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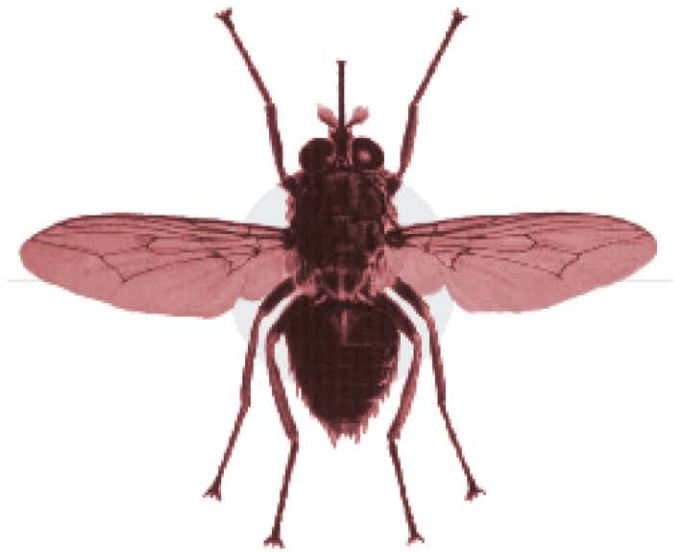

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TSETSE AND TRYPANOSOMIASIS INFORMATION

Numbers 14165–14340

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TSETSE AND TRYPANOSOMIASIS INFORMATION

The Tsetse and Trypanosomiasis Information periodical has been established to disseminate current information on all aspects of tsetse and trypanosomiasis research and control to institutions and individuals involved in the problems of African trypanosomiasis. This service forms an integral part of the Programme Against African Trypanosomiasis (PAAT) and is jointly sponsored by the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA), the Inter-African Bureau for Animal Resources of the African Union (AU-IBAR), the World Health Organization (WHO), the Research Department for Livestock Production and Veterinary Medicine of the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD-EMVT), the British Government's Department for International Development (DFID) and the Institute of Tropical Medicine (ITM), Antwerp.

The half-yearly periodical is prepared for publication, in both English and French editions, by the Food and Agriculture Organization of the United Nations. Each annual volume consists of two parts and an index. Subscription is free for all recipients engaged in trypanosomiasis research and control, and requests for enrolment may be sent to: Ms Maria Grazia Solari, AGAH, FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy (fax +39 06 5705 5749; e-mail MariaGrazia.Solari@fao.org).

Since the value of this information service depends to a great extent on the receipt of relevant material from research workers, campaign planners and organizers and field workers themselves, readers are requested to submit news items and copies of scientific papers and reports to the Editor: Dr James Dargie, Brunnstubengasse 43, 2102 Bisamberg, Austria (tel. +43 2262 61735; e-mail j.dargie@aon.at).

We regret that we are unable to supply photocopies of the papers quoted in the periodical.

Distribution dates and copy deadlines

	Copy deadline for news items	Distribution (English and French editions)
Part 1	15 April	July/August
Part 2	15 October	January/February

The Index will be distributed as soon as possible after the completion of each volume.

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ABBREVIATIONS USED IN *TTI*

AAT	animal African trypanosomiasis
a.i.	active ingredient
ACTH	adrenocorticotrophic hormone
ALAT	alanine aminotransaminase
ARI	advanced research institute
ASAT	aspartic acid aminotransaminase
b.w.	body weight
BIIT	blood incubation infectivity test
CATT	card agglutination test for trypanosomiasis
CD_{50}	median curative dose
CNS	central nervous system
CSF	cerebrospinal fluid
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
HAT	human African trypanosomiasis
HCT	haematocrit centrifugation technique
GIS	geographic information system(s)
GPS	global positioning system(s)
IPM	integrated pest management
i.m.	intramuscular(ly)
i.p.	intraperitoneal(ly)
i.v.	intravenous(ly)
IFAT	indirect fluorescent antibody test
KIVI	kit for in vitro isolation of trypanosomes
LC	land cover
LCCS	land cover classification system
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LPI	livestock policy initiative
M	molar
mAEC	miniature anion-exchange centrifugation technique
McAb	monoclonal antibody
MDGs	millennium development goals
MoU	memorandum of understanding
MW	molecular weight
NARS	National Agricultural Research Services/Systems
NGO	non-governmental organization
PAAT-IS	programme against animal trypanosomiasis-information system
PAG	PAAT advisory group coordinators
p.i.	post-infection
PCR	polymerase chain reaction
PCV	packed cell volume
ppb	parts per billion (10^9)
ppm	parts per million
r.h.	relative humidity
RNA	ribonucleic acid
SARD	sustainable agricultural and rural development
SAT	sequential aerosol technique
SIT	sterile insect technique
sp(p).	species (plural)
ssp(p).	subspecies (plural)
STEP	southern tsetse eradication project
TC	technical cooperation
T&T	tsetse and trypanosomiasis
TTI	tsetse and trypanosomiasis information bulletin
UV	ultra-violet
VAT	variable antigen type
VSG	variant surface glycoprotein
WBC	white blood cell

Organizations

AfDB	African Development Bank
ANDE	Agence Nationale de Développement de l'Elevage
AU	African Union
AU/STRC	African Union/Scientific, Technical and Research Commission
BICOT	Biological Control of Tsetse by the Sterile Insect Technique
CEBV	Communauté Economique du Bétail et de la Viande
CEMV	Centre Universitaire de Formation en Entomologie Médicale et Vétérinaire
CGIAR	Consultative Group on International Agricultural Research
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CIRAD-EMVT	Département d'Elevage et de Médecine Vétérinaire des Pays Tropicaux du CIRAD

CIRDES	Centre International de Recherche-Développement sur l'Elevage en Zone Subhumide
CNERV	Centre National d'Elevage et de Recherches Vétérinaires
CNRS	Centre National de Recherche Scientifique
COCTU	Coordinating office for contriol of trypanosomiasis in Uganda
CREAT	Centre de Recherche et d'Elevage, Avétonou, Togo
CRSSA	Centre de Recherches du Service de Santé des Armées Emile Pardé
CTVM	Centre for Tropical Veterinary Medicine
DFID	Department for International Development (UK)
DSE	German Foundation for International Development
EC/EU	European Community/European Union
EDF	European Development Fund
ESTA	Ethiopian Science and Technology Agency
FAO	Food and Agriculture Organization of the United Nations
FIND	Foundation for Innovative New Diagnostics
FITCA	Farming in Tsetse Control Areas of Eastern Africa
GFAR	Global Forumon Agricultural Research
GTZ	Deutsche Gesellschaft für Technische Zusammenarbeit
IAEA	International Atomic Energy Agency
IBAR	Interafrican Bureau for Animal Resources
ICIPE	International Centre of Insect Physiology and Ecology
ICPTV	Integrated Control of Pathogenic Trypanosomes and their Vectors
IFAD	International Fund for Agricultural Development
IFAH	International Federation for Animal Health
ILRI	International Livestock Research Institute
INRA	Institut National de Recherche Agronomique
IPR	Institut Pierre Richet
IRD	Institut de Recherche et de Développement (formerly ORSTOM)
ISCTRC	International Scientific Council for Trypanosomiasis Research and Control
ISRA	Institut Sénégalais de Recherches Agricoles
ITC	International Trypanotolerance Centre
ITM	Institute of Tropical Medicine
KARI	Kenya Agricultural Research Institute
KETRI	Kenya Trypanosomiasis Research Institute
LCV	Laboratoire Central Vétérinaire
LNERV	Laboratoire National de l'Elevage et de Recherches Vétérinaires
LRE	Laboratoire Régional de L'Elevage
LSHTM	London School of Hygiene and Tropical Medicine
MRC	Medical Research Council
MRU	Mano River Union
NITR	Nigerian Institute for Trypanosomiasis Research
NRI	Natural Resources Institute
OCCGE	Organisation de Coopération et de Coordination pour la Lutte contre les Grande Endémies
OCEAC	Organisation de Coordination pour la Lutte contre les Endémies en Afrique Centrale
OGAPROV	Office Gabonais pour l'Amélioration de la Production de la Viande
OIE	Office International des Epizooties
OMVG	Organisation pour la Mise en Valeur du Fleuve Gambie
PAAT	Programme against African Trypanosomiasis
PATTEC	Pan-African Tsetse and Trypanosomiasis Eradication Campaign
PRCT	Projet de Recherches Cliniques sur la Trypanosomiase

RDI	Rural Development International
RUCA	Rijksuniversitair Centrum Antwerpen
SADC	Southern African Development Community
SIDA	Swedish International Development Authority
SODEPRA	Société pour le Développement des Productions Animales
TDR	UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases
TDRC	Tropical Diseases Research Centre
TPRI	Tropical Pesticides Research Institute
TTRI	Tsetse and Trypanosomiasis Research Institute
UCLT	Unité Centrale de Lutte contre la Trypanosomiase
UNDP	United Nations Development Programme
UNEP	United Nations Environment Programme
UNIDO	United Nations Industrial Development Organization
UNTFHS	United Nations Trust Fund for Human Security
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
UTCC	Uganda Trypoanosomiasis Control Council
UTRO	Uganda Trypanosomiasis Research Organisation
WHO	World Health Organization

CONTENTS

SECTION A – NEWS

AfDB/AU-PATTEC

Special Donors Meeting 1-2 September 2006, Addis Ababa, Ethiopia.

The African Union-Pan African Tsetse and Trypanosomiasis Eradication Campaign (AU-PATTEC) organized a special donors meeting on 1-2 September 2006 in Addis Ababa, Ethiopia. AU-PATTEC and AfDB (African Development Bank) estimate that the total funding required to free 37 sub-Saharan countries from the tsetse and trypanosomosis (T&T) problem within the next fifteen years would amount to about US$ 3,150 million, of which AfDB has made available for the six 'list-1' countries (Burkina Faso, Mali, Ghana, Ethiopia, Kenya and Uganda) loans amounting to US$ 80.2 million and has earmarked for the twelve 'list-2' countries some additional US$ 76 million.

At the meeting, the WHO highlighted their intensified efforts in providing relevant training and in stepping-up sleeping sickness surveillance, drug supply (with the support and donations from the private sector) and respective treatments, which resulted in a decline of new cases of human African trypanosomosis (HAT) by 57 percent. Colleagues from WHO also covered in their report relevant activities under its special programme for research and training in tropical diseases (TDR).

Project counterparts presented summary reports on the status of activities under the six 'list-1' projects, and also on the work done by some of the twelve 'list-2' countries, which are preparing for sub-regional AfDB supported T&T intervention campaigns under the AU-PATTEC initiative. Particularly impressive was the presentation on the joint work already done by Angola, Botswana, Namibia and Zambia. Following the 2001/2002 sequential aerosol technique (SAT) campaign in the Okavango Delta, Botswana's aerial spraying operations were expanded in mid 2006 to cover the remaining tsetse habitats in Botswana (some 5700 km²) and adjacent tsetse-infested areas in the Namibian Caprivi strip and in Southern Angola (4700 and 200 km², respectively). During the next three years the four countries envisage an expansion of the transboundary SAT operations into some 16 000 km² tsetse-infested areas of southern Angola and southwest Zambia, thus attempting to free in total some 40 000 km² of land with open vegetation from the tsetse-transmitted trypanosomosis problem. Other tsetse-infested areas in northern Angola, where sleeping sickness is wide-spread, are scheduled to be treated as of 2011. More north, the fly habitats are denser than in Botswana and complete eradication of tsetse flies may not be achievable as easily as this appears to have been possible as a result of the 2001/2002 SAT operations in the Okavango Delta.

The Vice-President of AfDB, Dr. Zeinab El Bakri, requested Heads of Delegations of T&T affected countries, as well as, donor countries and organizations represented at the Special Conference to make announcements on intended national and international contributions. Besides a) the envisaged US$ 76 million of AfDB loans to the 12 'list-2' countries; b) some countries' re-confirmation of national contributions to planned T&T interventions; and c) assured continued support from the mandated UN organizations, no major additional pledges were made at the meeting.

REPORT OF THE TWELFTH PAAT ADVISORY GROUP CO-ORDINATORS MEETING

Foreword

The twelfth PAAT Advisory Group (PAG) Coordinators' meeting was held in Kasane, Botswana from 18-19 October 2006.

Mr R.C. Mattioli, Focal Point of the PAAT Secretariat, gave a brief introductory note and highlighted PAAT mandate and activities.

Mr A.A. Ilemobade, PAAT Chairman, welcomed the participants and thanked the PAAT Secretariat for convening the meeting and the Botswana authorities for assisting the PAAT Secretariat in the meeting organization. The PAAT Chairman recalled the objectives of the Programme and of the meeting. PAAT's scope and interest are broad, embracing also the related dimensions of rural and animal health, land use, natural resources and socio-economic development. These public goods are all interconnected and brought together under one single PAAT umbrella. Doing so, PAAT creates the best opportunities to benefit from the inter-agency PAAT alliance and opens the door to a comprehensive landscape based approach, addressing arthropod borne diseases, farming systems, integrated pest management (IPM), environment, sleeping sickness and other human and animal health constraints to rural development. The action programmes drawn up under the auspices of PAAT are aimed at changing the disease stricken landscape into healthy rural development environments. PAAT supports this process directly through the analysis of landscape dynamics, applying satellite imagery, land cover maps, livestock distribution maps, spatial epidemiology and rural income distributions, all applied in order to prioritise where and how disease affected rural areas may best be turned into healthy agricultural production environments. With the landscape scale studies gaining in importance, PAAT paves the way for a more rational approach to rural development in tsetse affected regions starting with the protection of people and their livestock. At the end of his introductory note, Mr Ilemobade welcomed the Deputy Permanent Secretary, Ministry of Agriculture to open the meeting.

The meeting was officially opened by Mr M.C. Chimbombi, Deputy Permanent Secretary. He expressed the honour to host the meeting and thanked the Government of Botswana and national institutions for providing the resources for the meeting. Mr Chimbombi confirmed the commitment of his Government to eliminate the problem that tsetse and trypanosomiasis (T&T) pose to livestock, agriculture and tourism (sleeping sickness) industry development. He attributed the poor performance of the livestock-agriculture sector in Botswana to the presence of T&T. In fact, in the National Poverty Reduction Strategy, the elimination of trypanosomiasis is an area that needs attention. The Deputy Permanent Secretary mentioned the successful tsetse elimination operations undertaken in 2001-02 over 16 000 km^2 and an additional operation on 10 000 km^2 initiated in 2005 funded by Botswana with Namibia providing logistical support. At the end of his intervention, Mr Chimbombi declared the meeting officially open.

Brief and discussion on the last PAG meeting report – A.A. Ilemobade

The Agenda of the meeting was agreed to by members of PAG. Consequently, the conclusions and recommendations of the last meeting, held in Addis Ababa, Ethiopia, September 2006, were discussed and endorsed.

Report of the PAAT Secretariat and FAO/PAAT activities – R.C. Mattioli

The participants were informed on FAO and PAAT activities since the last PAG meeting. Normative and technical assistance has been provided to PAAT partner countries.

FAO/IAEA/WHO/PAAT developed and presented a document which outlines possible assistance of the UN PAAT mandated organizations to tsetse affected countries along a phased, conditional approach. A joint IAEA-FAO project (US$1.7million) to support T&T intervention in the Southern Rift Valley of Ethiopia has been approved by the Japanese Government through the UNTFHS.

In partnership with DFID, FAO published a paper entitled "Mapping the benefits: a new decision tool for T&T interventions" which links quantitative economic variables to a GIS spatial framework in order to provide new insights and reinforce decision making process for intervention. Technical advice has been provided to the STEP project in Ethiopia and to planning T&T intervention in the six countries (Burkina Faso, Ethiopia, Ghana, Kenya, Mali, Uganda) benefiting from the AfDB financial support for T&T intervention.

Technical visits were paid to the above mentioned six countries, international research institutes based in Africa and Europe, and NARES to discuss and assess, *inter alia*, available facilities, human resource development and training modules.

Distance learning training packages, in particular for e-conference moderators, have been disseminated. Also in relation to training activities, WHO trained on the spot, hands on, several staff from the Ministry of Health and Veterinary Services Department of various sleeping sickness affected countries on HAT control methods. WHO also organized an international training course on African trypanosomiasis in Tunisia, October 2005. Sixteen participants from HAT endemic countries and research laboratories attended the course. A regional training course on "Standardized baseline data collection for area-wide T&T management" was jointly organized by IAEA and FAO, March-April 06, Nairobi, in collaboration with Kenya authorities, and with substantial coordination assistance from ICIPE and support from ILRI and AU-PATTEC. Twenty-six participants from T&T affected countries attended the course.

With regard to publications, in addition to the regularly published bi-annual Tsetse and Trypanosomiasis Information (TTI) bulletin, FAO is working on the standardisation of land cover mapping for T&T intervention. This activity will provide the basis for a PAAT Technical and Scientific Series publication. A draft document on guidelines for declaring areas T&T free was distributed for comment and further development, with a view to eventually produce a PAAT position paper. Another publication in the pipeline relates to a

study which defines guidelines for sustainable human and animal African trypanosomiasis control and rural development strategies.

In relation to partnership with the private sector and following negotiations, UNIDO has agreed to participate in the FAO-IFAH initiative on Quality Control/Quality Assurance of trypanocides. FAO and UNIDO confirmed, at DG level, their support to develop joint activities on the matter within the framework of PAAT. A joint (FAO / IAEA / UNIDO / IFAD / IFAH) project proposal has been drafted and circulated among involved parties for comments.

The PAAT Chairman led a two-man panel to carry out a review of ISCTRC and its Secretariat. The report and formulated recommendations were adopted by the ISCTRC Executive Committee at its 31st meeting.

FAO/PAAT activities also included active participation in international policy, scientific and technical fora, including:

- Regional Harmonization Workshop, Nairobi, Kenya, October 2005.
- Regional meeting of National Coordinators of AfDB and IAEA-TC funded projects, Vienna, Austria, December 2005;
- Consultants meeting on the "Role of pathogens and symbionts in tsetse sterile technique", organized by FAO/IAEA, Vienna, Austria, March 2006;
- 31st ISCTRC Executive Committee meeting, Addis Ababa, Ethiopia, September 2006.

FAO/PAAT convened the 10th PAAT Programme Committee meeting, Florence, Italy, April 2006. The six AfDB beneficiary countries, PAAT mandated organizations, UNIDO, ARIs, donor representatives and other national, regional and international stakeholders attended the meeting. Country representatives outlined the implementation of the workplans of the AfDB supported projects.

At the end of this session, Mr Mattioli informed PAG of the end of the chairship tenure (three years) of Mr Ilemobade in November 2006. Members of PAG endorsed the renewal for another three years of Mr Ilemobade as Chairman of PAAT.

Report from IAEA – U. Feldmann

Mr Feldmann gave an overview of current IAEA activities. These included the normative activities and building partnerships related to the production of "Generic design, technical guidelines and optimal location of tsetse fly mass-rearing facilities". This was complemented with the draft of a spreadsheet enabling Member States to identify size of tsetse factories, specify equipment needed and provision of cost estimates. Standard Operating Procedures (SOPs) for advanced mass-rearing of tsetse flies were also produced. Within this framework, a meeting was convened in Vienna in July 2006 to advise on developing architectural blueprints for national and sub-regional tsetse mass-rearing facilities focusing mainly on Burkina Faso. Another consultants' meeting concerned the assessment of the minimal size of area-wide integrated pest management programmes, including SIT. The development of a mathematical model for planning and efficiency-assessment of different options of integrated

area-wide tsetse control strategies have been initiated. Two e-learning modules, one related to SIT-relevant irradiation dosimetry and a second one to tsetse strain compatibility testing have been developed.

Research and method development actions focused on improving and developing quality assurance of tsetse-mass rearing, such as advancement in facilitated/automated sexing of tsetse and research on salivary gland hypertrophic virus. A collaborative research programme has been initiated on "Improved and harmonized quality control for expanded tsetse production, sterilization and field application", and a new one to be initiated in 2007 will concern "Improving SIT for tsetse flies through research on their symbionts and pathogens".

The Agency's TC activities have provided support to the PATTEC Plan of Action through nine national technical cooperation projects (Botswana, Burkina Faso, Ethiopia, Kenya, Mali, Senegal, South Africa, Tanzania, Uganda) amounting approximately to US$3.4M in 2006 (foreseen 10 projects in 2007), and one regional technical cooperation project. Support was also given to a national workshop in Uganda (June 2006) to define a detailed action plan for the collection of entomological base line data in the Lake Victoria Basin. A feasibility study for creating a zone free of remaining two tsetse fly species in KwaZulu natal, South Africa was funded.

From October 2005 to October 2006, 66 person-months of scientific visits and fellowships relevant to T&T were supported by the Agency.

Other activities were mentioned previously in Mr Mattioli's presentation.

Report from WHO – P. Simarro

Recently, WHO has intensified its support to HAT affected countries in disease control activities and capacity building. As far as HAT control activities are concerned, 20 endemic countries have received assistance for screening (reagents for serological tests, equipment for diagnosis, financial support for mobile teams and free drugs for treatment) and surveillance network (monitoring and reporting).

Capacity building activities focused on training in diagnosis, case and programme management.

Mr Simarro provided an update of the epidemiological situation of sleeping sickness (SS). In eleven countries in which disease surveillance was not carried out, no cases of SS were reported, absence of cases of SS was also reported in four countries where surveillance action was implemented; ten countries reported less than 50 cases per year, between 50 and 1000 cases per year were reported in eight countries and only three countries reported more than 1000 cases/year.

A new partnership was established between WHO and FIND to improve diagnosis of HAT. This partnership is a result of a grant of US$ 10 million spread over five years from the Gates Foundation. Similarly, a consortium was created with a view to develop new drugs to

treat parasitic diseases. The consortium, of which WHO is a member, has received in September 06 US$ 23 million to develop new drugs for second stage disease status and for the development of a new drug, orally administered, for the first stage of the disease. The collaboration between WHO and Sanofi-Aventis for free drug supply, amounting to US $4 million and support to control activities (US 12$ million) continues. A platform for capacity building to develop clinical trials has been established.

Cooperation has also been activated with:

- CIRDES (Bobo Dioulasso, Burkina Faso) for SS surveillance and treatment in Dubreka and Island of Loos in Guinea;
- CTVM (Edinburgh, Scotland) to control SS in Uganda;
- PATTEC for increasing awareness on the PATTEC initiative and production and dissemination of information.

Mr Simarro concluded that WHO continued to provide support to PAAT and in particular to co-fund part of the PAAT Information System activities.

PAAT Information System: new features and future activities – G. Cecchi

An update of progress made in the development and management of the PAAT-IS was presented. The PAAT website has been revised, expanded and made available also in CD-ROM format. Renewed impetus has been given to the use of GIS techniques; datasets of national and regional predictions of tsetse distributions have been made available for downloading from the website and new standardized metadata have been generated and disseminated. The new website structure now includes a section on “Disease and vector control”, “Trypanotolerance”, “Area-wide integrated pest management”, “Integrated disease management” and “Guiding principles for decision making”. Sections on “Donors” and “Activities” related to ongoing T&T interventions have been added to the website. A link has been created with GeoNetwork (the FAO’s Spatial Data and Information Portal). This link allows sharing and disseminating trypanosomiasis-related GIS-datasets on an equal basis within a wider group of stakeholders, well beyond the present T&T community. It has to be mentioned that data and metadata in GeoNetwork comply with international standards (ISO 19115).

Within the framework of the PAAT-IS activities, technical visits have been made to the six AfDB beneficiary countries (Burkina Faso, Ghana and Mali in West Africa, Ethiopia, Kenya and Uganda in East Africa) with a view to assessing strengths and weaknesses in GIS and Information Systems (IS) management. The main common weak point in these six countries is (i) the absence of a centralized database for storage and analysis of entomological datasets and (ii) limited skills in GIS and IS management. Possible future support of PAAT-IS to AfDB funded national projects has been identified as follows:

- To update predictive maps of tsetse absence/presence and abundance;
- To produce standardized land cover maps, customised for different activities related to T&T intervention (e.g. collection of baseline entomological datasets; implementation of T&T intervention; environmental monitoring of the impact of T&T intervention);

- To provide assistance to AfDB supported project activities to develop environmental monitoring procedures (land use change; biodiversity) and guidelines for land use planning and natural resources management.

