

- In Sudan no isolation was possible last year.
- Compare isolation success in immuno-suppressed *Mastomys* to *Grammomys* and immuno-suppressed *Grammomys* in DRC to decide on the best isolation protocol.
- Everybody uses standardized protocol using Triladyl. Philippe Buescher will communicate his results.
- Where transport of animals is unpractical, instead of taking animals to the field, patient blood should be preserved in liquid nitrogen and injected into mice in the lab. This yielded good results in DRC.
- For Tanzania, isolate parasites in Tbr/Tbg overlap area
- John Enyaru was successful by isolating parasites when feeding patient blood to tsetse and then isolate the parasite out of the tsetse. He will communicate it via Marcel.

#### B) Monkey model for *T.b. gambiense*

Contrary to last year's recommendation, the protocol is not established yet. Work has to be continued.

TRC needs a more virulent parasite isolate from DRC (Philippe Büscher) for the model.

#### C) Molecular characterisation

- In Uganda the techniques for distinction between Tbg and Tbr are close to be established but validation is continuing.
- Molecular characterisation of vaccine targets needs to be looked into.
- The amplification of LAMP will be tested in collaboration with Dr. Sugimoto. A broad range of isolates have to be made available to him.
- Isolates of relapse patients before treatment and after relapse should be genotyped using microsatellites in collaboration with Oliver Balmer.
- Allele-specific PCR for drug resistance identification should be validated in all member countries.

#### D) Drug resistance

- When isolating parasites from relapse patients a sample of patient blood should be taken to determine the patient factors involved in immune response. A further sample should be injected into an immune-suppressed mouse to determine if the parasite growth depends on the mouse immune-status.
- Characterize *T.b. rhodesiense* out of chancres in monkeys after tsetse infection.

#### E) Capacity building

- Points 2 and 3 of last year's protocol are not done yet.
- Tanzania and Malawi need to be trained in molecular techniques.
- Member countries should identify people who might receive thorough bioinformatics training.



## Summary of Group Discussions & Recommendations

### Group 3: Epidemiology of Human African Trypanosomiasis

#### Recommendations (2004)

- Awareness creation has been initiated in most EANETT member countries, however this needs to be undertaken at all levels (community, national, district, policy)
- Programmes to be established for continuous education and awareness in individual countries to increase case detection
- Policy to be drafted – format to be designed by EANETT

#### Capacity building

WHO support to countries within the year was appreciated

Uganda: Initiated, equipped and trained personnel in two centres  
At national level supported two officers for training

Tanzania: Equipped and trained personnel in three centres

Sudan: Trained 3 technicians, 6 officers, provided equipment plus vehicle

It is acknowledged that Tbg protocol is already harmonized and standardized, however, the same needs to be done for Tbr

Currently WHO setting guidelines for Tbr diagnosis & treatment, to be discussed in Jan 2006

#### Improvement of coordination

Uganda: Support for Uganda on case detection  
In new Tbr areas in case detection and awareness creation

Tanzania: Support for active case detection

Kenya: Draft strategy for sustained surveillance and early warning systems

#### Issues to be considered for effective Coordination:

Ownership of control programmes by districts (This will facilitate coordination of various actors)  
e.g. Sudan: Opening southern Sudan (situation different, therefore need to work with other actors already in the area)

Intensify search for reservoirs: Wildlife, livestock, increased participation by all EANETT countries

#### Recommendations for 2005

Following discussions on the papers presented at this meeting, the following issues were raised which need to be addressed

- Need for continuous education and awareness at all levels (community, national, district, policy) (Seek help of WHO to provide posters and brochure for education programmes)
- Collate and analyze existing data for policy briefs (advocacy)
- Policy briefs to be drafted – format to be designed by EANETT
- Need for accurate data for risk assessments
- Transmission dynamics (fly/man contact)
- Peri-domestic transmission/peri-urban
- Review literature on placental transmission and report at the next meeting
- The role of goats in disease transmission
- Sampling and sample size
- Find ways of assisting Malawi to build capacity, country to provide needs (Due to high personnel turnover, it is suggested that a training for trainers be considered for Malawi)