Standardising land cover mapping for T&T intervention – G. Cecchi

A draft paper was presented dealing with standardization of land cover (LC) classification for T&T intervention. Land cover datasets are essential in planning and monitoring T&T intervention activities. The available land cover maps are not necessarily produced for the needs of tsetse intervention and often apply heterogeneous classification systems. Hence, not all existing tsetse habitats can be described with the available classes and no clear boundaries exist between classes. The FAO/UNEP Land Cover Classification System (LCCS) overcomes this problems and it is expected to be adopted as the international standard by the International Organization for Standardization (ISO). Standardization of LC classification for T&T intervention will allow:
(i) inter-operability with LC datasets and maps produced by external sources;
(ii) easy development of customized manuals on land cover survey for field operators;
(iii) promotion and facilitation of regional and international cooperation.

The position paper (being proposed for publication in the PAAT Technical and Scientific Series) will deal with:

- Standardized land cover of tsetse habitats: analysis at continental level;
- Customization of a national, LCCS compliant dataset: the Africover map of Uganda for T&T intervention;
- Customization of multi-national, LCCS compliant datasets: the Africover map of East Africa for T&T intervention.

A standard description of LC classes of tsetse suitability has been developed and applied for tsetse distribution maps. Although improvement in mapping tsetse distribution has been achieved, the proposed values for tsetse suitability of the standardized LCCS classes should undergo a thorough examination by experienced entomologists and other T&T specialists and, where available, field datasets could be used to perform proper validation.

The IGAD Livestock Policy Initiative project: the T&T component – T. Robinson and A. Shaw

The IGAD-LPI, which comprises Djibouti, Eritrea, Ethiopia, Kenya, Somalia, Sudan, Uganda, implemented by FAO's Animal Production and Health Division (AGA), has included T&T as a component of its policy for livestock development. In IGAD's area, 80 percent of land is arid or semi-arid with high levels of poverty and food insecurity. A large proportion of the population depends on livestock for its livelihood and there is an increased demand for livestock and livestock products due to the demographic growth. Hence, there is an evolving requirement for both livestock services and the roles of actors involved. Policy and institutional framework need adjustment to accommodate trends in privatisation and decentralisation of livestock services, together with harmonisation of legislation and improved transboundary disease management.

The objective of the IGAD-LPI is to enhance the contribution of the livestock sector to sustainable food security and poverty reduction in the region. The purpose is to strengthen the IGAD capacity, member states, other regional organizations and other stakeholders to formulate and implement livestock sector and related policies that sustainably reduce food insecurity and poverty. The project is funded by the European Commission for a period of 4 years (2002-2005, budget US$7.5 million).

As far as T&T is concerned, the basic questions requiring attention are:

- Where to control;
- How to control: which control strategies (i.e. control vs. eradication) and which control methods (e.g. drugs, pour-ons, baits, SAT, SIT);
- Whether to integrate work on animal trypanosomiasis with HAT control activities.

Points to be considered in the formulation of T&T intervention strategy:

- 17 percent of IGAD cattle are at risk (16.5 million cattle);
- Countries with large numbers of cattle at risk are Ethiopia [4.8 million (15 percent of the national stock)], Kenya [4.5 million (40 percent)], Sudan [4.4 million (11 percent)] and Uganda [2.2 million (43 percent)];
- Agro-ecological, climatic conditions and livestock production systems.

Building on previous experience and work ("Mapping the benefits..." in West Africa) the plan is to produce cost maps and benefit maps for the IGAD Region to assist policy makers and advisors in PATTEC and IGAD members states to make informed decisions about the "where" and "how" to control trypanosomiasis. The "Mapping the benefits" work integrates three models with economic variables mapped for the first time. This template has been demonstrated useful in West Africa but it is also of general applicability. The IGAD "mapping the benefits" model will follow a step analysis which includes:

- The definition of the production systems and map their location;
- The development of herd models for each production system;
- Adding price, information about performance with/without trypanosomiasis;
- The calculation of losses/head of cattle applied to population/system.

Information will be collected on:

(i) location of production systems;
(ii) distribution of draught cattle;
(iii) distribution of dairy cattle;
(iv) livestock production parameters;
(v) prices of livestock outputs and inputs.

Each of these packages requires more detailed sub-sets of information for an accurate analysis of costs and benefits. For this purpose, a questionnaire will be soon distributed to national collaborators to acquire the necessary information. This study will be jointly carried out by FAO-IGAD LPI and FAO/PAAT.

Comparable costing of alternatives for dealing with tsetse: estimates for Uganda – A. Shaw, S. Torr, C. Waiswa, and T. Robinson

The last two decades have seen a significant decline in both the veterinary and tsetse control services throughout sub-Saharan Africa. Sleeping sickness has re-emerged as an important health problem, with both *gambiense* and *rhodesiense* forms reaching epidemic levels. Dealing with animal trypansomiasis has been left almost entirely to farmers, who mostly rely on trypanocides, spending some US$30-40 million a year. Against this background, since 2000, there has been a movement to implement large scale "area-wide" programmes to control the vector, under the aegis of PATTEC. PATTEC has been extremely successful in mobilising support for dealing with T&T, especially among African leaders, and in mobilising funds; currently AfDB is lending some US$ 67 million to six countries for the creation of 180,000 km^2 of tsetse-free zones. It is important to those in the field of T&T that the planning and execution of this programme runs as smoothly as possible. Decision-making on choice of technique for suppression and elimination as well as on other issues (monitoring, accompanying measures, etc.) needs to be as informed as possible. This work deals with one of the key issues, the economic aspects of choice of technique within the context of PATTEC's initiative.

In Uganda, Zone 1 consists of four blocks of 10,000 km^2 each. On the many possible approaches to deal with the vector the following were considered:

- Use of bait technology with insecticide, in this case with traps;
- Use of bait technology using insecticide-treated cattle (ITC);
- Aerial spraying using fixed wing aircraft and the sequential aerosol technique (SAT) spraying five cycles;
- Use of the SIT following suppression of the fly population by one of the above means.

A tsetse population dynamics model was used to calculate the impact of the four techniques on fly population reduction rate. In order to provide a level playing field for testing and comparing all techniques a 10,000 km^2 (100 x 100 km) block was used as the basis for calculation. The timing of each technique was carefully worked out and then figures were discounted at 10 percent per annum to their present value in the year tsetse elimination started. Field costs, administrative overheads and necessary studies (tsetse surveys, sleeping sickness surveys, surveys of trypanosomiasis in cattle, environmental and tsetse monitoring) were all included (based largely on PATTEC proposal). Accompanying measures to deal with sleeping sickness and animal trypanosomiasis are crucial but being common to all strategies were therefore not costed. For simplicity, all costs are given per km^2 of tsetse infestation.

The results for tsetse isolated populations pointed out that ITC is the less expensive (from US$ 134 to US$ 392), followed by traps (range US$ 373-496), SAT (US$ 502-593) and SIT (SIT+25 percent ITC US$ 1,015, SIT+80 percent SAT US$ 1305. SIT cannot be used in isolation, i.e. without previous tsetse suppression campaign). A model for non-isolated tsetse population was also developed. For both isolated and non-isolated populations the basic cost-hierarchy of ITC, traps (for savannah flies), SAT and SIT is maintained. Both SAT and SIT results are very sensitive to the cost of flying time, e.g. field cost for SIT falls

from US$ 761 to US$ 694 if cost of flying time down from US$ 700 to US$ 500. In the case of non-isolated tsetse populations, the barrier cost estimation is relatively modest (invasion from one side and only for three years). Barriers through the use of ITC are much cheaper but need testing.

Some conclusions can be drawn from this work:

- Cost hierarchy is confirmed and this ranking is robust;
- Cost differentials are far greater at field cost level – as published studies have long emphasised;
- The high cost of SIT reflects its being additional to the cost of suppression;
- Combinations of techniques may, however, be the most cost effective approach in some circumstances, especially against *G.fuscipes*, so more combined strategies need costing and investigations.

The modelling approach has produced realistic *ex-ante* cost calculations to guide decision-makers, but raises questions which need to be confirmed by field work. Studies are needed to collect more field evidence of scale on which cheaper techniques can be deployed, trials of what works best with specific flies. High cost of accompanying measures (administration, monitoring, socio-economic and environmental studies) needs to be questioned. There may be lessons to be learnt from past projects (strengths and weaknesses).

The AfDB funded T&T intervention in Uganda: update on the technical implementation and anticipated PAAT support – L. Semakula

The AfDB funded (loan) project ("Creation of sustainable T&T free areas in East and West Africa: the Uganda component") is foreseen to be executed in three phases, each phase corresponding to a zone to be freed from T&T. The project is implemented by the Ministry of Agriculture, Animal Industry and Fisheries and coordinated by COCTU with the support of the PCMU (Project Coordination and Management Unit). The PCMU is composed of a Project Coordinator, a Project Entomologist, a GIS Specialist, a Monitoring and Evaluation Officer and an Accountant. The project was officially signed by the Government in May 2005 and the loan was received in January 2006, with the first disbursement obtained in April 2006 (last disbursement foreseen in December 2011). A National Steering Committee and a Procurement Contracts Committee were created in April 2006. Additional administrative and financial arrangements necessary for starting the project implementation and executing field activities have been partially completed in September 2006.

The project coordination and management are assured by the PCMU which is supervised by the National Steering Committee and the Uganda Trypanosomiasis Control Council (UTCC). PCMU has requested the Auditor General to appoint an Audit firm to audit the project. Technical training was provided to the GIS specialist; PCMU and AfDB convened a resident planning session (10-13 October 2006) to develop a comprehensive training needs action plan and community awareness creation for the period 2006-2010. A MoU has been signed with the private sector (CEVA, Industrial Capital), CTVM, University of Edinburgh and Makere University to stamp out sleeping sickness using mass treatment of cattle with trypanocides and epicutaneous application of insecticides. For this a grant of US$ 500,000 has been provided of which US$ 300,000 is for drugs and insecticides and US$

200,000 for field operations. The Uganda T&T intervention plan foresees, *inter alia*, the use of SIT to eliminate the flies from the project area and relies on the Kaliti (Ethiopia) tsetse mass-rearing facility for tsetse supply. This is still a critical issue since the rate of production of the tsetse colony of the Ethiopian fly factory does not allow it to produce and deliver needed/requested quantities of tsetse sterile males in the short/medium term. Hence, PAAT support is requested to technically explore the feasibility of using SAT as suppression/elimination technique of tsetse flies.

The AfDB funded T&T intervention in Ethiopia: update on the technical implementation and anticipated PAAT support – T. Alemu

The loan provided by AfDB (US$ 14.6 million for a period of six years) to the Ethiopian Government is supporting in the on going STEP which aims at eliminating the T&T threat from an area of 25,000 km^2 in the Southern Rift Valley of Ethiopia using an area-wide, integrated pest management approach. The project, implemented by the ESTA, has as ultimate objective to enhance the national agricultural and poverty reduction efforts and reduce the pressure on the highland resources by improving the conditions necessary for sustainable agricultural and rural development.

The removal of tsetse from the area follows a phased approach with the full participation of the communities. The management of the project is assured by ESTA, a STEP Steering Committee, a STEP Technical and Advisory Committee and a Project Coordination and Management Unit (PCMU). PAAT and PAAT mandated organizations (e.g. FAO, IAEA) are among STEP's partners. Current project staff comprises 41 technicians and 54 auxiliaries. A community based tsetse suppression activity is on going using insecticide treated cattle and impregnated targets; monitoring of vector density and disease occurrence is carried out. The implementation of the AfDB funded project has not started yet. In addition to the AfDB loan, a joint FAO-IAEA project, funded by the Government of Japan (US$ 1.7 million), through the UNTFHS, and jointly executed by STEP, FAO and IAEA has been approved and is about to start. This project focuses on providing support of on going STEP activities for AW-IPM (vector and disease removal), information management, environmental monitoring, land use planning, socio-economics and training activities.

The Ethiopian Government has established a tsetse rearing and irradiation centre in Kaliti (approximately 40 km from Addis Ababa). The colony of *Glossina pallidipes* has been successfully established and mass rearing is in progress. The present colony size is estimated in 66,000 females with a growth in pupae production of 17,000/week. Adult fly mortality is below 1 percent. An embryonic colony of *G. fuscipes fuscipes* has been established through shipment of flies from Bratislava. The full foreseen capacity production of the tsetse fly factory in Kaliti is estimated at 1 million sterile males/week.

Major difficulties in project implementation concern insufficient staff to supervise and provide quality assurance of field activities, lack of training of communities involved in tsetse suppression activities and lack of direct income from continued tsetse control, particularly outside communal areas.

Issues that require particular attention can be summarized as follows:

- To ensure long term technical assistance to enhance tsetse rearing and sterile male management;
- To make available skilled experts to guide and monitor AW-IPM including SAT application and sterile male release;
- To provide adequate training to local staff to meet project needs;
- To establish workable management structure and systems;
- To identify proper institutions that could collaborate on land use management and environmental aspects of the project;
- To urgently solve the issue of purchasing/providing an industrial irradiator for Kaliti Tsetse Rearing and Irradiation Centre.

The AfDB funded T&T intervention in Mali: update on the technical implementation and anticipated PAAT support – A. Djiteye

Three species of tsetse fly (*Glossina morsitans submorsitans*, *G. palpalis gambiensis*, and *G. tachinoides*), infesting about 240,000 km^2 (20 percent of the total land), are present in the country. According to Mr Djiteye, in Mali approximately 20 percent of the total population (12 million) is exposed to sleeping sickness and about 2.7 million cattle are at risk of trypanosomiasis. Every year more than one million trypanocide treatments are administered to cattle. This amount represents over 50 percent of the total sale of veterinary drugs.

Tsetse control campaigns, with the support of IAEA, were conducted from 2003 to 2005: fly population was reduced over an area of 4,500 km^2 of the Niger river basin. However, following a disruption of the tsetse reduction campaign, the last control revealed an increase of the fly population in the peri-urban area of Bamako. In order to eliminate once for all the tsetse problem, the Government foresees the use of SIT from an initial target area of 32,000 km^2 (i.e.15,500 km^2 in the Niger basin and peri-urban area of Bamako, and further 16,500 km^2 in the Bani basin from the northern limit to the border with Burkina Faso). This project will be executed with the financial contribution (loan and grant) of AfDB and the Malian Government. Project components are:

(i) suppression and eradication;
(ii) capacity building;
(iii) sustainable land management;
(iv) co-ordination and management.

These components will be complemented with thematic maps generated with the use of GIS. Data to be collected regard tsetse fly distribution and population dynamics, animal and human trypanosomiasis prevalence, socio-economics, environment (for environmental impact studies). In the tsetse suppression campaign, the participation of the rural communities is envisaged.

The use of SIT, for tsetse elimination, targets mainly *G. p. gambiensis* along the river basins; under study is the possibility to establish a tsetse colony in the country. The total budget of the fly elimination operation is estimated at US$11.5 million (AfDB loan: US$ 9.5 million; AfDB grant: US$ 0.4 million; Malian Government contribution: US$1.6 million). The support requested to PAAT concerns equipment and chemicals, studies on land use and

socio-economic analysis, providing expert services for GIS and capacity building (training courses).

The Botswana experience with tsetse intervention (e.g. SAT) and related environmental issues – Nlingisisi Babayani, Casper Bonyongo, Sikhumbuzo Modo, Kefentze Motshegwa, Portia Otlaadisa, and Dominic Mazvimavi

The tsetse fly aerial spray operation against *G. morsitans centralis* in the Kwando and Linkati areas was reported. Aerial spray in Botswana started in the 1970s to replace ground spray with persistent pesticides. Following SAT tsetse distribution shrunk from 25,000 km^2 to 5,000 km^2. Last cases of HAT were recorded in 1981 and nagana (animal trypanosomiasis) limited to sporadic outbreaks. In 1991 SAT was discontinued due to environmental concerns and replaced by insecticide impregnated targets which, however, failed to suppress tsetse population. In 1998 tsetse density in the northern Okavango delta reached pre-spray levels with a resurgence of bovine trypanosomiasis in 1999 (300 cattle died). The Government of Botswana approved an integrated T&T control programme which involved the re-introduction of SAT followed by SIT as a contingency measure.

In July 2000 all cattle at risk of trypanosomiasis were treated with trypanocides every four months (more than 30,000 cattle treated). The treatment campaign ended in April 2002. Aerial spray started in June 2001 and was concluded in August 2002 with tsetse elimination as objective. In the work plan the use of SIT was foreseen to complete fly elimination. However, SIT was not required since SAT achieved the objective to eliminate tsetse. The SAT operation in 01 treated 7,180 km^2 in the northern Okavango and included high tsetse density areas like Mombo and Guai. In 2002 a further 8,600 km^2 were sprayed in the southern delta and included the district of Maun. At the end of the SAT cycles (6 cycles in 2001 and 5 cycles in 2002), no tsetse flies have been caught or reported by workers or visitors in the delta since the end of the second SAT cycle. Regular monitoring surveys confirmed the elimination of the flies (i.e. no flies caught) up to June 2006. Also, regular veterinary surveys confirmed the absence of cases of animal trypanosomiasis in cattle and horses around the Okavango delta. The Okavango 2001/2002 SAT operation seems to be a success story and hopefully play a role in the advocacy for the use of the technique elsewhere in sub-Saharan Africa. The cost of aerial spraying and insecticide alone (i.e. exclusive of recurrent expenditures) was US$ 270/km^2. Main factors which have contributed to a successful SAT campaign can be attributed to the following:

- The terrain is flat/undulating, perfect for SAT application;
- The distribution limits of fly population were geographically well circumscribed;
- Strong political support to eliminate T&T from the area;
- Well elaborated work plan;
- Flexible public procurement process;
- Little external influences, i.e. the project was wholly funded by the Government of Botswana.

Following the elimination of tsetse and trypanosomiasis from the Okavango delta it was logical to apply the same approach in northern Botswana to eliminate tsetse completely from the country. This could only be achieved successfully if tsetse along the border with

Namibia, in the Caprivi area, could also be removed. The 06 operation, therefore, became the first regional collaborative operation in the AU's PATTEC initiative.

Environmental monitoring studies carried out by the Harry Oppenheimer Okavango Research Centre, in association with BioTrack (Macquarie University, Australia) failed to detect any long term or irreversible impact due to SAT on non-target species.

Main points during the round table general discussion – Moderator P. Holmes

Country representatives expressed the desire to establish a harmonized mechanism for requesting technical assistance to PAAT and PAAT-mandated organizations for the planning and implementation of AfDB funded projects and for identification of other priority areas for T&T intervention.

There was a request to explore the feasibility of the application of SAT in other areas, particularly in the Southern Rift valley of Ethiopia. In addition, it was suggested to that PAAT should be asked to produce guidelines on the use of SAT and to publish them under the format of a PAAT Technical and Scientific Series publication.

Meeting participants were concerned over the lack of established, regular training programmes addressed to field operators for implementation and execution of field actions.

Recommendations

1. ***On the complexity of multiple funded and multi-institutional implemented projects:***
   - International institutions/organizations should assist in the formulation of practical guidelines for the implementation of field T&T intervention projects.

   Action: PAAT and PAAT mandated organizations.

2. ***On technical and field operational aspects of T&T intervention, UN – PAAT mandated organizations should provide technical assistance in:***
   - defining a set of baseline data to be collected;
   - tsetse rearing methodologies;
   - the application of area-wide integrated pest management;
   - land use;
   - livestock development programme(s);
   - human and animal trypanosomiasis control measures;
   - identifying institution(s) to collaborate on land use planning and environmental aspects of T&T projects.

   Action: PAAT and PAAT mandated organizations.

3. ***On FAO Liaison Officers' network***:
   - urgent measures to be taken to revitalize the FAO Liaison Officers' platform;
   - review of the Terms of Reference (TORs) of the Liaison Officers to be undertaken;

- The network explores effective harmonization with national PATTEC Co-ordinators.

Action: FAO Regional Office for Africa, Accra, Ghana; National PATTEC Co-ordinators.

4. On training:

- To evaluate currently available training capacities and identify training gaps and needs;
- To harmonize/co-ordinate ongoing training activities in different projects and link them to the PATTEC initiative.

Action: PATTEC, National AfDB-PATTEC Project Co-ordinators.

5. On Land Cover mapping:

- To continue in the refinement process of Land Cover mapping (higher resolution maps) and in the standardization process for decision support in T&T intervention.

Action: FAO.

6. On developing tsetse intervention costing models, PAG agrees on the urgent need:

- To develop guidelines on suitability and cost of various tsetse intervention techniques in different entomological/ecological situations for fly suppression and elimination.

Action: IAEA, FAO in collaboration with PAAT partners and stakeholders.

7. On re-orientation of PAAT's role, PAG agrees on the need:

- To consider enlarging PAAT role to embrace issues in tsetse infested areas related to rural development, poverty alleviation, and human health to further enhance PAAT contribution to the attainment of the MDGs.

Action: PAAT Secretariat.

8.On the use of Sequential Aerosol Technique (SAT) to suppress/eliminate tsetse fly:

- A position paper to be produced on SAT including its feasibility and limitations in different ecological situations and its potential environmental impacts.

Action: PAAT Secretariat.

9. On the possible risk of the merger of Trypanosoma brucei rhodesiense and T. b. gambiense zones in Uganda, PAG urges that:

- An advocacy be undertaken on assistance from the international community.

Action: Uganda Government and national concerned institutions/authorities.

10. On criteria for declaring an area free of tsetse and animal trypanosomiasis following intervention:

- The developed criteria be simplified and published in a form of a position paper.

Action: IAEA, FAO and PAAT.

Acknowledgement

The PAAT Advisory Group Coordinators expressed their thanks and appreciation to the Government and people of Botswana for the warm hospitality extended to the participants and for the excellent facilities placed at the disposal of the meeting.

FAO ADDRESS TO THE 29TH ISCTRC CONFERENCE

At this Conference, which was held from 1-5 October 2007 in Luanda, Angola, Mr. Raffaele Mattioli of FAO's Animal Health Service noted that since the last ISCTRC Conference, there has been intense planning and discussion, at all levels, concerning the elimination of the problem of tsetse and trypanosomiasis (T&T) from sub-Saharan Africa for the benefit of the rural poor and the whole process of sustainability of rural development. At the same time, global, and certainly African-oriented investments in agriculture and rural development, had fallen dramatically in the last 20 years, and that although poverty reduction and food security are the overarching priorities for FAO and UN sister agencies, there was a tendency to downplay or even ignore the role of agriculture in overcoming poverty. Also, there is insufficient recognition and international political commitment to the central role of livestock as the engine for agricultural development, income generation and economic growth in sub-Saharan Africa. This matter deserves great attention and it is central to the whole issue of making progress with the identification, prioritization and eventual elimination of key development problems in this vast part of the African continent. - In other words, key development constraints needed to be removed. He reminded participants that, FAO, a long time ago, identified the T&T problem as a major obstacle to be overcome for achieving Sustainable Agriculture and Rural Development (SARD), and that this view is shared by the Comprehensive Africa Agriculture Development Programme (CAADP) of the New Partnership for Africa's Development (NEPAD) initiative. However, the interaction between livestock and the multi-dimensional problem of poverty can not be seen independently from other external factors like food production, fuel, water, human health and markets. Hence, it is essential that efforts to reduce the problem of T&T are duly placed in the context of SARD, which is defined as a process that:

- Ensures that the basic nutritional requirements of present and future generations are met while providing a number of other agricultural-livestock products.

The problem posed by T&T and the promotion of SARD is vast and complex. The disease is present over approximately nine million km^2. Its impact, in monetary terms, on African agriculture Gross Domestic Product is valued at $US 4.5 billion per year. The overall impact extends to the restricted access to fertile and cultivable areas, imbalances in land use

and exploitation of natural resources and compromised growth and diversification of crop-livestock production systems. Given the magnitude of the problem and considering its complex and dynamic medical, veterinary, agricultural and rural development dimensions, the policy and the strategy to be implemented need to be comprehensive and, over and beyond the entomological and parasitological aspects of the disease, need to be oriented:

- to food security and poverty alleviation;
- to the conservation and protection of the environment;
- to policy assistance;
- to capacity building and institutional strengthening for enhanced decision making capacity.

In recognition of this complexity, the FAO General Conference in 1997 approved the Programme Against African Trypanosomiasis (PAAT), a forum which the AU/IBAR, FAO, IAEA and WHO use to concert and harmonize international efforts against T&T. The major objective of PAAT is to enable more effective T&T management and intervention programmes for improved livestock production and increased opportunities for SARD. Since its inception, other UN agencies, like UNIDO and IFAD, have adhered to the PAAT international forum and its activities. Recently, FAO/PAAT has developed partnerships with the African Livestock (ALive) initiative, the FAO/OIE Global Framework for Transboundary Animal Diseases for Africa and the Private Sector, such as the International Federation for Animal Health (IFAH) for the very important issue of Quality Control/Quality Assurance of trypanocides on the African markets.

The launch of the Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), established by the African Heads of State and Government in October 2001, underlined the recognition at the highest political level of the T&T problem as key constraint to African development, and the 31st session of the FAO Conference in November 2001 passed a resolution in support of PATTEC. In this regard, it should be recalled that both PAAT and PATTEC shared the long-term common goal of the elimination of the problem posed by the disease to the African continent.

PAAT and PATTEC are jointly working towards more general recognition of the T&T constraint as a key issue to be addressed at national, regional and international level for enhancing agricultural productivity and poverty alleviation in affected areas. One concrete example of the PAAT-PATTEC cooperation is the establishment of criteria and guiding principles for prioritising areas for T&T interventions in the context of SARD. These criteria and guidelines have been used and are going to be used by our colleagues responsible for the implementation of the projects supported by the African Development Bank in Burkina Faso, Ethiopia, Ghana, Kenya, Mali and Uganda. Also, PAAT is currently working with the FAO's Pro-Poor Livestock Policy Initiative (PPLPI), and with the Intergovernmental Authority on Development - Livestock Policy Initiative (IGAD's LPI) to further explore the costs and benefits of different control techniques. Having conducted a comparative analysis of the costs of various techniques in Uganda (with PPLPI), we are now building on work done in West Africa to map out the potential benefits of tsetse control in the IGAD Region of East Africa. This will help IGAD Member States and PATTEC to make informed decision on where and how to control T&T. Additional FAO/PAAT-IGAD LPI collaborative work includes the compilation and analysis of livelihoods zones in the Horn of Africa. We strongly believe that

the application of these criteria and guidelines are robust instruments which increase the feasibility and the efficacy of the interventions.

In addition, FAO/PAAT efforts and activity are directed to the harmonization of strategies and to the production of tools for T&T field programmes for policy makers and advisors, planners, scientific and technical staff. I would like to mention just a few of the most recent FAO/PAAT products and activity, such as:

- The standardization of land cover mapping for T&T intervention;
- The selection of global datasets for the management of trypanosomiasis problem: an environmental approach;
- The production of guidelines aiming at linking sustainable human and animal African trypanosomiasis control with rural development strategies;
- The study and publication of the book on mapping the benefits: developing a new decision tool for tsetse and trypanosomiasis intervention

An Interactive Training Workshop on "Harmonization of GIS-based decision support systems and information systems in T&T intervention" was organized late 2006 in FAO Rome. Among the field activities, mention should be made of the joint Ethiopian Government/IAEA/FAO project aiming at creating a zone free of tsetse and trypanosomiasis. This project is funded by the Japanese Government, through the UN Trust Fund for Human Security and it is executed in the southern part of the Rift valley in Ethiopia.

This was very short presentation of the problem posed by T&T to the development of agriculture and livestock in sub-Saharan Africa and of the role that FAO and PAAT play in support to FAO Member Nations and PATTEC in addressing the problem. However, in concluding, Mr. Mattioli stated that he was convinced that with the support and in collaboration with African colleagues, PATTEC and members of the international community, FAO, the PAAT alliance and others will successfully tackle the disease in sub-Saharan Africa, and thereby, contribute to SARD.

THE FAO/IAEA PROGRAMME

The FAO/IAEA Programme continued its active support of research, technology transfer and provision of science-based information to Member States of FAO and the IAEA relevant to the planning and implementation of area-wide integrated pest management activites involving the sterile insect technique (SIT). One major recent output from the programme was the publication of a textbook entitled "Area-Wide Control of Insect Pests: From Research to Field Implementation" and details of this are given in the Section on Book Publications. Full details on all activities, publications etc. can be obtained in the Insect Pest Control Newsletter which is published twice yearly (see http://www-naweb.iaea.org/nafa/index.html or http://www.fao.org/waicent/FAOINFO/AGRICULT/default.htm). Some recent highlights include:

Inauguration of the Tsetse Rearing and Irradiation Centre at Kaliti, Addis Ababa, Ethiopia (Technical Cooperation Project ETH5012).

The first two modules of the tsetse rearing and irradiation centre, located at Kaliti, Addis Ababa, Ethiopia of the Ethiopian southern tsetse eradication project (STEP) (supported under technical cooperation project ETH5012) was officially inaugurated on 3 February 2007. The inauguration benefited from being organized subsequently to the AU-PATTEC (African Union-Pan African Tsetse and Trypanosomiasis Eradication Campaign) Special Donors' Conference. Delegates from tsetse and trypanosomosis- (T&T) affected Member States, as well as, donor representatives were impressed by the facility. The event was well organized and numerous heads of delegations, including several ministers and ambassadors, participated in the meeting. As part of the opening ceremony, which was chaired by the Ethiopian Deputy Prime Minister, H.E. Addisu Legesse, Mr. Liang Qu, Director of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture made a brief statement on behalf of the Food and Agriculture Organization of the United Nations (FAO). All invited guests joined a tour through one of the fly production modules.

Third Research Coordination Meeting of the CRP on Improved and Harmonized Quality Control for Expanded Tsetse Production, Sterilization and Field Application 7-11 May 2007, Muguga, Kikuyu, Kenya.

The third research coordination meeting (RCM) of the CRP on Improved and Harmonized Quality Control for Expanded Tsetse Production, Sterilization and Field Application was held at the Trypanosomiasis Research Centre, Kenya Agricultural Research Organization (KARI-TRC), Muguga, Kikuyu, Kenya from 7-11 May 2007.

The meeting was attended by thirteen participants from twelve countries, three external observers and a number of observers from the host institute. The meeting was opened by the FAO Country Representative, Dr Castro Camarada, who recognized the burden caused by trypanosomosis and the role that the sterile insect technique could play in the eradication of tsetse flies for the control of African Human and Animal Trypanosomosis. The following presentations were divided into three groups on tsetse rearing, including blood collection and processing; tsetse development and behaviour; and methods to control the risk of released sterile flies picking up trypanosomes and transmitting the disease.

In the first group, improvements to the standard quality control parameters for rearing were presented and the problems of applying the standards under African rearing conditions were discussed. In particular the collection of sufficiently clean blood for tsetse diet is difficult in many locations. In the second group a detailed analysis of the genitalic structures in *Glossina pallidipes* and their relationship to mating behaviour and successful insemination was presented. A second presentation on flight muscle development under colony conditions and how this can be modified by enforced exercise demonstrated the possibility of relatively simple measures to improve the performance of released sterile flies. As part of this work the maintenance of a small population of flies in a greenhouse under essentially free-flying conditions was described; considerable progress has been achieved in this with flies feeding on an artificial lure and surviving for more than 30 days. In the third group of presentations work on the use of Samorin® as a prophylactic to prevent infection of released flies with

trypanosomes was presented. The results from Kenya and Belgium differed considerably, and the reasons for this were discussed.

Subsequent group discussion focused on developing harmonized work programmes for the remainder of the CRP. Many of the participants were able to contribute new ideas and insights to other participants and plans were prepared to resolve some of the differences highlighted by the presentations.

Research in Seibersdorf – Tsetse Flies

Colony Status

The improved performance of the *Glossina pallidipes* colony has continued through the first part of this year. The target colony size of 15 000 females was reached by week 13 and since week 11 a total of 12 000 surplus females have been added to the tsetse production unit (TPU3.2) (see below). The rate of salivary gland hypertrophy observed in the colony has fallen to about 7.5 percent from the peak of 11 percent seen late last year.

The colonies in Bratislava have also continued to grow since the beginning of the year. The *Glossina pallidipes* colony continued to decline for the first five weeks, but has now recovered to 10 692, 1300 higher than in week 1. The *Glossina fuscipes fuscipes* and *Glossina morsitans* have both grown continuously since the beginning of the year, such that the total holding in Bratislava exceeded 67 000 females by week 15. The colonies could now support some shipments to Ethiopia, but due to problems with the electrical installation in the Ethiopian rearing facility they have not been able to receive any material this year.

The improvements in the colony in both Seibersdorf and Bratislava have been attributed to the use of a new batch of blood late in 2006. Chemical analysis of the previous batch however has not revealed any significant contaminant and the actual cause of the improvement remains unknown.

TPU3.2

An initial test with one frame, holding 9 cages of *G. pallidipes* was started in week two, and when this reached 3.27 pupae per initial female (sufficient to ensure a growing colony) a number of frames of *G. pallidipes* were placed on the TPU3.2 from week 11, totalling 12 000 females. A number of problems continue to be encountered, but the most important issue of successful feeding now seems to be solved. Careful alignment of the feeding tray and membrane with the cages ensures that the cages all fit down flat on the membrane surface, coupled with adjustment of the feeding plate temperature, this has lead to effective engorgement of the flies.

We continue to make small adjustments to the design to improve the ease of use and efficacy of the system. The problem of aligning the feeding membranes with the cages is mostly due to the progressive shrinkage of the membranes with repeated washing and heat sterilizing. The membranes, which start at 640 x 640 mm, shrink by 20-25 mm over a period of three months. This shrinkage means that it is difficult to ensure that the cages (600 x 600

mm) will fit completely on the membrane. Increasing the membranes to 660 x 660 mm will ensure that sufficient tolerance is still available, even after the membranes have shrunk 25 mm. Some variation in the shrinkage was also noted with different types of netting bedded into the silicone to reinforce the membrane, leading to asymmetric shrinkage, but this is not enough to cause a problem once the large membranes are used. Other changes that have been made include changing the material for the cage arms to improve stability and for the pupal collection slopes to improve the strength and reduce weight, and providing an easier system for releasing the lock to allow the feeding trolley to move. Further improvements will be implemented as the opportunity arises.

Sexing of Pupae

As reported in the last newsletter (number 68), successive readings with the NIR (near infrared) spectrometer from a single pupa, vary considerable. To try to understand this variability we positioned pupae in the scanner in such a manner that we could rotate the pupa through a known angle about its long axis whilst keeping it in the spectrometer focal point. The pupae were mounted by gluing the anterior end to a metal rod in a holder with an index mark. This was mounted in a plate with an engraved scale, allowing the pupae to be re-positioned for repeat readings. Results from four male and four female pupae, with readings taken every 20 degrees showed that there was large variation in individual readings, which coupled with the variation with rotation, means that randomly aligned pupae will sometimes miss-classify, with female pupae sometimes giving higher values than male pupae. However in certain specific orientations (around 140 degrees in this case) the values for males and females are well separated.

Determining the position of pupae in relation to the dorso-ventral axis would be very difficult. The breathing lobes (apneustic lobes) are deflected towards the ventral aspect and in principle this could be used to determine the orientation, but the deflection is small and will be difficult to observe automatically at speed during the sorting. An alternative would be to take the reading from the anterior end of the pupa rather from the girth; this would avoid the rotational asymmetry, and it should be relatively simple to determine if the pupa is oriented with the anterior or posterior uppermost. Work will continue to determine the best conditions under which to run the sorting.

Salivary Gland Hyperplasia (SGH)

As reported in the last newsletter (no. 68), sequencing of the salivary gland hypertrophy virus (SGHV) was approached using two techniques; the shotgun method by fragmenting the genome with EcoRI restriction endonuclease resulted in 415 clones being sequenced totalling 60-90 kpb and the pyrophosphate sequencing by 454 Life Science in the USA, which gave more than 34 000 reads, assembled into 402 contigs. Intensive work has continued to combine these sequences with additional sequences extracted from the 454 data and new sequences from targeted PCR reactions, which has resulted in determining that the genome is circular with a sequence of 189 571 nucleotides. Final checking of the sequence is expected to be completed in the next few months, and the draft sequence has been submitted to GenBank (EF568108).

Two consultants group meetings were held on the analysis of the genome sequence, the first on 18-20 December 2006 under the title Genome Characterisation of the Tsetse Salivary Gland Hypertrophy Virus and the second on 11-13 April 2007 under the title "Finalizing the Genome Sequence of the Tsetse Salivary Gland Hypertrophy Virus". During the meetings the sequence data were discussed and the repeat regions found in the sequence were analyzed. Also a phylogenetic analysis of the DNA polymerase was carried out. The results of the phylogenetic analysis indicate that the predicted amino acid sequence of the DNA polymerase of SGHV was aligned with selected DNA polymerase of other large dsDNA viruses using Clustal W in BioEdit. Subsequently, a phylogenetic analysis (NJ) was performed using MEGA3.1 software.

The generated tree shows that the SGHV DNA polymerase does not cluster with the Baculoviruses or Nudiviruses, but with Iridoviruses, Herpesviruses and Phycodnaviruses. The bootstrap value for this position is very high (95 percent). Therefore it can be excluded that the SGHV DNA polymerase is phylogenetically closely related to Baculoviruses or Nudiviruses. SGHV might represent a new virus family. The virus has some genes which have homology with other insect viruses, especially the genes involved in the early infection steps like p74, pif-1 pif-2 and pif-3 of baculoviruses. Preliminary work has been undertaken to clone the p47 gene into baculovirus expression vector to produce this protein as a first step towards producing antibodies against this protein.

Another important aspect is the study of the impact of antiviral drugs on viral infection in the fly; four antiviral drugs were tested to determine their toxicity effect on tsetse. From this preliminary screening two drugs, Acyclovir and Valacyclovir, were selected for further work, the other two drugs being too toxic for use in tsetse feeding. To analyse the effect of the antiviral drugs on viral DNA replication a quantitative PCR test was established by choosing two primers and a preliminary test to quantify the number of viral DNA copies made. Work will continue on this aspect, principally using Valacyclovir.

THE INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE (ILRI)

Environment and sustainable land management in T&T intervention areas

Research on environmental and socio-economic impact assessment under the support of the United States State Department has generated a framework and methodological guidelines for assessing the impacts of T&T interventions. This work is being published in the ILRI Manuals or Guides Issue Nos, 4 and 5. This work was done with the collaboration of the PATTEC regional coordination office, PATTEC project coordinators in the six countries implementing the first phase of AfDB project, and AU-IBAR. The framework and guidelines are now being applied in Kenya to develop a baseline data base for environmental and land cover management in the PATTEC project areas, with the support of the Kenyan government through AfDB funds.

With funds expected from the National Institute of Health (NIH), ILRI in collaboration with Michigan State University will identify the linkages between climate, land use, land cover, socio-demographic factors and tsetse distribution. This work will include analysis of

the effects of climate change on vegetation and land use and land cover and how direct interventions impact tsetse distribution. These activities will provide an enhanced understanding of the impacts of climate change in tsetse systems and how these systems adapt, and develop appropriate planning and intervention scenarios for research and development to sustain livelihoods. This project will be implemented in Kenya in collaboration with the Kenya PATTEC office and will provide information replicable to other PATTEC regions.

ILRI is also working with four of the AfDB PATTEC countries to identify and apply best options for sustainable land management in tsetse-freed areas. The proposed project will identify appropriate land use practices that enhance sustainable natural resources management, agro-biodiversity, and land management strategies in tsetse-freed areas, considering the impacts of tsetse control / eradication interventions on environmental, social and economical systems and their consequences on rural development and poverty reduction. The sustainable forest, biodiversity and land management practices will provide a platform for sustainable utilization of land and economic growth to benefit rural communities where the trypanosomiasis constraint has been removed.

Twenty years of work by ILRI in the formerly tsetse-infested Ghibe Valley of South-western Ethiopia have recently been transformed into community-led livestock disease control via the formation of animal health "cooperatives". Members contribute money to a revolving fund used to buy veterinary drugs to control animal sleeping sickness. The scheme is highly successful. Hundreds of farmers line up every month to pay for the treatments and the drugs are greatly improving the health of their livestock. Farmer-to-farmer knowledge transfer is now speeding the scaling out of these community-based schemes to control livestock disease.

Improving the Management of Trypanocide Resistance

The Coordinated Regional Project on Improving the Management of Trypanocide Resistance in the Cotton Zone of West Africa which was summarized in TTI 29(1), pp. 44-45, has led to the publication of several working papers under ILRI's project publication series. Those published to date are listed below and are available from the ILRI website (www.ilri.org).

1. Grace D. Village Atelier and Participatory Rural Appraisal. Project Working Paper No. 1, February 2003.
2. Grace D. Participative trypanosomosis control in Burkina Faso: Lessons learned, ways forward. Project Working Paper No. 2, March 2003.
3. Grace D. Making Choices: Participatory planning for community trypanosomosis control. Project Working Paper No. 3, April 2003.
4. Grace D. Rational Drug Use for the management of trypanosomosis and trypanocide resistance. Project Working Paper No. 4, May 2003.
5. Grace D. Training Farmers in Rational Drug Use: Workshop Report. Project Working Paper No. 5, June 2003.
6. Grace D. Taking Stock: Monitoring and evaluation of community trypanosomosis control. Project Working Paper No. 6, December 2003.

7. Grace D. Managing trypanosomosis: Knowledge, Attitude and Practice in Upper Guinea. Project Working Paper No. 7, October 2004.

Student Theses

Affognon, H. D., 2007. Economic analysis of trypanocide use in villages under risk of drug resistance in West Africa. *PhD dissertation, University of Hanover.*

This thesis carries out an economic analysis of the use of drugs (isometamidium and diminazene) in controlling African Animal Trypanosomosis (AAT), a debilitating disease of cattle and small ruminants, in villages that exhibit resistance to isometamidium (ISMM) in Burkina Faso and Mali. We used a production function framework that integrates a damage control function to quantify cattle production losses, as well as the productivity effect of trypanocide use under different epidemiological conditions. The study was conducted from June 2003 to May 2004. Data were collected by a team of veterinary epidemiologists, agricultural economists and technicians. In all, 206 herds totalling 3565 cattle in eighteen villages were monitored during a period of 12 months. Input and output data were collected in villages for which epidemiological conditions were assessed throughout the study period. Additional price information was collected in local markets, abattoirs and through focus group discussions. The study confirms that trypanosomosis is an important disease in the cotton zone of West Africa. We found that the marginal value products of ISMM in all epidemiological conditions, and the marginal value product of diminazene (DIM) in high-prevalence-high-resistance conditions, reveal an underuse of trypanocidal drugs. The economic interpretation is that in the short term cattle farmers could increase the profitability of cattle rearing in those conditions by increasing trypanocide input beyond current levels. On the other hand, the static analysis applied in this study does not take into account the negative externality of trypanocide resistance in the future. If the use of trypanocide increases, cattle farmers will also be more likely to experience future losses from trypanocide resistance. Although drug resistance is increasing, trypanocidal drugs used are still effective against the disease. However, at the current sub-optimal level of ISMM use, output losses are much higher—9.8 percent to 22.7 percent of the value of output—than in a situation where ISMM use is optimal for all epidemiological conditions. When disease control effort reaches the optimum level, output losses are much lower—1.3 percent to 1.5 percent of output. At the current use of trypanocidal drugs, economic losses due to trypanosomosis range from €9.50 to €22.00 per TLU[1] per year. The costs of trypanosomosis at the current level of disease control effort, which include the control costs and the remaining loss after control are higher than they would be if ISMM use was at optimal levels, in all epidemiological conditions. Currently, trypanosomosis disease costs cattle farmers €13.30 to €26.00 per TLU/year; however, at optimal disease control efforts, costs would be reduced to €8.60 to €10.10 per TLU/year, depending on epidemiological conditions. While the current costs of the disease represent on average 12 percent to 28 percent of the output derived from cattle production in the study area, costs of the disease at optimal drug usage would represent only 7 percent to 8 percent of output depending on disease prevalence and drug resistance levels. Lower costs of the disease and the increasing productivity of trypanocide in conditions of high drug resistance may create an intractable situation in which cattle farmers' choices for

[1] TLU = Tropical Livestock Unit, corresponding to a bovine of 250 kg.

trypanosomosis control measures are guided by the phenomenon of path dependency. Once this occurs, the only options for controlling the disease would be the discovery of new drugs, for which the development is prohibitively expensive, or eradication of the tsetse flies, vectors of trypanosomosis—a strategy that has not been sustainable without external funding support. Maintaining the effectiveness of trypanocides is hence a priority for farming systems in West Africa.

Barry, A. M., 2006. La Trypanosomose Animale Africaine chez les Bovins N'Damas en Zone cotonnière de Haute Guinée (cas de la Préfecture de Mandiana). *Doctorat de spécialité, ISFRA, University of Mali.* [African animal trypanosomosis in N'dama cattle in the cotton zone of Guinea: The case of the Mandiana District]

In West Africa, failures in trypanocidal treatments have increased and have been found in cotton- producing regions. Chemical resistance to trypanocides has already been described in Mali and Burkina Faso. Because Guinea presents similar cattle rearing practices and has recently developed cotton production, resistance may also be suspected to exist. This hypothesis was tested in the north eastern region of Guinea by two studies. First, 1800 cattle from 30 villages located in Mandiana District were examined in a cross-sectional survey. The aim of the study was to assess trypanosomiasis risk linked to the host (cattle) and the vector (tsetse fly *Glossina*). Information on drug treatments permitted assessing the risk of resistance. The mean prevalence of infection in cattle in all the localities was 3 ± 3.84. The density of cattle was 0.7±1.08 in these villages. Concerning trypanocidal drugs, owners usually used diminazene aceturate and isometamidium chloride, both for cure and prophylaxis. However, they did not seem to be aware of their usage. One third of them treated cattle twice annually based on the symptoms. Only 40 percent of the recorded treatments were given by animal health professionals. Little information is available concerning trypanocidal drug supply, but formal and informal circuits seem to be equally involved. Second, block treatments with isometamidium chloride were performed to assess the importance and spatial trends of trypanocide resistance in Mandiana District. We chose 300 cattle in 3 villages. In each village, half of the animals (50) were treated with 1 mg/kg isometamidium chloride and the others (50) remained untreated. All the animals were monitored during 56 days and were checked by BCT method twice a month. At each control, positive animals were treated with 3.5 mg/kg diminazene aceturate for *T. congolense* and *T. vivax* infections and with 7 mg/kg for *T. brucei* infections. Early treatment failures were observed (the first two weeks of the monitoring) after treatment with isometamidium chloride for the three villages. Then, further block treatments were conducted on 1200 cattle in 10 villages around Saladou and 5 around Dialakoro, in order to assess the extent of chemical resistance in the region. In each village, half of the animals (40) were treated with 1 mg/kg isometamidium chloride and the others (40) remained untreated. All the animals were monitored during 28 days. Checking and treating positive animals were conducted as previously described. Among 15 villages, 4 failures were detected in different localities and might be attributed to treatment failure phenomena linked to the host. Third, 11 infected blood samples were collected during block treatments and 3 of them were reactivated in mice. These were *in vivo* tested for trypanocidal resistance on N'dama cattle. Among 13 calves, 9 (3 per sample) were infected and treated with 0,5mg/kg isometamidium chloride; 3 (1 per sample) were also infected but not treated (positive controls), and one remained uninfected (negative control). All calves were monitored during 100 days and no failure was

recorded in any treated calves. These results showed that treatment failures previously recorded in villages could be plausible attributed to new infections or to deficient immune status of animals and not to real resistance of trypanosome strains. Fourth, in an attempt to increase the sensitivity of diagnosis,, samples were also tested using PCR. While only 15 samples were positive in BCT, there were 78 positives using PCR indicating a 5-fold increase in sensitivity.