Summary of Group Discussions & Recommendations

**Group 4: Tsetse**

Based on recommendations of the 6<sup>th</sup> Annual EANETT conference, the following achievements have been made in the last year

| Recommendations of 2004                                      | Achievements/ progress in the past year                      |
|--|--|
| 1. Evaluation of PCR for trypanosome detection in tsetse     | -Sudan, Kenya, Tanzania: reported<br>-Uganda: ongoing        |
| 2. Role of biting flies in Tryp transmission - Sudan         | - being addressed under a PhD programme                      |
| 3. Representative tsetse sampling # >100                     | -Sudan, Kenya, Tanzania, Uganda: reported / SOP's            |
| 4. Web page accessible                                       | - Done, but protocols should be placed on webpage for access |
| 5. Standardization of collection and analysis of fly tissues | - Has been addressed by SOP's                                |
| 6. Tsetse population genetics                                | -Uganda has started studies                                  |
| 7. Trap efficiencies and tsetse vector ecology               | -Going on in all countries                                   |
| 8. GIS based tsetse distribution maps                        | -On going in all countries                                   |
| 9. Training  | -Not achieved  |

**Recommendations**

1. The group recommended strengthening of inter-country staff networking including site visits, technology utilization appraisals, attachments and personnel exchange programmes
2. It was recommended that there should be readiness in availing/sourcing catalogue information on materials and or finished control tools/products in vector work
3. Immediate inclusion of the protocols (SOPs) developed in the website for immediate access
4. It was recommended that further training needs in areas, such as bioinformatics should be addressed as an immediate priority
5. It was further recommended that areas identified in the research questions and problem table be looked in to by individual member states as may be practically relevant (see Appendix 1 A & B)

Appendix 1

A)

| RESEARCH QUESTION   | PROBLEMS  |
|---------------------|---|
| BIO-ECOLOGY         | Meteorological factors; Terrain; Vegetation; Hosts; Behavioural differences |
| SAMPLING TECHNOLOGY | Availability; Ease of use; Sensitivity; Standardization                     |
| SAMPLING PROCEDURE  | Design; Sampling frame  |
| SPECIES             | Identification; Importance; Purpose; Vectorial capacity                     |
| CONTROL             | Choice; Level; Sustainability   |

B)

| DESIGN  | APPROVAL   | USE  |
|---|--|--|
| <ol style="list-style-type: none"> <li>1. Research question</li> <li>2. Lack of training</li> <li>3. Lack of SOP's</li> <li>4. Methodology</li> <li>5. Timeframe</li> <li>6. Materials</li> </ol> | <ul style="list-style-type: none"> <li>• Delays</li> <li>• Conflict of interest</li> </ul> | <ul style="list-style-type: none"> <li>• Lack of seriousness in implementation</li> <li>• Apathy</li> <li>• Lack of support from administrative staff</li> <li>• Lack clear instruction</li> <li>• Lack of motivation</li> </ul> |



## Summary of Group Discussions & Recommendations

### Group 5: Human genetics and HAT

#### Rationale:

- Genetic influence on HAT infection by comparing cases and controls (differences in genes between cases and controls)
- Differences in environmental risk to become infected must be controlled for (eg fly density, parasite strains, human behavior)
- Initial stage in limited geographic areas, later stage with cases from other areas/countries as well

#### Controls:

- How do you define the controls
  - Controls should be individuals with similar risk exposure but not infected

#### Cases:

- First stage
  - From active surveillance
  - Add samples from passive surveillance
- Second stage:
  - Samples collected over time in various centres

#### Objectives:

- Finding new pathways important in HAT
- Finding target molecules for drugs or vaccines
- Identifying human genes important to control for in other studies (eg environmental risk factors)

#### Study stages:

- Active surveillance according to WHO guidelines for case finding and control samples in Tanzania and Sudan
- Entomological studies in selected area for modeling of risk effect
- Same for socio-economics and parasitology

#### Genetic analyses:

- Depending on funding
- Should be state of the art (compare malaria!)
- Can start small scale, depending on funding (candidate genes, small sample size)
- Extendable (information not lost)

#### Questionnaire:

- Should be developed from socio-economic experts from EANETT-countries together

#### Collaborations:

- Human sampling Sudan, Tanzania –standardized
- Samples from Uganda for later stages to be stored
- Parasitological expertise in Uganda as centre of gravity + outside training
- Socio-economic expertise from TRC/KARI



**Capacity building:**

- PCR in Tabora
- Training for PCR/molecular techniques for LIRI, TTRI, TMRI, TRC + Malawi from Europe + internally
- For active surveillance mobile freezer etc.
- Training on documentation, storage
- Standardisation of sampling and diagnostic procedures
- Control of parasitological diagnosis from screening by PCR in the lab (confirmatory testing) + LAMP ???

**Ethics:**

- Informed consent, follow national guidelines + WHO directive
- Anonymous data for research use
- Issue of sample storage for BIOBANK - bring it on to discussion

**Source of funding:**

- Initial funding for sample taking from Innsbruck Medical University (Start)
- Further funding to be applied for (EU, TDR, Bill-and-Melinda/Gates-Foundation? EANETT approval will help!)
- Funding for sample taking and genetic analyses might be stepwise



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