Dabiré, D., 2005. Sociological determinants of rural communication concerning Animal African Trypanosomosis among agropastoralists in Kenedougou Province, Burkina-Faso**.** *Maîtrise, University of Ouagadougou.*

As part of the activities of the project "Improved management of trypanocide resistance in the cotton zone of west Africa", a sociological study was conducted in 2004 in the south of Kenedougou, Burkina Faso. This study aimed at identifying and analysing socio-cultural factors that influence farmers' attitudes and strategies in their seeking information on Animal African Trypanosomosis (AAT). The methodology used was based on qualitative and quantitative surveys. In the face of persistent disease and frequent treatment failures with trypanocidal drugs, farmers and services providers have evolved a system based on multiple sources of information where drug sellers play a central role. Oral communication (95 percent) and images on the drug packaging (64 percent) are the main communication supports. Farmers use two communication strategies to acquire information: one at individual level and the other at collective level underpinned by socio-cultural habits in health and consumption management. Beyond these socio-cultural habits, three factors determine the recourse of farmers to a given source of information. These are:

- Its quality, meaning its credibility as perceived by the users (97.7 percent), proximity (88.4 percent) and availability/accessibility (84.8 percent);
- The socio-professional characteristics of the information provider: qualifications, education, experience and motivation;
- The sociological characteristics of farmers, such as ethnic group, religion, education, training, experience, and family status. Ethnic group and education appear to be the most significant factors.

However, certain socio-cultural, socio-economic, technical, professional, institutional and contextual problems encountered in daily life may bias the emitted or received information. The principal biases are due to restricted number or frequency of contacts and emerging sources that block, distort, retain and deform information. Consequently, farmers increasingly express the need for quality information on AAT.

Dao, D., 2005. Determinism of human factors in the control of Animal African Trypanosomosis: the case of the agro-pastoralists from the Department of Mandiana, Upper Guinea. *Maîtrise, University of Ouagadougou.*

This study, conducted as a Maîtrise thesis project, contributes to improving the control of African Animal Trypanosomosis (AAT) which is currently one of the major constraints to livestock development in sub-Saharan Africa. The target population was agro-pastoralists in Mandiana Department, Upper Guinea. The main objective was to understand socio-cultural

practices of agro-pastoralists and the influence of service providers in the control of AAT, and to analyse farmers' relevant knowledge, attitudes and practices. The following hypothesis emerged: the perception of AAT and its representation vary as a function of knowledge, attitudes and practices of the agro-pastoralists in Mandiana, depending particularly on whether they are small, medium or large producers and empirical experience in managing this disease. This study revealed that agro-pastoralists have partial knowledge about the causes of AAT. Of the surveyed farmers, 68 percent believe that tsetse flies are the main cause of trypanosomosis. More than 50 percent of farmers know some of the typical symptoms of AAT and 86 percent of treatments are with trypanocidal drugs exclusively. Farmer behaviour towards the disease is influenced by their experience, their cattle numbers, their level of education and access to service providers. Agro-pastoralists prefer using service providers from the formal sector, but their non availability and the high cost of their services lead the agro-pastoralists to seek for drugs and services from informal sector or treat animals themselves. Of the surveyed farmers 54 percent treat their animals themselves and 21 percent seek the services of non professionals. Nearly all treatments (99 percent) made by qualified service providers (veterinarians, technicians and paravets) were performed correctly. However, nearly half of the treatments (47 percent) performed by non professionals and by farmers were not successful. The study confirms that there is a common effort to control the AAT in this area, but many of the treatments made by non professionals are not successful. Direct training of non professionals and paravets is a crucial aspect of the problem not yet addressed.

THE LEVERHULME TRUST TSETSE RESEARCH NETWORK (LTTRN)

The Leverhulme Trust Tsetse Research Network (LTTRN) was formed in 2004 as an association of research scientists and control personnel with common interests in promoting activities in support of initiatives to control tsetse and interrupt the transmission of African trypanosomosis. The network has an underlying theme of promoting collaborative research and training to improve understanding of the biology and control of tsetse, and in support of control and surveillance activities directed against the disease and its insect vectors – especially in association with the PATTEC (Pan African Tsetse and Trypanosomiasis Eradication Campaign) initiative of the African Union.

The inaugural workshop of the LTTRN was held at the African Union (AU) Headquarters, Addis Ababa, Ethiopia, 5-6 February 2005, immediately followed by the 4th meeting of the AU-PATTEC Policy Committee during which the LTTRN was formally welcomed and adopted as the research and technical support arm of the AU-PATTEC initiative.

The network holds periodic meetings, the most recent of which was held in CIRAD/IRD Montpellier, France from 2-4 March, 2007. The objectives of the meeting were:

- To inform about current activities based in Europe that have actual or potential relevance to tsetse and trypanosomosis control (especially within the context of AU-PATTEC);
- To consider preparation of a summary paper for European funding organizations, particularly in relation to development of FP7 within the European Commission;

- To make best use of available knowledge and expertise to suggest areas of Africa where tsetse and trypanosomosis elimination might be operationally feasible, and recommend what additional research would be of significance for refining such concepts;
- To clarify the types of control interventions that are likely to be of greatest applicability over a large scale.

The meeting was attended by researchers from France, Belgium, the UK, Germany, Burkina Faso, Thailand and representatives from AU-PATTEC, TDR/WHO (Special Programme for Research and Training in Tropical Diseases of WHO) and the FAO/IAEA. Three commercial participants also attended.

Following the formal presentations several topics were discussed. Principal amongst these were: considering biological and physical conditions only in which areas was eradication most likely to be achieved; in which areas would eradication be most difficult; what effect do seasonal factors have on tsetse control, and where would these have most effect; which control techniques have the greatest applicability over the geographical scales envisaged by AU-PATTEC; in practical terms, what is the maximum area that can be controlled by the various available techniques within one season/year; what are the main geographical features that contribute to population structuring in tsetse, and if a population was removed to what extent would it be replaced by a neighbouring population; if tsetse can be eliminated from an area, how can this be confirmed; is there any evidence for genome erosion in tsetse; and given that reinfestation after local control has frequently occurred in the past, what would be the most informative markers to identify the likely source of immigration?

Amongst the results of these discussions, it was generally agreed that: isolated populations need to be identified if eradication is to be successfully maintained; isolation will be caused by topographic barriers or low rainfall areas; the scale of operation possible in one season with any technique will depend on the specific situation; the practical maximum area of an operation utilizing insecticide treated cattle would only reach the order of 10 000 km^2 in areas with good dipping infrastructure and veterinary services but could be as little as 1000 km^2 without this infrastructure; coordination and monitoring of community based control are likely to be practicable only over areas of 1-2000 km^2; and it is not clear that the necessary climatic, topographic and vegetation conditions for sustaining the effects of sequential aerial spraying, as formerly applied in Zimbabwe and recently in Botswana/Namibia/Zambia/Angola, would be found further north in Africa.

BOOK PUBLICATIONS

The Fatal Sleep

Peter Kennedy, Luath Press Ltd., The Royal Mile, Edinburgh EH1. Price approx. $US 40.
Sleeping sickness, also known as human African trypanosomiasis, is one of Africa's major killers. It puts 60 million people at risk of infection, occurs in 36 countries in sub-Saharan Africa, and claims the lives of many thousands of people every year.Transmitted by the tsetse fly, trypanosomiasis affects both humans and cattle. The animal form of the disease severely limits livestock production and farming, and in people the toxic effects of the treatment can

be as painful and dangerous as the disease itself.Existing in the shadow of AIDS and malaria, it is an overlooked disease, largely ignored by pharmaceutical companies and neglected by the western world. The Fatal Sleep traces a medical passion over 30 years, taking the reader on an exciting and captivating medical and scientific journey into Africa. Peter Kennedy has devoted much of his working life to researching sleeping sickness in Africa, and this first-hand account shares his trials and experiences, evoking our empathy with the affected patients, together with an explanation of the disease, including its history and its future. Interweaved with African geography and history, his compassionate story reveals what it is like to be a young doctor falling in love with Africa, and tells of his building of a vocation in the search for a cure for this cruel disease.

Area-Wide Control of Insect Pests: From Research to Field Implementation.

Editors: Vreysen, M. J. B., Robinson, A. S., & Hendrichs, J., 2007. Springer, Dordrecht, The Netherlands. 792 pp., 31 illustrations in colour. Hardcover ISBN: 978-1-4020-6058-8.

Price: around 190 Euro.

The world population is still growing at an alarming rate, requiring ever increasing productivity and less waste in agriculture to cope with the increasing demands to satisfy food security for all humans. Alleviation of poverty is in many countries hampered by a myriad of insect pests that cause enormous economic losses to agricultural commodities, both at the pre- and postharvest stages. Initially, most of these insect pests were controlled to a varying degree by the use of broad-spectrum insecticides. However, the indiscriminate use of these chemicals as a control tactic is no longer sustainable in view of increased development of resistance, pollution of soils and surface water, residues in food and the environment, representing risks to human health and biodiversity, etc. As a consequence, demands have been voiced at least since “Silent Spring” in 1962 for control tactics and approaches that are not only efficient, but also sustainable and friendlier to the environment. Integrated pest management (IPM) has been accepted since the 1960’s and 70s as a viable pest management strategy that aims at integrating control tactics to maintain damage levels below a certain economic threshold level whilst also protecting the environment by thriving to limit the use of pesticides. Classical IPM is however a localized approach, with the objective of protecting crops or livestock that is largely under the control of each farmer, with little collaboration or any coordinating structure. Control is exercised only in the areas of economic interest, often resulting in the main or residual pest population pockets remaining in the surrounding areas that have no economic value. These constitute permanent sources from where the commercial areas under control are re-invaded. A quite different, more efficient and sustainable approach is the integration of control tactics against an entire pest population, i.e. area-wide integrated pest management (AW-IPM) or total population management. The AW-IPM is a coordinated, sustainable and preventive approach that targets pest populations in all areas, including non-commercial urban settings, non-cultivated and wild host areas. The coordination required among farmers and all other stakeholders for an area-wide approach, makes AW-IPM programmes complex, management intensive, requiring long-term commitment and funding. Although they result in more sustainable control of insect pests, there is by no means a guarantee for success.

This new textbook on area-wide control of insect pests collates a series of selected papers that attempts to address various fundamental components of AW-IPM, e.g. the importance of relevant problem-solving research, the need for essential baseline data, the significance of adequate tools for appropriate control strategies, and the value of pilot trials, etc. Of special interest are the numerous papers on pilot and operational programmes that pay special attention to practical problems encountered during programme implementation. The book is a compilation of 66 papers that are authored by experts from more than 30 countries. Each paper was peer-reviewed by at least one, in most case two or more independent, outside experts and edited for the English language by Dr James Dargie, former Director of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture. We both thank the many reviewers and Jim whose meticulous work and suggestions improved many of the papers. In addition, the editors subjected each paper to an in-depth technical quality control process. As a result, we trust that the technical quality of the papers is optimal, the information provided accurate, up-to-date and of a high international standard. This process of peer-review, editing and formatting has taken considerable time and we appreciate the patience of the authors.

The book is organized into 8 Sections, the first of which contains two papers on "Scene Setting". This is followed by Sections dealing with Basic Research, Modelling and Methods Development, Feasibility Studies, Commercialization and Regulation, Pilot Programmes and Operational AW-IPM programmes, and finally with a Section on Lessons Learned. The book covers many insect pest species, scientific and technological developments ranging from mass rearing, cryogenics, GIS, aerial navigation technologies of pests through to transgenic approaches for their control and the challenges of commercialization and regulation of area-wide management operations. For the readers of TTI and those in general concerned with meeting the challenges posed by African trypanosomiasis, the introduction provided by Waldemar Klassen [14176] and the scene-setting papers of Hendrichs *et al*., [14173] and Pimental [14182] are well worth a read. For those more interested in basic research, the paper by Aksoy and Weiss dealing with tsetse symbionts [14189] will be valuable, while those with a bent for modeling and methods development will learn much from the papers by Cox [14168] and Boyer *et al*., [14203] to be very valuable. While there are many interesting papers dealing with the feasibility of embarking on an area-wide IPM programme involving the SIT, the papers by Kappmeier *et al*., [14211] and by Alemu *et al*., [14209] are particularly relevant for tsetse management. Finally, the paper by Vreysen *et al*., [14188] is really a "must read" for all interested in African trypanosomiasis although other species are also covered.

All these papers, as well as that by Devorshak [14169] dealing with regulation are abstracted in this issue of TTI as indicated in the square brackets. Since this book is just "hot off the press" and the editor of TTI was to some extent involved in its production, an independent review of this book must await the next volume of TTI. However, for those scientists and institutions in developing countries concerned about the price tag, the Editor suggests that contact is made with Marc Vreysen of the Joint FAO/IAEA Division (m.vreysen @iaea.org) for additional information.

DEATH FROM A FLY

By John P. Kabayo, PATTEC Coordinator

One bite,
One red site
You begin the fight
Win which you can't.
Lassitude and malaise
Fevers and a haze
For a year or more
Not letting go.
Insomnia in the night
Drowsier in the day
Pain in the head,
Might wake the dead
Rash on my trunk,
My chest and back;
More when it's hot
Or when it's not.
Muscles cramp
Glands swell
Pulses up, fevers up
Rigours and sweat.
Drowsiness to sleepiness,
Melancholy distress;
Memory fades and goes,
Coma sets in,
.....and then death.

SECTION B - ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

14165. **Berenger, J. M. & Pages, F., 2007**. *Triatominae*: growing trend to domesticity. *Médecine Tropicale (Mars),* **67** (3): 217-222.

Unité d'Entomologie médicale, Institut de Médecine Tropicale du Service de Santé des Armées, BP 46 Le Pharo, 13998 Marseille. [imtssa.entomo@wanadoo.fr].

Triatominae are biting hematophageous insects that have been wild vectors of the parasite *Trypanosoma cruzi* for thousands of years. The arrival of man with his cortege of domestic animals and impact on the natural environment led these insects to adapt to the human environment so well that many species are now domesticated. Insect extermination programmes have allowed satisfactory control of parasite transmission but have also promoted replacement of the exterminated species by species that were once semi-domestic or wild.

14166. **Beyrer, C., Villar, J. C., Suwanvanichkij, V., Singh, S., Baral, S. D. & Mills, E. J., 2007.** Neglected diseases, civil conflicts, and the right to health. *Lancet,* **370** (9587): 619-627.

Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA. [cbreyer@jhsph.edu].

Neglected diseases remain one of the largest causes of disease and mortality. In addition to the difficulties in provision of appropriate drugs for specific diseases, many other factors contribute to the prevalence of such diseases and the difficulties in reducing their burden. We address the role that poor governance and politically motivated oppression have on the epidemiology of neglected diseases. We give case examples including filariasis in eastern Burma and vector-borne diseases (Chagas' disease, leishmaniasis, and yellow fever) in Colombia, we show the links between systematic human rights violations and the effects of infectious disease on health. We also discuss the role of researchers in advocating for and researching within oppressed populations.

14167. **Chronaki, C. E., Berthier, A., Lleo, M. M., Esterle, L., Lenglet, A., Simon, F., Josseran, L., Lafaye, M., Matsakis, Y., Tabasco, A. & Braak, L., 2007**. A satellite infrastructure for health early warning in post-disaster health management. *Medinfo,* **12** (Pt 1): 87-91.

Institute of Computer Science, FORTH, Heraklion, Crete, Greece. [chronaki@ics.forth.gr].

The risk of epidemics and emerging or re-emerging diseases such as avian flu, tuberculosis, malaria and other vector-borne diseases, is rising. These risks can be contained with prevention, early warning, and prompt management. Despite progress in information technology, communication is still a bottleneck for health early warning and response

systems in post-disaster situations. This paper presents Satellites for Epidemiology (SAFE), a component-based interoperable architecture for health early warning that employs satellite, radio, and wireless networks, geographic information systems, integration technology, and data mining to promptly identify and respond to a disease outbreak. In a post-disaster situation, a mobile health emergency coordination center is established and integrated to public health services for health monitoring. The added-value of SAFE for post-disaster health management will be demonstrated as part of an earthquake readiness exercise regarding a typhoid fever epidemic, in the island of Crete. Advanced communication and data mining techniques in SAFE offer new tools to the "Epidemic Intelligence" and contribute to advanced preparedness and prompt response by lifting communication barriers, promoting collaboration, and reducing the isolation of affected areas.

14168. **Cox, J. St. H., 2007.** The role of geographic information systems and spatial analysis in area-wide vector control programmes. In: *Area –Wide Control of Insect Pests: From Research to Field Implementation*. Springer,The Netherlands, pp.199-211

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The success of area-wide interventions aimed at suppressing or eradicating insect populations rests largely on appropriate project planning and implementation - and this is as true in the context of vector-borne diseases as it is within the wider context of insect pest management. In either context, a successful control programme requires accurate knowledge of pre-existing distributions of insects (disease vectors) in time and space, on the appropriate design of insect control strategies, and on the development of suitable frameworks for monitoring and evaluation. Standard disease control operations, such as indoor residual spraying of insecticides or insecticide-treated bed nets for malaria, and the aerial application of insecticides or use of baited traps against the vectors of human and animal trypanosomosis, often include elements of area-wide planning because they target particular disease strata. Genetic control strategies (including the sterile insect technique (SIT)) are more intrinsically area-wide because they target specific vectors over delimited geographical areas delineated by biological criteria associated with colonization or dispersal potential. In either case it is argued that a strong geographical basis to planning and implementation is likely to improve the chances of programme success, as well as making more efficient use of resources and increasing cost effectiveness. Geographic information systems (GIS), global positioning systems (GPS) and remote sensing (RS) are allied technologies that together provide a means of gathering, integrating and analysing spatial data. To date, the application of these tools within traditional and area-wide programmes has been relatively limited, but this seems likely to change, particularly as GIS and GPS are already being used extensively in other areas of agroecological management and research. This paper examines potential areas for the application of GIS and associated spatial tools at various stages of planning and implementation of area-wide programmes integrating the SIT as a primary example, before going on to look beyond the SIT and to a number of examples of infectious diseases where GIS and spatial analysis have, to a greater or lesser extent, been employed within disease control efforts. With the help of these case studies the paper attempts to evaluate the extent to which the hype surrounding spatial tools has been (or can be) justified, and examines the barriers that remain in terms of further expansion of their use.

14169**. Devorshak, C., 2007.** Area-wide integrated pest management programmes and agricultural trade: Challenges and opportunities for regulatory plant protection. In: *Area –Wide Control of Insect Pests: From Research to Field Implementation*. Springer,The Netherlands, pp. 407-417.

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The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) entered into force for all member countries in 2000. It states that measures to protect human, animal and plant health or life shall be based on international standards where possible. These measures shall be based on a scientific risk assessment and should be implemented only to the extent necessary to achieve an appropriate level of protection. The International Plant Protection Convention (IPPC) is the international standard setting body for protecting plant health identified in the SPS Agreement. Both international treaties make provision for control of pests at regional levels (regionalization) and for identification of pest free areas. The IPPC provides guidance to countries, in the form of international standards, on the implementation of pest free areas and pest risk analysis (including systems approaches and other risk management measures). These standards can contribute to area-wide integrated pest management (AW-IPM) programmes for two main reasons. First, when AW-IPM programmes are implemented according to IPPC standards, trading partners should be prepared to recognize the results of a successful AW-IPM programme as meeting requirements, for example, of a pest free area or an area of low pest prevalence. Second, these standards provide scientific and technical guidance for the design and operation of key components of AW-IPM programmes. Therefore, countries that implement AW-IPM programmes that are in accordance with IPPC standards are better positioned to take advantage of liberalized trade while maintaining their phytosanitary security.

14170. **Dodd, R. Y., 2007**. Current risk for transfusion transmitted infections. *Current Opinion in Hematology,* **14** (6): 671-676.

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Blood safety is a topic of continuing concern, and much effort is expended on measures to decrease the risk for transmission of infectious agents via transfusion. At the same time, emerging infections may threaten this safety. A periodic review of risk is therefore appropriate. The risk for major transfusion transmissible infections continues to decline as a result of continually strengthening interventions and because of more general improvements in public health. More attention is being paid to emerging infections, and recently donor testing has been implemented for West Nile virus and *Trypanosoma cruzi*. Within the period covered by this review, the transmission of variant Creutzfeldt-Jakob disease by transfusion has been confirmed. Our understanding of other agents is improving. In summary, the estimated risk for transfusion transmitted hepatitis viruses and retroviruses is now vanishingly small, but clinicians should be alert to the possibility of infection with emerging infectious agents, because preventive measures may not be available in all cases.

14171. **Doumbia, S., Chouong, H., Traore, S. F., Dolo, G., Toure, A. M. & Coulibaly, M., 2007**. Establishing an insect disease vector functional genomics training center in Africa. *African Journal of Medecine and Medical Sciences,* **36 Suppl**: 31-33.

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The genome sequences for many insects vector of human diseases are now available and promise the development of a set of new, powerful tools that can be used to develop innovative approaches to control these diseases. The African continent, which is the most severely affected by vector borne diseases, lacks adequate infrastructures and personnel for rational use of genomic information. To fill this gap, the African Center for Training in Functional Genomics of Insect Vectors of Human Disease (AFRO VECTGEN) was initiated by WHO/TDR and the Department of Medical Entomology and Vector Ecology (DMEVE) of the Malaria Research and Training Centre (MRTC) in Mali. The aim of the AFRO VECTGEN programme is to train young scientists in functional genomics who will ultimately use genome sequence data for research on insect vectors of human diseases. The programme could trigger collaborative research and will benefit from an existing vector biology network in Mali, which was built around research grants funded by the National Institutes of Health, USA and WHO/TDR.

14172. **Franco-Paredes, C., Von, A., Hidron, A., Rodriguez-Morales, A. J., Tellez, I., Barragan, M., Jones, D., Naquira, C. G. & Mendez, J., 2007**. Chagas disease: an impediment in achieving the Millennium Development Goals in Latin America. *BMC International Health and Human Rights,* **7**: 7.

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Achieving sustainable economic and social growth through advances in health is crucial in Latin America within the framework of the United Nations Millennium Development Goals. Health-related Millennium Development Goals need to incorporate a multidimensional approach addressing the specific epidemiologic profile for each region of the globe. In this regard, addressing the cycle of destitution and suffering associated with infection with *Trypanosoma cruzi*, the causal agent of Chagas' disease of American trypanosomiasis, will play a key role to enable the most impoverished populations in Latin America the opportunity to achieve their full potential. Most cases of Chagas' disease occur among forgotten populations because these diseases persist exclusively in the poorest and the most marginalized communities in Latin America.

14173. **Hendrichs, J., Kenmore, P., Robinson, A. S., & Vreysen,M. J. B., 2007.** Area-Wide Integrated Pest Management (AW-IPM): Principles, Practice and Prospects. In: *Area –Wide Control of Insect Pests: From Research to Field Implementation*. Springer, The Netherlands, pp. 3-33.

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Integrated pest management (IPM) has remained the dominant paradigm of pest control for the last 50 years. IPM has been endorsed by essentially all the multilateral environmental agreements that have transformed the global policy framework of natural resource management, agriculture, and trade. The integration of a number of different control tactics into IPM systems can be done in ways that greatly facilitate the achievement of the goals either of field-by-field pest management, or of area-wide (AW) pest management, which is the management of the total pest population within a delimited area. For several decades IPM and AW pest control have been seen as competing paradigms with different objectives and approaches. Yet, the two "schools" have gradually converged, and it is now generally acknowledged that the synthesis, AW-IPM, neither targets only eradication, nor relies only on single control tactics, and that many successful AW programmes combine a centrally managed top-down approach with a strong grassroots bottom-up approach, and that some are managed in a fully bottom-up manner. AW-IPM is increasingly accepted especially for mobile pests where management at a larger scale is more effective and preferable to the uncoordinated field-by-field approach. For some livestock pests, vectors of human diseases, and pests of crops with a high economic value and low pest tolerance, there are compelling economic incentives for participating in AW control. Nevertheless issues of free riders, public participation and financing of public goods, all play a significant role in AW-IPM implementation. These social and managerial issues have, in several cases, severely hampered the positive outcome of AW programmes; and this emphasises the need for attention not only to ecological, environmental, and economic aspects, but also to the social and management dimensions. Because globalization of trade and tourism are accompanied by the increased movement of invasive alien pest species, AW programmes against major agricultural pests are often being conducted in urban and suburban areas. Especially in such circumstances, factors likely to shift attitudes from apathy to outrage, need to be identified in the programme planning stage and mitigated. This paper reviews the evolution and implementation of the AW-IPM concept and documents its process of development from basic research, through methods development, feasibility studies, commercialization and regulation, to pilot studies and operational programmes.

14174. **Hodgetts, A., Levin, M., Kroll, J. S. & Langford, P. R., 2007**. Biomarker discovery in infectious diseases using SELDI. *Future Microbiology,* **2**: 35-49.

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Surface enhanced laser desorption ionization-time of flight is a mass spectrometric-based method that requires a minimal amount of sample for analysis and can be used for high-throughput screening. It has been used to discover serum or tissue protein signatures and biomarkers for infectious diseases in the fields of virology (hepatitis B and C viruses, severe acute respiratory syndrome, HIV-1, human T-cell leukemia virus-1 and BK virus), parasitology (trypanosomiasis) and bacteriology (intra-amniotic inflammation, tuberculosis and bacterial endocarditis). The protein signatures, or biomarkers, can be used to diagnose

infection, predict disease states and to inform on disease processes. Careful attention to experimental design, sample handling and storage, and the use of appropriate internal controls are crucial to success.

14175. **Huho, B. J., Ng'habi, K. R., Killeen, G. F., Nkwengulila, G., Knols, B. G. & Ferguson, H. M., 2007**. Nature beats nurture: a case study of the physiological fitness of free-living and laboratory-reared male *Anopheles gambiae s.l. Journal of Experimental Biology,* **210** (Pt 16): 2939-2947.

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Laboratory experimentation forms the basis for most of our knowledge of the biology of many organisms, in particular insects. However, the accuracy with which laboratory-derived estimates of insect life history and behaviour can predict their fitness and population dynamics in the wild is rarely validated. Such comparison is especially important in cases where laboratory-derived information is used to formulate and implement strategies for the genetic control of insects in nature. We have conducted a comparative study of the reproductive potential and life history of male *Anopheles gambiae* Gilies *sensu lato* mosquitoes from both standardized laboratory conditions and from natural field settings. We measured three indirect indicators of male mosquito fitness: energetic reserves, body size and survival, in a bid to determine whether the demographics and energetic limitations of wild males can be correctly predicted from their laboratory counterparts. Crucially, the body size and lipid reserves of wild males were substantially greater than those reared under standard laboratory conditions. We caution that the energetic limitations of insects as identified in the laboratory may underestimate their resilience in the wild, and discuss the implications of this phenomenon with respect to vector-borne disease control programmes based on genetic control of mosquitoes.

14176. **Klassen, W., 2007**. Introductory remarks. In: *Area –Wide Control of Insect Pests: From Research to Field Implementation*. Springer, The Netherlands, pp. vii-ix.

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Since area-wide integrated pest management (AW-IPM) programmes almost always are social enterprises each with a diverse set of stakeholders reliant on advanced technology, they tend to be complex to implement, especially in terms of management. Therefore, the appearance of this book, “Area-wide control of insect pests: from research to field implementation” is most timely, and it will be invaluable for informing concerned scientists, leaders of private firms, commodity organizations and public agencies, bankers, legislators, students and those interested in placing management of major insect pest problems on a sustainable and environmentally acceptable footing. Although the need to develop and implement more effective strategies of combating pests and pathogens has always been dire, the urgency of this challenge has increased sharply for two reasons. The first reason is the rapid increase in the world population, which more than doubled from roughly 2.5 billion to 6

billion people in the second half of the 20th century; and the second reason is that the rapid globalization of travel and trade in agricultural and other products has dramatically increased the spread of pests, pathogens and other invasive harmful organisms. Many of the economically most damaging pests are invasive alien species that have escaped the constraints, which keep their populations in check in their regions of origin. In North America roughly one-half of the major pests originated abroad, and this seems also to be true in other continents. Major exotic pests and pathogens – many adapted for wide dispersal and high rates of reproduction – are becoming established with increasing frequencies on all continents and on many ecologically sensitive islands. Therefore, to facilitate the expansion of international agricultural trade while minimizing the further spread of some major pests, commodities which are hosts to these pests are increasingly produced for export in pest free areas or in areas of low pest prevalence that obtained their favourable phytosanitary status through AW-IPM approaches. Currently, for the most part, the control of many highly mobile and very destructive insect pests is still carried out by individual producers who rely heavily on the use of broad-spectrum insecticides. Although other control technologies are often incorporated into the producer's IPM system, these technologies, too, are usually applied by producers independently of other producers, and without due consideration of surrounding host and non-host areas. Such an uncoordinated farm-by-farm IPM pattern provides opportunities for the pest population to build up and to establish damaging infestations.
Consequently, on most farms insect pest populations increase to damaging levels each year, and the farmer is forced to apply fast-acting insecticides as a rescue treatment. This defeats the primary goal of the IPM system, which is to take maximum advantage of naturally occurring biological control agents. Similarly in combating pests and pathogens of concern to human and animal well-being, less than thorough treatment of the entire population fails to provide durable relief. Thus the key concept of the AW-IPM strategy is to address the whole pest population including all places of refuge or foci of infestation from which recruits could come to re-establish damaging densities of the pest population in areas of concern. The area-wide approach is not new, but originated several thousand years ago. In the Roman Empire it was recognized that some services carried out area-wide were more efficient and cheaper than when left to the action of individual citizens. As such, garbage was diligently removed from some cities, clean water was brought from distant sources and public baths were provided. The sudden appearance in 1347 of Black Death, a bacterial disease transmitted by the flea, *Xenopsylla cheopis* (Rothschild), led to the invention of quarantine to contain the epidemic and to stamp it out. Beginning in the late 1920s, when catastrophic locust plagues were widespread in Africa and southwest Asia, continent-wide campaigns have been organized to protect against highly devastating locust species; and these campaigns, now led by FAO's Locust Group, employ sophisticated technologies. During the past one-half century the area-wide application of the sterile insect technique in combination with other technologies against an array of major insect pests has served to focus the attention of scientists and administrators on ways of applying the area-wide approach in the combat against many other pests and diseases. The principles of AW-IPM are addressed in this book's introductory chapter. The chapter argues that each fundamental component of classical IPM, be it a cultural, biological or chemical control tactic, applied against an entire insect population (total population management) will lead in most cases to more sustainable pest control as compared to a localized farm-by-farm approach. Some fundamental management and strategic challenges of AW-IPM programmes are likewise addressed, including the make or break environmental and economic issues. Successful AW-IPM

programmes require basic research and preparatory activities including methods development, feasibility studies, pilot trials and a regulatory framework. These aspects are dealt with in subsequent sections. Section 2 covers and illuminates several important basic research areas including genetics, transgenesis, genetic sexing, cryobiology, physiology, insect symbionts and mating behaviour strategies. Ecological heterogeneity within field, within farm, and at broader spatial scales profoundly affects the population dynamics of pests and their natural enemies and other aspects of their ecology. Methods of systems analysis, mathematical modelling and a number of geo-spatial technologies (geographic information systems and global positioning system) have been adapted to cope with the spatio-temporal complexity in AW-IPM programmes and have contributed greatly to increased effectiveness and efficiency of programme activities. These and other methods development tools are described in section 3. Feasibility studies addressing economic, social and technical considerations are required prior to any major and costly field programme. A science-based analysis of these considerations will enable a judgment to be made as to whether the various control tactics can be applied on an area-wide basis and whether the envisioned control strategy is the most appropriate for the particular pest situation. Section 4 provides examples of how such elucidating studies have been conducted for different pest situations. AW-IPM programmes are dependent on the synergistic collaboration of many stakeholders. They require the entry onto private properties. They can affect the movement of goods, and they can also impact or inconvenience the non-farming community. Thus AW-IPM requires that a sensitive and effective regulatory framework be developed by the relevant national and international regulatory agencies. Commercialization of part or even entire AW-IPM programmes, a complex and sometimes contentious issue, holds the promise of properly capitalizing such programmes, introducing efficiencies and tackling pest problems that government cannot afford to address. These regulatory and privatization issues are discussed in section 5. Pilot field programmes are often carried out following a feasibility study with a favourable outcome. Such programmes are needed to evaluate and fine-tune various control tactics and field methodologies to increase their effectiveness and efficiency. Pilot programmes can vary in size and scope as is described in the chapters in section 6. Section 7 describes operational AW-IPM programmes against key pests such as the boll weevil, several lepidopteran pests, the bont tick, termites, mosquitoes, fruit flies, etc. Several of the chapters emphasize the technical and managerial difficulties encountered during the implementation of eradication, suppression, containment or preventive AW-IPM programmes and attempt to extract important lessons. As a concluding chapter (section 8) a critical review is provided of AW-IPM programmes in terms of their successes and failures, and key factors are identified which must be addressed in order to improve the chance of success. The chapters in this text book originate from papers and selected posters presented at the 2nd FAO/IAEA International conference on area-wide control of insect pests. To complete the book, several invited chapters have been included. This book is an invaluable compendium of reports on operational AW-IPM programmes. It will help to further develop the theory, technology and practice of such programmes. Graduate students will learn much about the history, accomplishments, problems and the great potential of the area-wide strategy. Entrepreneurs and policy-makers will gain in-depth perspective on aspects of commercialization. I am honoured greatly to have been asked to write these introductory comments in this text book devoted to the area-wide management of insect pests. The prodigious progress in AW-IPM made in recent decades confirms that the area-wide strategy has a far greater potential than any other approach to achieve sustainable management of many major insect pests. Truly we

are now at the beginning of an era of decidedly improved and sustainable insect pest management.

14177. **Lefevre, T. & Thomas, F., 2007**. Behind the scene, something else is pulling the strings: Emphasizing parasitic manipulation in vector-borne diseases. *Infection, Genetics and Evolution*. **In press: corrected proof.**

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Merging the field of epidemiology with those of evolutionary and behavioural ecology can generate considerable fundamental knowledge, as well as help to guide public health policies. An attempt is made here to integrate these disciplines by focusing on parasitic manipulation in vector-borne diseases. Parasitic manipulation is a fascinating strategy of transmission which occurs when a parasite alters phenotypic trait(s) of its host in a way that enhances its probability of transmission. Vector-borne parasites are responsible for many of the most harmful diseases affecting humans, and thus represent public health priority. It has been shown for several decades that viruses, bacteria and protozoa can alter important features of their arthropod vector and vertebrate host in a way that increases their probability of transmission. Here, we review these changes, including the feeding behaviour, survival and immune system of the vector, as well as attraction, defensive behaviour, blood characterictics and immune system of the vertebrate host. Based on the classic measure of vector-borne disease transmission R(0), additional changes, such as, vertebrate host choice by infected vectors or parasite development duration in the vector are expected. Reported or expected phenotypic changes are discussed in terms of costs and benefits to the parasite, its vector, and the vertebrate host. Introducing the parasitic manipulation concept into vector-borne diseases clearly highlights fruitful avenues not only for fundamental research, but also for developing strategies for disease control.

14178. **Macedo, P. A., Peterson, R. K. & Davis, R. S., 2007**. Risk assessments for exposure of deployed military personnel to insecticides and personal protective measures used for disease-vector management. *Journal of Toxicology and Environmental Health Part A,* **70** (20): 1758-1771.

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Infectious diseases are problematic for deployed military forces throughout the world, and, historically, more military service days have been lost to insect-vectored diseases than to combat. Because of the limitations in efficacy and availability of both vaccines and therapeutic drugs, vector management often is the best tool that military personnel have against most vector-borne pathogens. However, the use of insecticides may raise concerns about the safety of their effects on the health of the military personnel exposed to them. Therefore, our objective was to use risk assessment methodologies to evaluate health risks to deployed U.S. military personnel from vector management tactics. Our conservative tier-1, quantitative risk assessment focused on acute, subchronic, and chronic exposures and cancer risks to military personnel after insecticide application and use of personal protective

measures in different scenarios. Exposures were estimated for every scenario, chemical, and pathway. Acute, subchronic, and chronic risks were assessed using a margin of exposure (MOE) approach. Our MOE was the ratio of a no-observed-adverse-effect level (NOAEL) to an estimated exposure. MOEs were greater than the levels of concern (LOCs) for all surface residual and indoor space spraying exposures, except acute dermal exposure to lambda-cyhalothrin. MOEs were greater than the LOCs for all chemicals in the truck-mounted ultra-low-volume (ULV) exposure scenario. The aggregate cancer risk for permethrin exceeded 1 x 10^{-6}, but more realistic exposure refinements would reduce the cancer risk below that value. Overall, results indicate that health risks from exposures to insecticides and personal protective measures used by military personnel are low.

14179. **Nauen, R., 2007**. Insecticide resistance in disease vectors of public health importance. *Pest Management Science,* **63** (7): 628-633.

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Vector-borne diseases are a global problem-a trend that may only increase if global temperature rises and demographic trends continue-and their economic and social impact are enormous. Insecticides play a vital role in the fight against these diseases by controlling the vectors themselves in order to improve public health; however, resistance to commonly used insecticides is on the rise. This perspective outlines the major classes of disease vector control agents and the mechanisms of resistance that have evolved, arguing that effective resistance management strategies must carefully monitor resistance in field populations and use combinations of the limited modes of action available to best effect. Moreover, the development of novel insecticide classes for control of adult mosquitoes and other vectors becomes increasingly important.

14180. **Pearson, R. A. & Krecek, R. C., 2006**. Delivery of health and husbandry improvements to working animals in Africa. *Tropical Animal Health and Production,* **38** (2): 93-101.

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Problems have been identified in the delivery of extension messages about the maintenance of healthy and well-fed working animals. The different factors that need to be considered in developing effective disease control and prevention programmes for working oxen and equids including vector-borne diseases, helminth disease, and vaccination programmes have been reported and discussed and experiences in improving husbandry including footcare, harness, and worm management reported. Most draught animals are owned by people who lack the financial means to pay for or to access the information needed on nutritional supplements, vaccinations and drug treatment. Smallholder farms are often remote from veterinary services, thereby requiring greater emphasis on preventive measures and local remedies. Several NGOs have traditionally provided static and mobile treatment teams for equines and training courses for farriers and harness makers. The effectiveness and

sustainability of these services and ways in which delivery of health care and husbandry messages could be delivered to improve impact are discussed.

14181. **Pherez, F. M., 2007**. Factors affecting the emergence and prevalence of vector-borne infections (VBI) and the role of vertical transmission (VT). *Journal of Vector Borne Diseases,* **44** (3): 157-163.

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Vector-borne infections (VBI) are very common around the globe and they account for many devastating diseases. They are not found exclusively in the third world or tropical regions but spread to every corner of the planet. The factors driving these infections are many and interact in very complex ways. This review attempts to put into perspective the external factors-climate change and demographics, as well as the internal factors that drive these infections with particular attention to the role that vertical transmission (VT) plays in the prevalence and emergence of these infections. VT has been widely demonstrated, its role in the maintenance of disease in nature has been suggested, but whether this role has a positive or negative effect seems to vary from species to species. The incorporation of this mechanism of transmission into the classic cycle of infection/maintenance of disease seems to explain important aspects of the epidemiology of VBI.

14182. **Pimental, D., 2007.** Area-wide pest management: environmental, economic, and food issues. In: *Area –Wide Control of Insect Pests: From Research to Field Implementation*. Springer, The Netherlands, pp. 35-51.

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Insect pests destroy approximately 14 percent of all potential food production despite the yearly application of more than 3,000 million kg of pesticides. This contributes to rising human malnutrition which in 2004 was estimated by the World Health Organization to have reached 3700 million - the largest number in history. Several major insect pests of crops and livestock are effectively controlled using area-wide pest management practices. As an example, the New World screwworm fly *Cochliomyia hominivorax* (Coquerel) that attacks livestock, especially cattle, was successfully eradicated by releasing radiation-sterilized screwworm flies over large areas. Area-wide insecticide treatments in the USA have also proved effective in the control of the boll weevil, while timed crop-planting over wide areas enables crops like wheat to evade major pests and has also been proven highly successful against rice pests in the USA and Asia. Yet, when the basic ecology of the insect pests and crops are ignored, major crop losses can occur, as illustrated by the manipulation of corn production in the USA. Damages caused by invading insect pests that attack established crop, forest, and natural ecosystems continue to be challenges to pest management specialists. Approximately 40 percent of the insect and mite pests of crops grown in the USA are introduced species and they cause about US$ 100,000 million in damage and control costs each year. The most recent introductions include the long-horned beetle *Anoplophora glabripennis* (Motschulsky) and the emerald ash borer *Agrilus planipennis* Fairmaire that

were both accidentally introduced from Asia. Area-wide strategies to control these destructive forest pests are being implemented.

14183. **Serandour, J., Girel, J., Boyer, S., Ravanel, P., Lemperiere, G. & Raveton, M., 2007**. How human practices have affected vector-borne diseases in the past: a study of malaria transmission in Alpine valleys. *Malaria Journal,* **6** (1): 115.

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Malaria was endemic in the Rhône-Alpes area of eastern France in the 19th century and life expectancy was particularly shortened in Alpine valleys. This study was designed to determine how the disease affected people in the area and to identify the factors influencing malaria transmission. Demographic data of the 19th century were collected from death registers of eight villages of the flood-plain of the river Isere. Correlations were performed between these demographic data and reconstructed meteorological data. Archive documents from medical practitioners gave information on symptoms of ill people. Engineer reports provided information on the hydraulic project developments in the Isere valley. Description of fevers was highly suggestive of endemic malaria transmission in the parishes neighbouring the river Isere. The current status of anopheline mosquitoes in the area supports this hypothesis. Mean temperature and precipitation were poorly correlated with demographic data, whereas the chronology of hydrological events correlated with fluctuations in death rates in the parishes. Nowadays, most of the river development projects involve the creation of wet areas, enabling controlled flooding events. Flood-flow risk and the re-emergence of vector-borne diseases would probably be influenced by the climate change. The message is not to forget that human disturbance of any functioning hydrosystem has often been linked to malaria transmission in the past.

14184. **Sommerfeld, J. & Oduola, A. M., 2007**. Health-related biotechnologies for infectious disease control in Africa: Ethical, legal and social implications (ELSI) of transfer and development. *African Journal of Medicine and Medical Sciences,* **36 Suppl**: 1-5.

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The African continent is disproportionately affected by infectious diseases. Malaria, HIV/AIDS, tuberculosis, and more "neglected" diseases including African trypanosomiasis, Buruli ulcer, leishmaniasis, onchocerciasis and trachoma continue to dramatically impact social and economic development on the continent. Health biotechnologies provide potential to develop effective strategies for the fight against the vicious circle of poverty and infections by helping in the development and improvement of novel affordable drugs, diagnostics and vaccines against these diseases. As the prospects of this emerging biotechnology research and deployment of its products become a reality in Africa, there is a need to consider the ethical, legal and social implications of both the scientific and technological advances and their use in

the communities. The article provides a short overview of the potential values of biotechnology, issues involved in its transfer and presents the rationale, design and recommendations of the international workshop/symposium held in April 2005 at the International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria.

14185. **Sparagano, O. A. & De Luna, C. J., 2007**. From population structure to genetically-engineered vectors: New ways to control vector-borne diseases? *Infection, Genetics and Evolution*. **In press; corrected proof**.

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Epidemiological studies on vectors and the pathogens they can carry (such as *Borrelia burgdorferi*) are showing some correlations between infection rates and biodiversity highlighting the "dilution" effects on potential vectors. Meanwhile other studies comparing sympatric small rodent species demonstrated that rodent species transmitting more pathogens are parasitized by more ectoparasite species. Studies on population structure and size have also proven a difference on the intensity of the parasitic infection. Furthermore, preliminary results in genetic improvement in mosquitoes (genetic markers, sexing, and genetic sterilization) will also increase performance as it has already been shown in field applications in developing countries. Recent results have greatly improved the fitness of genetically-modified insects compared to wild type populations with new approaches such as the post-integration elimination of transposon sequences, stabilising any insertion in genetically-modified insects. Encouraging results using the Sterile Insect Technique highlighted some metabolism manipulation to avoid the viability of offspring from released parent insect in the wild. Recent studies on vector symbionts would also bring a new angle in vector control capabilities, while complete DNA sequencing of some arthropods could point out ways to block the deadly impact on animal and human populations. These new potential approaches will improve the levels of control or even in some cases would eradicate vector species and consequently the vector-borne diseases they can transmit. In this paper we review some of the population biology theories, biological control methods, and the genetic techniques that have been published in the last years that are recommended to control for vector-borne diseases.

14186. **Torto, N., Mmualefe, L. C., Mwatseteza, J. F., Nkoane, B., Chimuka, L., Nindi, M. M. & Ogunfowokan, A. O., 2007**. Sample preparation for chromatography: an African perspective. *Journal of Chromatogrphy Part A,* **1153** (1-2): 1-13.

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Africa as a continent has its unique challenges for analytical chemists in sample preparation for chromatographic analyses. The areas of agriculture, environment, food and health provide formidable challenges when it comes to method development, for example, drought can result in inadequate supplies of good quality water. The testing of water quality necessitates the development of assay methods that can be employed to not only determine the quantities of pesticides associated with malaria and tsetse fly eradication programmes, but also to monitor mycotoxins or neurotoxins. Urbanisation has also meant that endocrine

disruptors such as phthalate esters need to be monitored. This review will profile some of the activities by analytical chemists practising in the African continent, who seek to address some of the challenges in sample preparation for chromatographic analyses.

14187. V**an den Berg, H., von Hildebrand, A., Ragunathan, V. & Das, P. K., 2007**. Reducing vector-borne disease by empowering farmers in integrated vector management. *Bulletin of the World Health Organization,* **85** (7): 561-566.

Laboratory of Entomology, Wageningen University, Wageningen, the Netherlands. [henk.vandenberg@wur.nl].

Irrigated agriculture exposes rural people to health risks associated with vector-borne diseases and pesticides used in agriculture and for public health protection. Most developing countries lack collaboration between the agricultural and health sectors to jointly address these problems. We present an evaluation of an intersectoral project targeting rice irrigation systems in Sri Lanka that uses the "farmer field school" method to teach farmers how to manage vector-borne diseases and how to improve rice yields. Teaching farmers about these two concepts together is known as "integrated pest and vector management". Project partners developed a new curriculum for the field school that included a component on vector-borne diseases. Rice farmers in intervention villages who graduated from the field school took vector-control actions as well as improving environmental sanitation and their personal protection measures against disease transmission. They also reduced their use of agricultural pesticides, especially insecticides. The intervention motivated and enabled rural people to take part in vector-management activities and to reduce several environmental health risks. There is scope for expanding the curriculum to include information on the harmful effects of pesticides on human health and to address other public health concerns. Benefits of this approach for community-based health programmes have not yet been optimally assessed. Also, the institutional basis of the integrated management approach needs to be broadened so that people from a wider range of organizations take part. A monitoring and evaluation system needs to be established to measure the performance of integrated management initiatives.

14188. **Vreysen, M. J. B., Gerardo-Abaya, J., & Cayol, J. P., 2007**. Lessons from area-wide integrated pest management (AW-IPM) programmes with an SIT component: an FAO/IAEA perspective. In: *Area–Wide Control of Insect Pests: From Research to Field Implementation*. Springer, The Netherlands, pp. 723-745.

Insect Pest Control Sub-Programme, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, IAEA, Wagramerstrasse 5, A-1400 Vienna, Austria; Latin America Section 1, Division for Latin America, Department of Technical Cooperation, IAEA, Wagramerstrasse 5, A-1400 Vienna, Austria; Asia and the Pacific Section 2, Division for Asia and the Pacific, Department of Technical Cooperation, IAEA, Wagramerstrasse 5, A-1400 Vienna, Austria

Area-wide integrated pest management (AW-IPM) programmes that integrate the release of sterile insects are complex and very management intensive undertakings. Their

success depends upon continuous interactions between essential components of the system of pest suppression. Although there are many AW-IPM programmes that very effectively incorporate the release of sterile insects, success cannot be taken for granted. From an analysis of successful programmes and those beset with difficulties, several essential technical and managerial prerequisites for success were extracted. Technical requirements included: the availability of high-quality baseline data to develop an appropriate strategy, adequate competitiveness and mating compatibility between the strain used for release and that of the target population, persistence of the quality of the release strain, and sound monitoring. On the managerial side, the prerequisites of success were: commitment of all stakeholders, adequate funding, a flexible and independent management structure with dedicated full-time staff, independent peer reviews and consistency in the implementation of critical programme components taking into account differences in local ecological, socio-economic and political conditions.

TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

[See also 30: nos. 14175, 14209].

14189. **Aksoy, S., & Weiss, B. L., 2007.** Symbiosis-based technological advances to improve tsetse *Glossina* spp. SIT application. In: *Area –Wide Control of Insect Pests: From Research to Field Implementation*. Springer, The Netherlands, pp. 137-149.

Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06510, USA.

Tsetse flies, *Glossina* spp. are the sole vectors of the parasitic African trypanosomes, which cause devastating diseases in humans and animals. The sterile insect technique (SIT) is one pest control tool that, when integrated on an area-wide basis, is highly effective against tsetse populations. Several molecular techniques have the potential to enhance the application of this approach. In particular, the ability to engineer refractoriness into released strains would enhance the efficacy of this approach, especially in human disease endemic areas. In addition, natural mating incompatibilities between some populations could be exploited to enhance the fitness of released males, as the irradiation dose could be reduced to that required for female sterilization without compromising overall male sterility. The viviparous reproductive nature of tsetse flies has made direct germ-line transformation impossible. However, the symbiotic microorganism *Sodalis glossinidius* that lives in tsetse midgut tissue can be cultured and transformed. Because these symbionts live in close proximity to where parasites differentiate and replicate, gene products expressed and secreted by these microbes could have immediate impact. Fly midguts have been successfully repopulated with symbionts engineered to express foreign gene products. The complete genome sequence of *S. glossinidius* together with information on its population dynamics during fly development is now available. Gene expression experiments currently in progress now aid in identifying an expression system that does not reduce the fitness of engineered flies. This is done by making use of midgut-specific promoters. In addition to midgut symbionts, various field populations

of tsetse harbour *Wolbachia* spp. In many insect species the presence of *Wolbachia* induces cytoplasmic incompatibility, a phenomenon that causes reproductive incompatibilities between infected and uninfected insects. To understand the impact of *Wolbachia* infections on tsetse, lines with and without *Wolbachia* should be developed for formal mating experiments. Field populations are heterogeneous for the presence/absence of the bacteria, and it may be possible to develop such lines directly from the field to evaluate the potential role of cytoplasmic incompatibility. In an attempt to identify such field populations, information was obtained on *Wolbachia* infections in *Glossina fuscipes fuscipes* Newstead.

14190. **Enserink, M., 2007**. Welcome to Ethiopia's fly factory. *Science,* **317** (5836): 310-313.

No abstract available.

14191. **Terblanche, J. S. & Chown, S. L., 2007.** Factory flies are not equal to wild flies. *Science*, **317** (5845): 1678.

No abstract available.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

[See also 30: nos. 14200, 14223]

14192. **Briceno, R. D., Eberhard, W. G. & Robinson, A. S., 2007**. Copulation behaviour of *Glossina pallidipes* (Diptera: *Muscidae*) outside and inside the female, with a discussion of genitalic evolution. *Bulletin of Entomological Research,* **97** (5): 471-488.

Escuela de Biologia, Universidad de Costa Rica, Ciudad Universitaria, Costa Rica.

If species-specific male genitalia are courtship devices under sexual selection by cryptic female choice, then species-specific aspects of the morphology and behaviour of male genitalia should often function to stimulate the female during copulation. The morphology and behaviour of the complex, species-specific male genitalia of the tsetse fly, *Glossina pallidipes* Austen, were determined from both direct observations and dissections of flash-frozen copulating pairs; we found that some male genitalic traits probably function to stimulate the female, while others function to restrain her. The male clamps the ventral surface of the female's abdomen tightly with his powerful cerci. Clamping does not always result in intromission. Clamping bends the female's body wall and her internal reproductive tract sharply, posteriorly and dorsally, and pinches them tightly. The male performed sustained, complex, stereotyped, rhythmic squeezing movements with his cerci that were not necessary to mechanically restrain the female and appeared instead to have a stimulatory function. Six different groups of modified setae on and near the male's genitalia rub directly against particular sites on the female during squeezing. The designs of these setae correlate with the force with which they press on the female and the probable sensitivity of the female surfaces that they contact. As expected under the hypothesis that these structures are under

sexual selection by female choice, several traits suspected to have stimulatory functions have diverged in *G. pallidipes* and its close relative, *G. longipalpis*. Additional male non-genitalic behaviour during copulation, redescribed more precisely than in previous publications, is also likely to have a courtship function. The elaborate copulatory courtship behaviour and male genitalia may provide the stimuli that previous studies showed to induce female ovulation and resistance to remating.

14193. **Jurenka, R., Terblanche, J. S., Klok, C. J., Chown, S. L., & Krasfur, E. S., 2007.** Cuticular lipid mass and desiccation rates in *Glossina pallidipes*: interpopulation variation. *Physiological Entomology*, **2**: 287-293.

Department of Entomology, Iowa State University, Ames, IA 50011, USA. [ekrafsur@iastate.edu].

Tsetse flies, *Glossina pallidipes* (Diptera: Glossinidae) are said to have strong dispersal tendencies. Gene flow among these populations is estimated to be the theoretical equivalent of no more than one or two reproducing flies per generation, thereby raising the hypothesis of local regimes of natural selection. Flies were sampled from four environmentally diverse locations in Kenya to determine whether populations are homogeneous in desiccation tolerance and cuticular lipids. Cuticular hydrocarbon fractions known to act as sex pheromones do not differ among populations, thereby eliminating sexual selection as an isolating mechanism. Cuticular lipid quantities vary among populations and are not correlated with prevailing temperatures, humidities, and normalized density vegetation indices. Females demonstrate a stronger correlation than males between cuticular lipid mass and body weight. Desiccation rates also vary among populations, but are not correlated with the amounts of cuticular lipid. Chemical analysis of cuticular hydrocarbons by gas chromatography-mass spectroscopy shows that one of the four populations has more 11,15- and 11,21-dimethyl-31 hydrocarbon on females. These results are discussed in the context of population differences and estimates of gene flow.

14194. **Lehane, M. J., Gibson, W. & Lehane, S. M., 2007**. Differential expression of fat body genes in *Glossina morsitans morsitans* following infection with *Trypanosoma brucei brucei*. *International Journal for Parasitology*. **In press; corrected proof.**

Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.

To determine which fat body genes were differentially expressed following infection of *Glossina morsitans morsitans* with *Trypanosoma brucei brucei* we generated four suppression subtractive hybridisation (SSH) libraries. We obtained 52 unique gene fragments (SSH clones) of which 30 had a known orthologue at E-05 or less. Overall the characteristics of the orthologues suggest: (i) that trypanosome infection has a considerable effect on metabolism in the tsetse fly; (ii) that self-cured flies are mounting an oxidative stress response; and (iii) that self-cured flies are displaying increased energy usage. The three most consistently differentially expressed genes were further analysed by gene knockdown (RNAi). Knockdown of *Glossina* transferrin transcripts, which are upregulated in self-cured

flies compared with flies infected with trypanosomes, results in a significant increase in the number of trypanosome infections establishing in the fly midgut, suggesting transferrin plays a role in the protection of tsetse flies from trypanosome infection.

14195. **Nayduch, D. & Aksoy, S., 2007**. Refractoriness in tsetse flies (Diptera: *Glossinidae*) may be a matter of timing. *Journal of Medical Entomology,* **44** (4): 660-665.

Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06510, USA. [dnayduch@georgiasouthern.edu].

Tsetse flies (Diptera: *Glossinidae*: *Glossina* spp.) are vectors for African trypanosomiasis, a devastating disease that kills both people and animals in sub-Saharan Africa. Trypanosomes ingested with an infected blood meal reside within the gut of tsetse and eventually move to the salivary glands where they become transmissible during blood feeding. Although tsetses are efficient vectors for disease transmission, infection prevalence in the field is surprisingly low, a trait referred to as refractoriness. Refractoriness is relatively more pronounced in *palpalis* subgroup flies, although certain species within the susceptible *morsitans* species complex are also highly refractory, such as *Glossina pallidipes* Austen. We examined the role of the humoral immune response in refractoriness to infection by comparing the expression of the antimicrobial peptide gene attacin across three species with varied vector competence. Gene expression was measured both temporally (time after feeding and fly age) and spatially (tissue specificity). Although microbial immune challenge induces attacin expression in all three species, "refractory" fly species showed an uninduced, baseline level of systemic (fat body) attacin, whereas the "susceptible" flies did not. In addition, refractory species had a higher level of attacin expression in the proventriculus and midgut. We also found that blood feeding alone up-regulated attacin expression in refractory species but not in the susceptible species. Finally, reverse genetics showed that repression of attacin by double-stranded RNA-mediated RNA interference increased susceptibility to trypanosome infection in *G. pallidipes*. The role of early, uninduced attacin expression, and its role in relative refractoriness in tsetse, is discussed.

14196. **Petersen, F. T., Meier, R., Kutty, S. N. & Wiegmann, B. M., 2007**. The phylogeny and evolution of host choice in the *Hippoboscoidea* (Diptera) as reconstructed using four molecular markers. *Molecular Phylogenetics and Evolution,* **45** (1): 111-122.

Zoological Museum, University of Copenhagen, Universitetsparken 15, DK - 2100 Copenhagen O, Denmark.

Hippoboscoidea is a superfamily of Diptera that contains the *Glossinidae* or tsetse flies, the *Hippoboscidae* or louse flies, and two families of bat flies, the *Streblidae* and the *Nycteribiidae*. We reconstruct the phylogenetic relationships within *Hippoboscoidea* using maximum parsimony and Bayesian methods based on nucleotide sequences from fragments of four genes: nuclear 28S ribosomal DNA and the CPSase domain of CAD, and mitochondrial 16S rDNA and cytochrome oxidase I. We recover monophyly for most of the presently recognized groups within *Hippoboscoidea* including the superfamily as a whole, the Hippoboscidae, the *Nycteribiidae,* the bat flies, and the Pupipara

(=*Hippoboscidae+Nycteribiidae+Streblidae)*, as well as several subfamilies within the constituent families. *Streblidae* appear to be paraphyletic. Our phylogenetic hypothesis is well supported and decisive in that most competing topological hypotheses for the *Hippoboscoidea* require significantly longer trees. We confirm a single shift from a free-living fly to a blood-feeding ectoparasite of vertebrates and demonstrate that at least two host shifts from mammals to birds have occurred. Wings have been repeatedly lost, but never regained. The hippoboscoid ancestor also evolved adenotrophic viviparity and our cladogram is consistent with a gradual reduction in the motility of the deposited final instar larvae from active burrowing in the soil to true pupiparity where adult females glue the puparium within the confines of bat roosts.

14197. **Terblanche, J. S., Clusella-Trullas, S., Deere, J. A. & Chown, S. L., 2007**. Thermal tolerance in a south-east African population of the tsetse fly *Glossina pallidipes* (Diptera: *Glossinidae*): Implications for forecasting climate change impacts. *Journal of Insect Physiology*. **In press; corrected proof.**

Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa.

For tsetse (*Glossina* spp.), the vectors of human and animal trypanosomiases, the physiological mechanisms linking variation in population dynamics with changing weather conditions have not been well established. Here, we investigate high- and low-temperature tolerance in terms of activity limits and survival in a natural population of adult *Glossina pallidipes* from eastern Zambia. Due to increased interest in chilling flies for handling and aerial dispersal in sterile insect technique control and eradication programmes, we also provide further detailed investigation of low-temperature responses. In wild-caught *G. pallidipes*, the probability of survival for 50 percent of the population at low-temperatures was calculated at 3.7, 8.9 and 9.6^{O}C (95 percent CIs: +/-1.5 degrees C) for 1, 2 and 3h treatments, respectively. At high temperatures, it was estimated that treatments at 37.9, 36.2 and 35.6^{O}C (95 percent CIs: +/-0.5^{O}C) would yield 50 percent population survival for 1, 2 and 3h, respectively. Significant effects of time and temperature were detected at both temperature extremes (GLZ, $p<0.05$ in all cases) although a time-temperature interaction was only detected at high temperatures ($p<0.0001$). We synthesized data from four other Kenyan populations and found that upper critical thermal limits showed little variation among populations and laboratory treatments (range: 43.9-45.0^{O}C; 0.25^{O}C/min heating rate), although reduction to more ecologically relevant heating rates (0.06^{O}C/min) reduce these values significantly from approximately 44.4 to 40.6^{O}C, thereby providing a causal explanation for why tsetse distribution may be high-temperature limited. By contrast, low-temperature limits showed substantial variation among populations and acclimation treatments (range: 4.5-13.8^{O}C; 0.25 ^{O}C/min), indicating high levels of inter-population variability. Ecologically relevant cooling rates (0.06^{O}C/min) suggest tsetses are likely to experience chill coma temperatures under natural conditions (approximately 20-21^{O}C). The results from acute hardening experiments in the Zambian population demonstrate limited ability to improve low-temperature tolerance over short (hourly) timescales after non-lethal pre-treatments. In flies which survived chilling, recovery times were non-linear with plateaus between 2-6 and 8-12^{O}C. Survival times ranged between 4 and 36h and did not vary between flies which had undergone chill coma by comparison with flies which had not, even after

factoring body condition into the analyses ($p>0.5$ in all cases). However, flies with low chill coma values had the highest body water and fat content, indicating that when energy reserves are depleted, low-temperature tolerance may be compromised. Overall, these results suggest that physiological mechanisms may provide insight into tsetse population dynamics, hence distribution and abundance, and support a general prediction for reduced geographic distribution under future climate warming scenarios.

14198. **Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C. & Chown, S. L., 2007**. Critical thermal limits depend on methodological context. *Proceedings. Biological Sciences,* **274** (1628): 2935-2942.

Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, Republic of South Africa.

A full-factorial study of the effects of rates of temperature change and start temperatures was undertaken for both upper and lower critical thermal limits (CTLs) using the tsetse fly, *Glossina pallidipes*. Results show that rates of temperature change and start temperatures have highly significant effects on CTLs, although the duration of the experiment also has a major effect. Contrary to a widely held expectation, slower rates of temperature change (i.e. longer experimental duration) resulted in poorer thermal tolerance at both high and low temperatures. Thus, across treatments, a negative relationship existed between duration and upper CTL while a positive relationship existed between duration and lower CTL. Most importantly, for predicting tsetse distribution, *G. pallidipes* suffer loss of function at less severe temperatures under the most ecologically relevant experimental conditions for upper (0.06 ^{O}C min^{-1}; 35 ^{O}C start temperature) and lower CTL (0.06 ^{O}C min^{-1}; 24 ^{O}C start temperature). This suggests that the functional thermal range of *G. pallidipes* in the wild may be much narrower than previously suspected, approximately 20-40 ^{O} C, and highlights their sensitivity to even moderate temperature variation. These effects are explained by limited plasticity of CTLs in this species over short time scales. The results of the present study have broad implications for understanding temperature tolerance in these and other terrestrial arthropods.

14199. **Van Den Abbeele, J., Caljon, G., Dierick, J. F., Moens, L., De Ridder, K. & Coosemans, M., 2007**. The *Glossina morsitans* tsetse fly saliva: General characteristics and identification of novel salivary proteins. *Insect Biochemistry and Molecular Biology,* **37** (10): 1075-1085.

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The tsetse fly (*Glossina* spp.) is an obligate blood-sucking insect that transmits different human-pathogenic and livestock threatening trypanosome species in Africa. To obtain more insight in the tsetse salivary function, some general aspects of the tsetse fly saliva and its composition were studied. Direct pH and protein content measurements revealed a moderately alkaline (pH approximately 8.0) salivary environment with approximately 4.3mµg soluble proteins per gland and a constant representation of the major saliva proteins throughout the blood-feeding cycle. Although major salivary genes are

constitutively expressed, upregulation of salivary protein synthesis within 48h after the blood meal ensures complete protein replenishment from day 3 onwards. Screening of a non-normalised *Glossina morsitans morsitans* lambdagt11 salivary gland expression library with serum from a saliva-immunized rabbit identified three full-length cDNAs encoding for novel salivary proteins with yet unknown functions: a 8.3kDa glycine/glutamate-rich protein (*G. morsitans morsitans* salivary gland protein *Gmm*sgp1), a 12.0kDa proline-rich protein (*Gmm*sgp2), and a 97.4kDa protein composed of a metallophosphoesterase/5'nucleotidase region with a glutamate/aspartate/asparagines-rich region (*Gmm*sgp3).

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

14200. **Abd-Alla, A., Bossin, H., Cousserans, F., Parker, A., Bergoin, M. & Robinson, A., 2007**. Development of a non-destructive PCR method for detection of the salivary gland hypertrophy virus (SGHV) in tsetse flies. *Journal of Virological Methods,* **139** (2): 143-149.

Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria. [a.m.m.abd-alla@iaea.org].

A PCR based diagnostic method to detect salivary gland hypertrophy virus (SGHV) in tsetse flies is described. Two sets of primers GpSGHV1F/GpSGHV1R and GpSGHV2F/GpSGHV2R were selected from a virus-specific sequence. Both primer sets can detect specifically the virus in individual tsetse flies by generating an amplicon of 401 bp. Attempts were made to develop a simple and reliable non-destructive virus detection method in live flies. PCR reactions were performed on either crude or purified tsetse DNA from saliva and legs. While saliva can be an indicator for the presence of the virus in flies, the method is laborious. Crude extract from an excised middle leg resulted in a positive PCR reaction equivalent to crude extract from whole fly. However, sensitivity could be significantly increased when purified DNA was used as the template. In conclusion, PCR using a purified DNA template from a single tsetse leg represents an efficient, non-destructive method for virus diagnosis in live tsetse flies.

14201. **Bouyer, J., 2007**. Les tsétsé, mouches intelligentes ? (1ere partie) Comportement alimentaire des glossines. *Insectes* **145**: 29-32.

CIRAD-EMVT/CIRDES, 01 BP 454, Bobo-Dioulasso 01, Burkina Faso.

Les études comportementales sur les insectes vecteurs sont aujourd'hui mises en avant pour mettre en place de meilleures méthodes de prévention des maladies qu'ils véhiculent. Car on aurait tort, par exemple, de penser que seule la faim guide l'insecte hématophage – ici la Tsé-tsé - vers sa victime Et mieux comprendre pourquoi et comment elle choisit qui - et où - elle va piquer, c'est mieux pouvoir l'en empêcher.

14202. **Bouyer, J., S., Ravel, S., Dujardin, J. P., de Meeüs, T., Vial, L., Thévenon, S., Guerrini, L., Sidibé, L. & Solano, P., 2007**. Population structuring of *Glossina palpalis gambiensis* (Diptera: *Glossinidae*) according to landscape fragmentation in the Mouhoun river, Burkina Faso. *Journal of Medical Entomology* **44**(5): 788-795.

CIRAD-EMVT/CIRDES, 01 BP 454, Bobo-Dioulasso 01, Burkina Faso.

The impact of landscape fragmentation due to human and climatic mediated factors on the structure of a population of *Glossina palpalis gambiensis* (Diptera: *Glossinidae*) was investigated in the Mouhoun river basin, Burkina Faso. Allele frequencies at five microsatellite loci, and metric properties based on 11 wing landmarks, were compared between four populations. The populations originated from the Mouhoun river and one of its tributaries. The average distance between samples was 72 km with the two most widely spaced populations being 216 km apart. The sampling points traversed an ecological cline in terms of rainfall and riverine forest ecotype, along a river enlarging from downstream to upstream and oriented south to north. Microsatellite DNA comparison demonstrated structuring between the populations, but not complete isolation, with an overall $Fst = 0.012$ ($P< 0.001$). Wing geometry revealed significant centroid size and shape differences between populations, especially between the two most distant populations. There was no significant correlation between gene flow and geographic distance at this scale, but there was a positive correlation in females between metric distances (wing shape differences) and geographic distances that might be attributed to the cline of environmental conditions. The impact of the fragmentation of riparian landscapes on tsetse population structure is discussed in the context of control campaigns currently promoted by Pan African Tsetse and Trypanosomosis Eradication Campaign.

14203. **Bouyer, J., Siberti, A., Desquesnesi, M., Cuisance, D., & de la Roque, S., 2007**. A diffusion model for *Glossina palpalis gambiensis* in Burkina Faso. In: *Area –Wide Control of Insect Pests: From Research to Field Implementation*. Springer, The Netherlands, pp. 137-149.

Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Département d'Elevage et de Médecine Vétérinaire, BP 5035, 34032 Montpellier, France; Centre International de Recherche-développement sur l'Elevage en Zone Subhumide, BP 454, Bobo Dioulasso, Burkina Faso; Institut Sénégalais de Recherches Agricoles, Laboratoire National d'Etudes et de Recherches Vétérinaires, BP 2057, Dakar-Hann, Senegal; Conseil Général Vétérinaire, 251 rue de Vaugirard, 75732 Paris Cedex 15, France

The dispersal of *Glossina* species is of interest to pest control personnel since these flies are the biological vectors of human and animal trypanosomes in Africa. The design of control and/or eradication programmes requires an accurate knowledge of the ecological characteristics of tsetse flies and the geographic structure of their populations. The present study attempts to model the dispersal process of a riverine tsetse species, i.e. *Glossina palpalis gambiensis* Vanderplank in Burkina Faso along an apparent homogeneous gallery forest. While for savannah species, dispersal is usually modelled as a two-dimensional

random walk (in time and space) or diffusion (its continuous analogue), for riverine species, dispersal can be viewed more simply as a one-dimensional random walk. The data reported here show that the topology of the habitat, which is a system of tributaries rather than a straight line, has a great impact on the dispersal process. Moreover, since only a part of the river system can be observed in practice, the effect of partial observation when estimating dispersal parameters can be quantified. The results reported here were obtained using a data set from a mark-release-recapture experiment carried out with *G. p. gambiensis* on a tributary of the Mouhoun River in Burkina Faso. The model was fitted to field data and used to estimate the displacement of a fly during 10 percent of its lifespan (13 kilometres) and the probability of it dispersing more than 10 kilometres from its initial position ($p > 0.1$). The analysis was carried out by either taking into account, or ignoring, the fact that only part of the river system was observed during the mark-release-recapture protocol.

14204. **Cano, J., Descalzo, M. A., Ndong-Mabale, N., Ndongo-Asumu, P., Bobuakasi, L., Buatiche, J. N., Nzambo-Ondo, S., Ondo-Esono, M., Benito, A. & Roche, J., 2007**. Spatial and temporal variability of the *Glossina palpalis palpalis* population in the Mbini focus (Equatorial Guinea). *International Journal of Health Geographics,* **6**: 36.

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Human African Trypanosomiasis is a vector-borne parasitic disease. The geographical distribution of the disease is linked to the spatial distribution of the tsetse fly. As part of a control campaign using traps, the spatial and temporal variability of the *Glossina* populations present in the Mbini sleeping sickness foci (Equatorial Guinea) is analysed. A significant drop in the annual mean *G. p. palpalis* apparent density was noted from 2004 to 2005, although seasonal differences were not observed. The apparent density (AD) of *G. p. palpalis* varies significantly from one biotope to another. The fish dryers turned out to be zones with the greatest vector density, although the AD of *G. p. palpalis* fell significantly in all locations from 2004 to 2005. Despite the tsetse fly density being relatively low in fish dryers and jetties, the population working in those zones would be more exposed to infection. The mono-pyramidal traps in the Mbini focus have been proven to be a useful tool to control *G. p. palpalis*, even though the activity on the banks of the River Wele needs to be intensified. The application of spatial analysis techniques and geographical information systems are very useful tools to discriminate zones with high and low apparent density of *G. p. palpalis*, probably associated with different potential risk of sleeping sickness transmission.

14205. **Fukatsu, T., Koga, R., Smith, W. A., Tanaka, K., Nikoh, N., Sasaki-Fukatsu, K., Yoshizawa, K., Dale, C. & Clayton, D. H., 2007**. Bacterial endosymbiont of the slender pigeon louse, *Columbicola columbae*, allied to endosymbionts of grain weevils and tsetse flies. *Applied Environmental Microbiology,* **73** (20): 6660-6668.

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The current study focuses on a symbiotic bacterium found in the slender pigeon louse, *Columbicola columbae* (Insecta: Phthiraptera). Molecular phylogenetic analyses indicated that the symbiont belongs to the gamma subdivision of the class Proteobacteria and is allied to *Sodalis glossinidius*, the secondary symbiont of tsetse flies (*Glossina* spp.) and also to the primary symbiont of grain weevils (*Sitophilus* spp.). Relative-rate tests revealed that the symbiont of *C. columbae* exhibits accelerated molecular evolution in comparison with the tsetse fly symbiont and the weevil symbiont. Whole-mount *in situ* hybridization was used to localize the symbiont and determine infection dynamics during host development. In first- and second-instar nymphs, the symbionts were localized in the cytoplasm of oval bacteriocytes that formed small aggregates on both sides of the body cavity. In third-instar nymphs, the bacteriocytes migrated to the central body and were finally located in the anterior region of the lateral oviducts, forming conspicuous tissue formations called ovarian ampullae. In adult females, the symbionts were transmitted from the ovarian ampullae to developing oocytes in the ovarioles. In adult males, the bacteriocytes often disappeared without migration. A diagnostic PCR survey of insects collected from Japan, the United States, Australia, and Argentina detected 96.5 percent (109/113) infection, with a few uninfected male insects. This study provides the first microbial characterization of a bacteriocyte-associated symbiont from a chewing louse. Possible biological roles of the symbiont are discussed in relation to the host nutritional physiology associated with the feather-feeding lifestyle.

14206**. Guerrini, L., & Bouyer, J., 2007.** Mapping African animal trypanosomosis risk: the landscape approach. *Veterinaria Italiana* **43**(3): 643-654.

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African animal trypanosomosis (AAT) is a major hindrance to cattle breeding in the Mouhoun River Basin of Burkina Faso. The authors describe a landscape approach that enables the mapping of tsetse densities and AAT risk along the Mouhoun River loop (702 km long) in Burkina Faso. Three epidemiological landscapes were described: the first and most dangerous corresponded to protected forests and their border areas, with a 0.74 apparent density of infectious fly per trap per day (ADTi), the second to a partially disturbed vegetal formation, with a 0.20 ADTi and the third to a completely disturbed landscape with a 0.08 ADTi. Using this risk indicator, the first landscape was 3.92 more risky than the second which was 3.13 more risky than the last. Similar infectious rates were found in all landscapes (approximately 8 percent) but tsetse apparent densities dropped significantly ($p<0.001$) in half-disturbed (2.66) and disturbed landscapes (0.80) in comparison to the natural and border landscapes (11.77). Females were significantly younger (mean physiological age of 29 days) only in the most disturbed landscape ($p<0.05$) than in the two other ones (41 days). According to these results, practical implications of stratifying AAT risk and mapping tsetse densities in vector control campaigns are discussed.

14207. **Peacock, L., Ferris, V., Bailey, M. & Gibson, W., 2007**. Dynamics of infection and competition between two strains of *Trypanosoma brucei brucei* in the tsetse fly observed using fluorescent markers. *Kinetoplastid Biology and Disease,* **6**: 4.

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Genetic exchange occurs between *Trypanosoma brucei* strains during the complex developmental cycle in the tsetse vector, probably within the salivary glands. Successful mating will depend on the dynamics of co-infection with multiple strains, particularly if intraspecific competition occurs. We have previously used *T. brucei* expressing green fluorescent protein to study parasite development in the vector, enabling even one trypanosome to be visualized. Here we have used two different trypanosome strains transfected with either green or red fluorescent proteins to study the dynamics of co-infection directly in the tsetse fly. The majority of infected flies had both trypanosome strains present in the midgut, but the relative proportion of red and green trypanosome strains varied considerably between flies and between different sections of the midgut in individual flies. Colonization of the paired salivary glands revealed greater variability than for midguts, as each gland could be infected with red and/or green trypanosome strains in variable proportions. Salivary glands with a mixed infection appeared to have a higher density of trypanosomes than glands containing a single strain. Comparison of the numbers of red and green trypanosomes in the proventriculus, salivary exudate and glands from individual flies showed no correlation between the composition of the trypanosome population of the proventriculus and foregut and that of the salivary glands. For each compartment examined (midgut, foregut, salivary glands), there was a significantly higher proportion of mixed infections than expected, assuming the null hypothesis that the development of each trypanosome strain is independent. Both the trypanosome strains used were fully capable of infecting tsetse, but the probabilities of infection with each strain were not independent, there being a significantly higher proportion of mixed infections than expected in each of three compartments examined: midgut, proventriculus and salivary glands. Hence there was no evidence of competition between trypanosome strains, but instead co-infection was frequent. Infection rates in co-infected flies were no different to those found routinely in flies infected with a single strain, ruling out the possibility that one strain enhanced infection with the other. We infer that each fly is either permissive or non-permissive of trypanosome infection with at least 3 sequential checkpoints imposed by the midgut, proventriculus and salivary glands. Salivary glands containing both trypanosome strains appeared to contain more trypanosomes than singly-infected glands, suggesting that lack of competition enhances the likelihood of genetic exchange.

14208. **Ravel, S., Meeus, T. de., Dujardin, J. P., Zeze, D. G., Gooding, R. H., Dusfour, I., Sane, B., Cuny, G., & Solano, P., 2007.** The tsetse fly *Glossina palpalis palpalis* is composed of several genetically differentiated small populations in the sleeping sickness focus of Bonon, Cote d'Ivoire. *Infection, Genetics and Evolution*, **7**: 116-125.

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Glossina palpalis is the main vector of human African trypanosomosis (HAT, or sleeping sickness) that dramatically affects human health in sub-Saharan Africa. Because of the implications of genetic structuring of vector populations for the design and efficacy of control campaigns, *G. palpalis palpalis* in the most active focus of sleeping sickness in Cote d'Ivoire was studied to determine whether this taxon is genetically structured. High and statistically significant levels of within population heterozygote deficiencies were found at each of the five microsatellite loci in two temporally separated samples. Neither null alleles, short allele dominance, nor trap locations could fully explain these deviations from random mating, but a clustering within each of the two samples into different genetic sub-populations (Wahlund effect) was strongly suggested. These different genetic groups, which could display differences in infection rates and trypanosome identity, were composed of small numbers of individuals that were captured together, leading to the observed Wahlund effect. Implications of this population structure on tsetse control are discussed.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also 30: nos. 14168, 14173, 14178, 14179, 14185, 14186, 14187, 14188, 14202, 14203, 14204]

14209. **Alemu, T., Kapitano, B., Mekonnen, S., Aboset, G., Kiflom, M., bancha, B., Woldeyes, G., Bekele, K., & Feldmann, U., 2007**. Area-wide control of tsetse and trypanosomosis: Ethiopian experience in the Southern Rift Valley. In: *Area – Wide Control of Insect Pests: From Research to Field Implementation*. Springer, The Netherlands, pp. 325-337.

Southern Rift Valley Tsetse Eradication Project (STEP) National Coordination Office, PO Box 19917, Addis Ababa, Ethiopia; STEP Field Coordination Office, PO Box 474, Awassa, Ethiopia; Insect Pest Control Sub-Programme, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Wagramerstrasse 5, PO Box 100, A-1400 Vienna, Austria.

In 1997, the Ethiopian Government – assisted by the International Atomic Energy Agency (IAEA) – initiated a project in the Southern Rift Valley called the Southern Tsetse Eradication Project (STEP). Its long-term objectives are: (1) to create a tsetse-free zone in a 25 000 km^2 area under agricultural development, and (2) to develop adequate national capacity for applying the concept of area-wide integrated pest management (AW-IPM) with a sterile insect technique (SIT) component to other parts of the country affected by the tsetse and trypanosomosis (T and T) problem. This project will require consistent commitment and inputs by major stakeholders over a period of at least 15 years. The project was initiated with the collection and evaluation of entomological, veterinary, environmental and socio-economic baseline data which reconfirmed the presence of only one species, i.e. *Glossina*

pallidipes Austen in the main valley, and the positive socio-economic and agro-ecological impact anticipated. This situation generated international acceptance of the Southern Rift Valley as a high priority area for the control of T and T and for related sustainable agriculture and rural development. A colony of *Glossina pallidipes* Austen originating from the Southern Rift Valley was also initiated. In 2002, community-based tsetse suppression was initiated in localized areas using insecticides on cattle and on blue-black-blue fabric targets that attract tsetse flies. These localized tsetse suppression activities have been expanded to all operational grids of the 10500 km^2 STEP block-1 area. Limited entomological and veterinary monitoring in 15 sites suggests that the apparent density of *G. pallidipes* in these localized control sites may have been reduced by 92 percent, while the prevalence of trypanosomes in livestock in those areas decreased by 58 percent. An analysis using geographic information systems (GIS) has indicated that the community-based tsetse suppression does not cover all of the tsetse-infested areas in the STEP block-1 and it is therefore assumed that some cattle herds remain with high disease prevalence in areas that were not adequately covered by the community fly control measures. The operational programme will include the introduction of a set of implementation rules and regulations conducive to the special needs of an operational AW-IPM campaign, i.e. an efficient management structure and the provision of adequate financial flexibility.

14210. **Baumgärtner, J., Gilioli, G., Tikubet, G., & Gutierrez, A.P., 2007.** Eco-social analysis of an East African agro-pastoral system: Management of tsetse and bovine trypanosomiasis**.** *Ecological Economics***. In press; corrected proof.**

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A key constraint for development of many East African agro-pastoral communities is African animal trypanosomiasis or nagana caused by *Trypanosoma* spp. and vectored by species of tsetse flies (*Glossina* spp.). Suppression of trypanosomiasis through trapping of tsetse fly populations was conducted from 1995 to 2005 at and near Luke, Southwest Ethiopia. Odour baited mass trapping technology was used to suppress adult fly populations to very low levels while trypanocidal drugs were used to treat trypanosome infections in cattle. Data on ecological, economic and social variables were collected and analyzed in the context of eco-social dynamics in the community. The bio-economic model of Regev *et al.* [Regev, U., Gutierrez, A.P., Schreiber, S.J. & Zilberman, D. 1998. Biological and Economic Foundations of Renewable Resource Exploitation. Ecological Economics 26, 227-242] and Gutierrez and Regev [Gutierrez, A.P. & Regev, U. 2005. The bioeconomics of tritrophic systems: applications to invasive species. Ecological Economics 52, 382-396] was used as a methodological framework for qualitative evaluation of the effects of tsetse/trypanosomiasis suppression on ecological, economic and social aspects. An objective function for single farmers was formulated to determine the optimal harvesting level of cattle, exposed to high and low levels of risk from tsetse/trypanosomiasis, as measured by the discount rate (δ) for a

given base level pastoral resource (R = pasture or forage for cattle). The socially optimal objective function for resource exploitation by all farmers is that which maximizes the present value of utility of individuals expending revenues (*consumption*) from the revenue stream in ways that enhance the quality of life and yet assures the persistence of the resource base over an infinite time horizon (i.e., renewable resource sustainability). The bio-economic model predicts that reducing risk (δ) from tsetse and disease increased the cattle populations and their marginal value. The model also predicts that the interaction of δ and increased productivity (θ) can lead to increased human and cattle populations and hence to over-exploitation of base resources (pastures) that lower environmental carrying capacity and reduced sustainability. Trap catches indicated that tsetse populations were reduced to very low levels, while the disease prevalence decreased from 29 percent to 10 percent. This led to a substantial increase in cattle including oxen populations, increased calving rates, increased milk production and increased the per-capita income. The availability of oxen allowed an increase in cultivated land from 12 ha in 1995 to 506 ha in 2005. Revenues (*consumption*) were invested in the purchase of more cattle and the establishment of a school for educating village children. Increases in land allocated to crops and other sources of income were also found. The bioeconomic model predicts the solution of the trypanosomiasis problems so transforms the East African agro-pastoral communities that new social structures will be required to cope with the ecological, economic and social consequences of this technological changes on sustainable development (*sensu* [Goodland, R., 1995. The concept of environmental sustainability. Annual Review of Ecology and Systematics 26, 1-24]). This insight should not be lost in international rural development programmes.

14211. **Kappmeier Green, K., Potgieter, F. T., & Vreysen, M. J. B., 2007**. A strategy for an area-wide control campaign with an SIT component to establish a tsetse-(*Glossina austeni* and *Glossina brevipalpis*) free areas in South Africa. In: *Area – Wide Control of Insect Pests: From Research to Field Implementation*. Springer, The Netherlands, pp. 309-325.

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A strategy is proposed to create an area free of *Glossina brevipalpis* Newstead and *Glossina austeni* Newstead in the southern-most tsetse fly belt in the province of KwaZulu-Natal, South Africa. The concept is based upon an area-wide integrated pest management (AW-IPM) approach that integrates several tsetse suppression techniques, such as insecticide impregnated odour-baited targets, mobile targets, the sequential aerosol technique (SAT), and the release of sterile insects (sterile insect technique (SIT)). The prerequisites for the proposed programme are described and include the development of sampling and control tools, ecological studies, entomological field surveys, feasibility studies and the development of adequate tsetse rearing capacity. The proposed AW-IPM strategy suggests the division of the 12000 km^2 tsetse-infested area into four zones of manageable size and the successive implementation of four phases (pre-suppression, suppression (population reduction), release of sterile males and post-eradication activities) in each of these zones following the "rolling carpet principle". Assuming a minimum release density of 100 sterile males per square

kilometre, tsetse colonies of around 4.5 million producing *G. brevipalpis* females, and 5.5 million *G. austeni* would be required to sustain the releases. The entire programme would require an annual budget of USD 3.35 million for the duration of eight years. The creation of a tsetse fly-free area in South Africa and southern Mozambique would result in significant improvements to the livelihood of communal farmers owning around 350000 cattle.

14212. **Pendleton, F. N., & Baldwin, A.H., 2007.**The effects of spraying deltamethrin for tsetse fly control on insectivorous bird populations in the Okavango Delta, Botswana. *African Journal of Ecology*, 45: 566-576.

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We investigated the effects of spraying deltamethrin for tsetse fly control on bird populations in the Okavango Delta, Botswana. The northern part of the Delta was sprayed five times in 2001, and the southern part was sprayed five times in 2002. While deltamethrin is not particularly toxic to vertebrates, it is highly toxic to many types of insects. Therefore, we hypothesized that birds could be affected indirectly through reductions in their insect food supplies. We monitored resident bird populations using point counts at four sites (two sprayed in 2001 and two sprayed in 2002). We conducted 22 analyses of insectivorous bird numbers and four (18 percent) of these showed declines, five (23 percent) showed increases, and thirteen (59 percent) showed no change. No insectivorous species declined at more than one study site. When insectivorous species were lumped as a guild, there were weak declines at one site but no changes at the other three sites. Our results indicate that spraying deltamethrin did not cause catastrophic population declines in insectivorous birds within a year. However, foraging behaviour, physiology, breeding success and longer-term population effects were not investigated and cannot be ruled out.

14213. **Saini, R. K., & Hassanali, A., 2007.** A 4-alkyl-substituted analogue of guaiacol shows greater repellency to savannah tsetse (*Glossina* spp.). *Journal of Chemical Ecology*, **33**: 985-95.

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The responses of *Glossina morsitans morsitans* Westwood to guaiacol (2-methoxyphenol), a mild repellent constituent of bovid odours, and seven analogues comprising 2-methoxyfuran, 2,4-dimethylphenol, 2-methoxy-4-methylphenol (4-methylguaiacol), 4-ethyl-2-methoxyphenol (4-ethylguaiacol), 4-allyl-2-methoxyphenol (4-allylguaiacol; eugenol), 3,4-methylenedioxytoluene, and 3,4-dimethoxystyrene were compared in a two-choice wind tunnel. The 4-methyl-substituted derivative (2-methoxy-4-methylphenol) was found to elicit stronger repellent responses from the flies compared with guaiacol. None of the other analogues showed significant repellent effects on flies. 4-methylguaiacol, guaiacol, and eugenol (which was included because of previous reports of its repellency against a number of arthropods) were further evaluated in the field with wild populations of predominantly *Glossina pallidipes* Austen. The presence of guaiacol or

eugenol near odour-baited traps caused some nonsignificant reduction in the number of tsetse catches at relatively high release rates (approximately 50 mg/h). In contrast, the 4-methyl derivative at three different release rates (2.2, 4.5, and 9.0 mg/h) reduced trap catches of baited traps in a dose-response manner. At 10 mg/h release rate, it reduced the catches of baited and unbaited traps by approximately 80 and approximately 70 percent, respectively. In addition, the compound not only reduced the number of tsetse attracted to natural ox odour (approximately 80 percent), but also had an effect on their feeding responses, reducing the proportion that fed on an ox by more than 80 percent. Our study shows that the presence of a methyl substituent at the 4-position of guaiacol enhances the repellency of the molecule to savannah tsetse and suggests that 4-methylguaiacol may represent a promising additional tool in the arsenal of techniques in trypanosomiasis control.

14214. **Somda, J., Kamuanga, M. & Tollens, E., 2006**. Prospective analysis for community participation in trypanosomosis control in The Gambia. *Tropical Animal Health and Production,* **38** (2): 103-111.

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The shift towards community participation in the eradication of trypanosomosis calls for investigation the underlying incentive structure for individuals in the community to cooperate in the provision of various control methods. Survey data were used to assess patterns of the community's demand for insecticide pour-ons and trypanocidal drugs and factors affecting individual demand in The Gambia. The results show that insecticide pour-on is strongly preferred. Similarly, farmers revealed a preference for community-based provision scheme. Factors affecting an individual farmer's decision to invest in either pour-on or trypanocidal drugs were highlighted. While there are many factors associated with farmer's decisions to invest in trypanosomosis control methods and to participate in collective actions, the results indicate that farmers are ready to anticipate complete privatization of veterinary services through community-based schemes.

14215**. Symeonakis, E., Robinson, T., & Drake, N., 2007.** GIS and multiple-criteria evaluation for the optimisation of tsetse fly eradication programmes. *Environmental Monitoring and Assessment*, 124: 89-103.

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Tsetse flies are the vectors of trypanosomes, the causal agent of trypanosomiasis, a widespread disease of livestock and people in Africa. Control of tsetse may open vast areas of land to livestock-keeping, with the associated benefits of developing mixed crop-livestock production systems. However, as well as possible positive impacts there are also risks: bush clearing would accelerate and cattle numbers would rise, leading to a reduction of vegetation cover, and an increase in runoff and erosion; there may also be increased pressure on conserved areas and reductions in biodiversity. The objective of this study is to show how remotely sensed and other environmental data can be combined in a decision support system to help inform tsetse control programmes in a manner that could be used to limit possible detrimental effects of tsetse control. For Zambia, a methodology is developed that combines a

tree-based decision-support approach with the use of Multiple-Criteria Evaluation (MCE), within a Geographical Information System (GIS), in order to target areas for tsetse control. The results show clear differentiation of priority areas under a series of hypothetical scenarios, and some areas (e.g. northwest of Petauke in the Eastern Province of Zambia) are consistently flagged as high priority for control. It is also demonstrated that priority areas do not comprise isolated tsetse populations, meaning that disease control using an integrated approach is likely to be more economically viable than local eradication.

4. EPIDEMIOLOGY: VECTOR-HOST AND VECTOR-PARASITE INTERACTIONS

[See also 30: nos. 14167, 14170, 14179, 14181, 14183, 14194, 14195, 14198, 14207, 14232, 14233]

14216. **Adams, E. R., Hamilton, P. B., Malele, II & Gibson, W. C., 2007.** The identification, diversity and prevalence of trypanosomes in field caught tsetse in Tanzania using ITS-1 primers and fluorescent fragment length barcoding. *Infection, Genetics and Evolution*. **In press; corrected proof.**

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We report on the development of two generic, PCR-based methods, which replace the multiple species-specific PCR tests used previously to identify the trypanosome species carried by individual tsetse flies. The first method is based on interspecies size variation in the PCR product of the ITS-1 region of the ribosomal RNA (rRNA) locus. In the second approach, length variation of multiple fragments within the 18S and 28S rRNA genes is assayed by PCR amplification with fluorescent primers; products are subsequently sized accurately and rapidly by the use of an automated DNA sequencer. Both methods were used to identify samples collected during large-scale field studies of trypanosome-infected tsetse in Tanzania in the National Parks of Tarangire and Serengeti, and the coastal forest reserve of Msubugwe. The fluctuations of trypanosome prevalence over time and two different field seasons are discussed. As well as facilitating the identification of trypanosome species with increased speed, precision and sensitivity, these generic systems have enabled us to identify two new species of trypanosome.

14217. **Courtin, D. & Garcia, A., 2007.** Human African trypanosomiasis: involvement of host genetics. *Médecine Tropicale (Mars)*, 67 (2): 131-133.

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Two genetic epidemiological studies were carried out in the Ivory Coast and Democratic Republic of Congo to assess the role of human genetic diversity in susceptibility to human African trypanosomiasis (HAT). Findings showed that four single DNA polymorphisms located on genes coding for cytokines were correlated with a variable risk for development of the disease. Whereas presence of the rare A and T alleles for IL10 592 C/A

and IL6 4339 C/T polymorphisms appeared to protect against HAT, presence of the T allele and AA genotype for IL1 5417 C/T and TNFalpha-308 G/A polymorphisms were correlated with an increase in HAT risk. These results will improve understanding of the host-parasite interaction and, ultimately, assist the development of new therapeutic and prophylactic tools.

14218. **Dedet, J. P., 2007.** Edmond Sergent's discoveries on the vectorial transmission of agents of human and animal infectious diseases. *Bulletin de la Société de Pathologie Exotique*, **100** (2): 147-150.

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Edmond Sergent has been head of the Institut Pasteur dispar (now *T. annulata*) by the tick *Hyalomma mauritanicum* (1928).in Algeria from 1910 to 1963. During these years, he carried out an impressive scientific production and studied a lot of agents responsible for human, animal and plant diseases. In the field of vectorial transmission of infectious diseases, he made two essential discoveries: the transmission of cosmopolitan relapsing fever by human body louse in 1908, a year before Charles Nicolle discovered the transmission of the classical exanthematic typhus by the same insect, and the transmission of cutaneous leishmaniasis by the phlebotomine sandfly. Moreover he made other discoveries in similar fields, such as the transmission of dromedary trypanosomiasis by Tabanids and later by *Stomoxys calcitrans*, or the transmission of the pigeon *Haemoproteus* by *Lynchia maura*. Finally he described the transmission of *Theileria dispar* (now *T.annulata*) by the tick *Hyalomma mauritanicum* (1928)

14219. **Herder, S., Votypka, J., Jirku, M., Radrova, J., Janzen, C. J. & Lukes, J., 2007**. *Trypanosoma brucei* 29-13 strain is inducible in but not permissive for the tsetse fly vector. *Experimental Parasitology*, **117** (1): 111-114.

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Using green fluorescent protein as a reporter, we have shown that the strain 29-13 of *Trypanosoma brucei*, widely used for inducible down-regulation of mRNA, is inducible in, but not permissive for the tsetse flies *Glossina palpalis gambiensis* and *Glossina morsitans morsitans*. Within two weeks post-infection, 42 percent males and females of teneral and non-teneral tsetse flies harboured intestinal infections, yet not a single infection progressed into the salivary glands.

14220**. Kokwaro, E. D., Okoth, S. O., Kiragu, J. M., & Murila, G. A., 2007.** Influence of socio-economic and cultural activities on vector-host interaction and risk of rhodesian sleeping sickness at Busia and Nguruman areas of Kenya. *Trends in Applied Sciences. Research . Academic Journals, Faisalabad, Pakistan*: 2: 28-38.

Trypanosomiasis Research Center, Agricultural Research Institute, Po Box 362, Kikuyu, Kenya.

This study assessed the influence of socio-economic, cultural and demographic variations on human-fly contact i.e. interaction between human beings and tsetse flies and their potential role in transmission risk of rhodesian sleeping sickness among the Teso and Maasai communities of Kenya. Results indicated that farming was the economic activity with the highest risk, exposing 84 percent males and 75 percent females to tsetse bites at Busia. Level of formal education influenced choice of occupation, with farming absorbing 85 percent of those with primary education. Ritual bathing was the riskiest cultural activity exposing 45.97 percent of the population into contact with tsetse flies. Grazing pattern favoured contact avoidance thus minimizing risk of disease trypanosomiasis to livestock. However at Nguruman, livestock keeping was the riskiest economic activity exposing 60 percent of the population to tsetse flies. Level of formal education did not influence choice of occupation and over 90 percent of the land was under bush and was tsetse infested. Ol pull and Moranism were the most important cultural activities exposing 85.7 percent of the population into contact with tsetse flies. Vector-host contact was highest at watering points and men and women were at risk with risk indices of 47.73 and 26.5 percent, respectively. Management of transmission risk in both Busia and Nguruman would therefore be partly aided by conducting socio-cultural practices in tsetse free areas and scheduling economic activities such as herding as to avoid intense contact with the tsetse flies.

14221. **Kubi, C., Billiouw, M. & Van Den Bossche, P., 2007.** Age prevalence of trypanosomal infections in female *Glossina morsitans morsitans* (Diptera: *Glossinidae*) on the plateau area of eastern Zambia. *Onderstepoort Journal of Veterinary Research*, **74** (3): 223-229.

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Trypanosomal infections in female *Glossina morsitans morsitans* were investigated in an area in the Eastern Province of Zambia between 1992 and 1994. A total of 4416 flies were captured, aged using the ovarian ageing method and screened for trypanosomal infections in both the mouthparts, salivary glands and the midgut. *Congolense*-type infections were identified in 4.8 percent of the flies. *Vivax*-type and immature infections were identified in 1.8 percent and 6.8 percent of the flies, respectively. The prevalence of *congolense*-type, *vivax*-type and immature infections increased with age. For *vivax*-type infections the age-prevalence relationship could be described by a model assuming a constant per capita rate of infection. For *congolense*-type and midgut infections, a polynomial term was added to the model significantly improving the fit. The *per capita* rate at which flies become infected was significantly higher for immature compared to mature infections. Observations strongly suggest that tsetse acquire new midgut infections at any age and that maturation of these infections is not limited to those obtained during the first blood meal.

14222. **Lefevre, T., Thomas, F., Ravel, S., Patrel, D., Renault, L., Le Bourligu, L., Cuny, G. & Biron, D. G., 2007.** *Trypanosoma brucei brucei* induces alteration in the head proteome of the tsetse fly vector *Glossina palpalis gambiensis*. *Insect Molecular Biology.* **In press; corrected proof.**

GEMI, Montpellier, France.

Parasitic manipulations of host behaviour are known from a wide range of host-parasite associations. However, the understanding of these phenomena is far from complete and detailed investigation of their proximate causes is needed. Many studies report behavioural modifications, such as altered feeding rates in tsetse fly (*Glossina*) infected with the mature transmissible stage (i.e. metacyclic) of the trypanosomes. Here, bidimensional (2D) gel electrophoresis and mass spectrometry were employed to analyse and compare the head proteome between four *Glossina palpalis gambiensis* categories (uninfected, refractory, mature infection, immature infection). Twenty-four protein spots specifically present or absent in the head of metacyclic-infected flies were observed. These protein spots were subsequently identified and functionally classified as glycolitic, neurotransmiter synthesis, signalling, molecular chaperone and transcriptional regulation proteins. Our results indicate altered energy metabolism in the head of metacyclic-infected tsetse flies. Some of the proteins identified, such as casein kinase 2 and jun kinase have previously been shown to play critical roles in apoptosis in insect neurones. In addition, we found two pyridoxal-dependent decarboxylases (dopa decarboxylase and alpha methyldopa hypersensitive protein), suggesting a modification of serotonin and/or dopamine in the brain of metacyclic-infected tsetse flies. Our data pave the way for future investigation of the alteration of the *Glossina* central nervous system during infection by trypanosomes.

14223. **MacLeod, E. T., Maudlin, I., Darby, A. C. & Welburn, S. C., 2007.** Antioxidants promote establishment of trypanosome infections in tsetse. *Parasitology*, **134** (Pt 6): 827-831.

Centre for Infectious Diseases, College of Medicine and Veterinary Medicine, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian EH25 9RG, UK.

Efficient, cyclical transmission of trypanosomes through tsetse flies is central to maintenance of human sleeping sickness and nagana across sub-Saharan Africa. Infection rates in tsetse are normally very low as most parasites ingested with the fly bloodmeal die in the fly gut, displaying the characteristics of apoptotic cells. Here we show that a range of antioxidants (glutathione, cysteine, N-acetyl-cysteine, ascorbic acid and uric acid), when added to the insect bloodmeal, can dramatically inhibit cell death of *Trypanosoma brucei brucei* in tsetse. Both L- and D-cysteine invoked similar effects suggesting that inhibition of trypanosome death is not dependent on protein synthesis. The present work suggests that antioxidants reduce the midgut environment protecting trypanosomes from cell death induced by reactive oxygen species.

14224. **Merid, N., Melaku, G. & Emiru, S., 2007**. Epizootiological importance of *Glossina morsitans submorsitans* (Diptera: *Glossinidae)* (Newstead) in the Ghibe River Valley, Southwest Ethiopia. *Acta Tropica*, **102** (2): 100-105.

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The epizootiological importance of *Glossina morsitans submorsitans* in Ghibe River Valley was investigated from October 2000 to September 2001. The flies were collected

using baited monoconical traps. *G. m. submorsitans* occurred with a mean apparent density of 4.26+/-0.49 flies/trap/day and the apparent density was characterized by an increase during the wet season and a decrease during the dry season. Among 450 *G. m. submorsitans*, approximately 5 percent were found to be infected with trypanosome. Of these infected flies, 76 percent were female. *Nanomonas, Duttonella* and *Trypanozoon* were the three trypanosome subgenera detected and occurred in the proportions of 57.1 percent, 38.1 percent and 4.8 percent, respectively. Among 139 blood meals of *G. m. submorsitans* collected, 54.68 percent were identified to group or species levels. Accordingly, 36.84 percent, 25 percent, 11.84 percent and 10.53 percent accounted for cattle, kudu, suidae (warthog and/or wild pig) and human, respectively and others such as goats (6.58 percent), bovidae (5.26 percent), baboon (2.63 percent) and water buck (1.32 percent). While 21.05 percent of the blood meals were found to be out of detection range.

14225. **Okoth, S. O., Kokwaro, E. D., Kiragu, J., & Murila, G. A., 2007.** *Glossina pallidipes* and host interactions: implications of host preference on transmission risk of rhodesian sleeping sickness in Kenya. *Trends in Applied Sciences Research. Academic Journals, Faisalabad, Pakistan*: **2**: 386-394.

Trypanosomiasis Research Center-Kenya, Agricultural Research Institute, P.O. Box 362, Kikuyu, Kenya.

Host preference by tsetse flies, tsetse-host interaction and host diversity and abundance were evaluated in relation to transmission risk of rhodesian sleeping sickness in two tsetse subpopulations in Kenya. *Bovidae* provided the highest proportion of blood meals (58 percent) to tsetse at Busia while that from humans was 4.9 percent. Contrastingly, the highest proportion of blood meals at Nguruman (35 percent) was from Warthogs, while no blood meals were obtained from humans at Nguruman. The bushbuck *Tragelaphus criptus*, Pallas, an important reservoir host of *T. b. rhodesiense*, provided 2.5 percent of blood meals at Busia and 5 percent of blood meals at Nguruman. Hosts were more diverse and abundant at Nguruman than Busia. Host activity did not significantly influence vector activity at both Busia and Nguruman during the dry season. However, there was a significant influence of host activity on vector activity ($F_{10,11}$=7.27; $p<0.022$) at Nguruman during the wet season. The diversity and abundance of reservoir hosts at Nguruman is a potential risk in maintenance of sleeping sickness, unlike at Busia where the reservoir hosts are fewer and less diverse. The occurrence of *Bovidae*, especially livestock, as the major alternative source of blood meal at Busia pose higher risk to humans as the livestock are constantly in close contact with humans. Risk control would therefore aim at contact avoidance and sustained suppression of vector population.

14226. **Sindato, C., Malele, II, Mwalimu, C., Nyingilili, H. S., Kaboya, S., Kombe, E., Msumary, C. & Manoza, A., 2007**. Seasonal variation in human African trypanosomiasis in Tarangire National Park in Babati district, Tanzania. *Tanzanian Health Research Bulletin*, **9** (2): 136-139.

National Institute for Medical Research, P.O. Box 482, Tabora, Tanzania. [kndato@yahoo.co.uk].

A survey was carried out to determine seasonal epidemiological variation of human African trypanosomiasis (HAT) in Tarangire National Park and villages around it in Babati District, Tanzania. Concentration and Field's stain techniques were employed to examine the presence of trypanosomes in human blood samples. Tsetse flies were collected using traps and dissected under light microscope to examine for presence of trypanosomes. Retrospective data on HAT were sought from health facilities. Blood samples were collected from a total 509 individuals (306 during the dry and 203 during wet seasons). None of the individuals was infected with trypanosomes in the area. A total of 766 tsetse flies were collected. Of these, *Glossina swynnertoni* accounted for 94.6 percent and *G. pallidipes* for 5.4 percent of the total collection. The largest proportion (63.8 percent) of the tsetse flies was collected during the wet season. *Glossina swynnertoni* was most abundant tsetse species during both wet and dry seasons. Salivary gland examination revealed the presence of *Trypanosoma brucei* type of infection in 3.2 percent of tsetse flies collected. All infective trypanosomes were found during the dry season. This study concludes that the transmission and prevalence of HAT among human population in Tarangire National Parks and its surrounding villages are low despite the recent reports on tourists acquiring the infection during their visits to the Park. However, disease surveillance needs to be strengthened to monitor any impending epidemic.

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE

[See also 30: no. 14226]

14227. **Airauhi L. U., Airauhi, E. S., & Adesina, K. A., 2003.** Sleeping Sickness Surveillance in the Abraka sleeping sickness focus (ASSF) Nigeria**.** *Annals of Biomedical Sciences,* **2(1):** 20 –29.

Department of Medical Microbiology, School of Medicine, University of Benin, Nigeria. [yununica@yahoo.com]

Humans are the main reservoir hosts for *gambiense* sleeping sickness. They are therefore essential for the sustenance of its endemicity and reemergence of epidemics in many disease foci within sub-Saharan Africa. To investigate the epidemiological and clinical significance of reservoir hosts in the Abraka Sleeping Sickness Focus (ASSF), this survey was conducted between 8th April and May 11th 2002 in seven endemic villages with an overall population of 13,683. Pretested structured questionnaires were administered following informed consent on 2437 participants (1061 males and 1376 females) to assess knowledge and beliefs about the disease. Card Agglutination Test for Trypanosomiasis (CATT) screening was performed using whole blood from 1568 (11.6 percent) subjects of the overall population. Study participants were aged between less than 1 year and over 61 years. Confirmation of sleeping sickness (ss) was by the detection of trypanosomes in blood, body fluids and biopsy tissues. Thirteen (0.8 percent) seropositive subjects were parasitologically confirmed and treated with melarsoprol at the Baptist Medical Centre (BMC) in Eku. One (0.06 percent) patient died during the course of treatment. Forty-two (0.3 percent) subjects dropped out from the study while 128 (0.9 percent) seropositive and aparasitaemic subjects

are currently being followed up. Five hundred and twenty (21.3 percent) of the 2,437 subjects interviewed reported having heard about the disease, while only 316 (12.9 percent) had correct knowledge about the vector for the disease. Three hundred and sixty six (15.0 percent) believe those with the disease should remain in hiding, 422 (17.3 percent) believe the disease is a taboo while only 592 (25.3 percent) believe the disease is treatable by orthodox means. Our result provides data on active case detection and suggests the need for the formulation of health policies aimed at promoting compatibility of beliefs and knowledge about sleeping sickness with appropriate treatment seeking behaviour in the area. This approach will highlight acceptable levels of effective disease suppression with the involvement and cooperation of the affected communities.

(b) PATHOLOGY AND IMMUNOLOGY

[See also 30: nos. 14217, 14249]

14228. **Blum, J. A., Schmid, C., Hatz, C., Kazumba, L., Mangoni, P., Rutishauser, J., la Torre, A. & Burri, C., 2007**. Sleeping glands?-The role of endocrine disorders in sleeping sickness (*T. b. gambiense* Human African Trypanosomiasis). *Acta Tropica,* **104** (1): 16-24.

Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland.

Symptoms consistent with hypothyroidism or adrenal insufficiency, such as lethargy, anorexia, cold intolerance, weakness, hypotension or paraesthesia, are frequently reported in the literature in patients with Human African Trypanosomiasis (HAT), but an endocrine origin for these symptoms has not yet been demonstrated. Thyroid and adrenocortical function were assessed in 60 patients with late-stage HAT and compared to those in 60 age- and gender-matched healthy controls. Clinical assessment and endocrine laboratory examinations were performed on admission, within 2 days after the end of treatment and at follow-up 3 months later. Signs and symptoms of hypothyroidism, such as fatigue, cold sensation, constipation, paraesthesia, peripheral oedema and dry skin, were significantly more frequent in HAT patients than in the controls. However, these signs and symptoms could not be attributed to hypothyroidism due to the lack of supporting laboratory data, and thus empirical replacement therapy for the clinically suspected hypothyroidism was not warranted. Signs and symptoms consistent with adrenal insufficiency, such as weakness, anorexia, weight loss or hypotension, were significantly more frequent in HAT patients than in controls, but they could not be associated with an insufficiency of the adrenocortical axis. Higher basal levels of cortisol were found in HAT patients than in controls, which can be viewed as a stress response to the infection. However, a transitory adrenal insufficiency was suspected in 8 percent of HAT patients at admission and in 9 percent at discharge. All values were normal at follow-up 3 months later.

(c) TREATMENT

14229. **Barrett, M. P., Boykin, D. W., Brun, R. & Tidwell, R. R., 2007**. Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. *British Journal of Pharmacology*. **In press; corrected proof.**

Division of Infection and Immunity, Institute of Biomedical and Life Sciences, The Glasgow Biomedical Research Centre, University of Glasgow, Glasgow, UK.

This review discusses the challenges of chemotherapy for human African trypanosomiasis (HAT). The few drugs registered for use against the disease are unsatisfactory for a number of reasons. HAT has two stages. In stage 1 the parasites proliferate in the haemolymphatic system. In stage 2 they invade the central nervous system and brain provoking progressive neurological dysfunction leading to symptoms that include the disrupted sleep wake patterns that give HAT its more common name of sleeping sickness. Targeting drugs to the central nervous system offers many challenges. However, it is the cost of drug development for diseases like HAT, that afflict exclusively people of the world's poorest populations, that has been the principal barrier to new drug development and has led to them becoming neglected. Here we review drugs currently registered for HAT, and also discuss the few compounds progressing through clinical trials. Finally we report on new initiatives that might allow progress to be made in developing new and satisfactory drugs for this terrible disease.

14230. **Kagira, J. M. & Maina, N., 2007**. Occurrence of multiple drug resistance in *Trypanosoma brucei rhodesiense* isolated from sleeping sickness patients. *Onderstepoort Journal of Veterinary Research,* **74** (1): 17-22.

Kenya Agricultural Research Institute-Trypanosomiasis Research Centre (KARI-TRC), P.O. Box 362, Kikuyu, Kenya. [jkagira@yahoo.com].

The occurrence of cross-resistance among melarsoprol-resistant *Trypanosoma brucei rhodesiense* isolates was investigated in this study. The isolates, *T. b. rhodesiense* KETRI 237, 2538, 1992, 2709, 2694 and 3530, had been obtained from sleeping sickness patients in Kenya and Uganda between 1960 and 1985. Five groups consisting of six mice each were inoculated intraperitoneally with 10(5) parasites of each isolate, and 24 h later treated with either melarsoprol, homidium chloride, diminazene aceturate or isometamidium chloride. The control group comprised infected but untreated mice. The mice were monitored for cure for a period of 60 days post-treatment. The mean prepatent period in the control mice was 5 days while the mean survival period was 22 days. Five of the stabilates, KETRI 237, 2538, 2709, 2694, and 3530, were confirmed to be melarsoprol resistant. Cross-resistance was observed, with the majority of the isolates being resistant to homidium chloride (5/6) and diminazene aceturate (5/6), but all were sensitive to isometamidium chloride (6/6). However *T. b. rhodesiense* KETRI 1992, which was previously considered as melarsoprol resistant, was sensitive to all the drugs tested. In conclusion, our study has revealed the existence of cross-resistance among the melarsoprol resistant isolates which could only be cured by isometamidium.

14231. **Woodrow, C. J., Abel, P. M. & Krishna, S., 2007**. Randomized, controlled trial of treatments for second-stage sleeping sickness. *Journal of Infectious Diseases,* **196** (4): 650-651.

No abstract available.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

[See also 30: nos. 142o6, 14209, 14224]

14232. **Herrera, H. M., Rademaker, V., Abreu, U. G., D'Andrea, P. S. & Jansen, A. M., 2007**. Variables that modulate the spatial distribution of *Trypanosoma cruzi* and *Trypanosoma evansi* in the Brazilian Pantanal. *Acta Tropica,* **102** (1): 55-62.

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An evaluation was made on how the landscape and cattle ranching affect the transmission cycles and the patterns of trypanosomatid infection (*Trypanosoma cruzi* and *Trypanosoma evansi*) of small wild mammals in the Pantanal. This region comprises a large natural environment with a multiplicity of habitats and a wide variety of biodiversity besides the presence of livestock. *T. cruzi* and *T. evansi* infections were evaluated by parasitological and serological methods in one preserved and one cattle ranching area. The diversity of the small mammal fauna were found to be the same in the two studied areas, however, their relative abundance was different. Distinct enzootiological scenarios of both trypanosomatids could be observed. Transmission of *T. cruzi* occurred mainly in forested areas within the two study areas, while *T. evansi* occurred dispersed among all habitats studied in the unpreserved area. The arboreal rodent *Oecomys mamorae*, the most abundant species in both areas, displayed high *T. cruzi* and *T. evansi* serum prevalence and parasitaemias. Also, the caviomorph rodent *Thrichomys pachyurus* was shown to be an important host due to its relative abundance, prevalence of infection by both trypanosomatid species and its broad range of habitats. The role of small mammal fauna in the transmission cycle of both trypanosomes species seems to be distinct according to land use since we found a broad range of *T. evansi* infected hosts in the protected area in contrast to the cattle ranching area and half the number of rodent species infected with *T. cruzi* in the unpreserved in comparison to the protected area. The present study showed that cattle ranching in this study area did not enhance overall prevalence of *T. cruzi* infection among small wild mammals. Together with the observation that small mammal diversity in the forested areas is similar to that in the ranching area, this suggests that ranching activity may also not necessarily contibute to biodiversity loss or to risk of Chagas' disease.

14233. **Mamoudou, M., 2007.** Trypanosomosis and trypanocidal drug resistance on the Adamaoua Plateau in Cameroon. *Thesis, Freie Universität Berlin, Berlin, Germany: 2007. 100 pp.*

Freie Universität Berlin, Kaiserswerther Str. 16-18, 14195 Berlin Germany [international.office@fu-berlin.de].

Trypanosomosis is among the most devastating diseases in sub-Saharan Africa and according to FAO it is at the root of poverty, while the tsetse fly is considered to be one of the most serious pest problems in the world today. African Animal Trypanosomosis (AAT) has a severe impact on African agriculture. To control AAT multiple strategies are available including keeping naturally resistant cattle and vector control, but the most commonly used strategy is no doubt the use of trypanocidal drugs. However, resistance to trypanocidal drugs is rapidly emerging and has been reported in many countries in Africa. A study was carried out on the Adamaoua Plateau in Cameroon: firstly, to assess the trypanosomosis risk using the combination of entomological, parasitological and serological methods; secondly to determine the tsetse distribution using traps and fly rounds; thirdly, to assess the prevalence of trypanocidal drug resistance in the study area. To assess the trypanosomosis risk a longitudinal survey of trypanosomosis in 9 sentinel herds was carried out in the 3 study zones, i.e. the plateau, the buffer zone and the valley. A seroconversion study was also carried out in cattle during transhumance in the valley. To determine the tsetse distribution an entomological survey was organised over a period of one year along 4 transects traversing the 3 zones of the study area using tsetse traps as well as a longitudinal survey using fly rounds along two transects in the tsetse infested valley. To assess trypanocidal drug resistance a questionnaire survey was carried out on knowledge, attitude and practice of trypanosomosis management in the study area. Furthermore, a field test was done in two cattle herds to assess the presence of drug resistant trypanosomes and a study of the prevalence of drug resistance in trypanosome isolates of the Adamaoua using the standard test in mice. We found that the trypanosomosis risk on the plateau was significantly lower than in the buffer zone and the valley. This may be explained by the regular insecticide treatment of the cattle herds in the buffer zone which is probably contributing to prevent reinvasion of the plateau by tsetse flies. Entomological surveys have demonstrated the presence of *G. m. submorsitans* and *G. tachinoides* in the buffer zone and in the valley whereas no tsetse flies could be captured on the plateau. The distribution of tsetse in the valley undergoes substantial seasonal changes depending on the presence or absence of cattle. In the presence of cattle (dry season) large areas are reinvaded. In the absence of cattle, tsetse distribution is confined to areas where game is present. We have shown for the first time the presence of trypanosomes resistant to isometamidium and diminazene in Cameroon using various techniques (field trial, mouse test). An alarmingly high prevalence of trypanocidal drug resistance was found in the study area. The data collected during this study allowed to formulate recommendations for the sustainable control of African Animal Trypanosomiasis in the Adamaoua region.

14234. **Simukoko, H., Marcotty, T., Phiri, I., Vercruysse, J. & Van den Bossche, P., 2007**. Heterogeneity in the trypanosomosis incidence in Zebu cattle of different ages and sex on the plateau of eastern Zambia. *Acta Tropica,* **103** (2): 98-101.

University of Zambia, School of Veterinary Medicine, Zambia.

On the plateau of eastern Zambia, trypanosomosis is endemic. *Glossina morsitans morsitans* Westwood (Diptera: *Glossinidae*), the only tsetse species present, is almost entirely dependent on livestock as its source of food with cattle being the most preferred host. To determine if tsetse challenge is distributed equally over the various age categories and sexes within a cattle herd, a longitudinal study of trypanosomosis incidence was conducted during the rainy season. A total of 354 head of cattle consisting of 40 percent oxen, 30 percent cows, 15 percent young stock, 13 percent calves and 2 percent bulls were sampled for three consecutive months and their infection statuses determined using the PCR-RFLP technique as diagnostic method. Results indicated that there were significant differences ($P<0.001$) in the proportion of infected animals between the various categories. In oxen, the risk of infection was 5.6 times higher than in calves. Those results suggest heterogeneity in the challenge by tsetse flies and are in line with entomological observations on the feeding preference of tsetse on cattle. The implications of these results for the control of trypanosomosis in Eastern Province and other epidemiologically related areas are discussed.

(b) PATHOLOGY AND IMMUNOLOGY

14235. **Gow, A. G., Simpson, J. W. & Picozzi, K., 2007**. First report of canine African trypanosomosis in the UK. *Journal of Small Animal Practice***. 48** (11) 658-661.

Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Hospital for Small Animals, Easter Bush Veterinary Centre, Roslin, Midlothian EH25 9RG, UK.

A six-year-old neutered male Jack Russell terrier was presented two years after importation into the UK from southern Africa with severe anaemia and abdominal distension. Abdominal ultrasonography revealed the presence of hepato-splenomegaly and ascites. A diagnosis of trypanosomosis was made by blood smear examination. Shortly after admission the dog collapsed and died. PCR analysis revealed a single infection with *Trypanosoma congolense* savannah type. This is the first reported case of canine African trypanosomosis in Europe and suggests that chronic trypanosomosis may allow importation of the disease in apparently asymptomatic animals, even with extended quarantine periods.

14236. **Reglero, M., Vicente, J., Rouco, C., Villafuerte, R. & Gortazar, C., 2007**. *Trypanosoma* spp. infection in wild rabbits (*Oryctolagus cuniculus*) during a restocking program in Southern Spain. *Veterinary Parasitology,* **149** (3-4): 178-184.

Instituto de Investigacion en Recursos Cinegeticos (IREC, CSIC-UCLM-JCCM), Ronda de Toledo S/N, Ciudad Real 13071, Spain.

The effect of parasites on managed rabbit populations may prove crucial to develop sanitary strategies during restocking programmes of such key prey species. We investigated natural infection of European wild rabbits (*Oryctolagus cuniculus*) with *Trypanosoma* spp. in Spain. By fencing part of the warrens during a rabbit restocking program, we induced host variation in rabbit density across these socio-spatial units. We aimed (i) to compare

Trypanosoma spp. infection spread between fenced and open warrens and (ii) to assess the relationship between body condition and infection. *Trypanosoma* spp. parasitaemia peaked in juveniles and decreased onwards. Adult females showed statistically higher infection rates than males. Rabbits from fenced warrens presented statistically higher infection rates than those from open ones, but did not differ in body condition. Parasite abundance negatively correlated with body condition in adults. Sex differences could account for increased susceptibility to infection in females as a cost of reproduction and/or a higher exposition inside the warrens. Future studies should clarify whether aggregation caused enhanced exposure to intermediate hosts (fleas) and subsequent transmission of the parasite, and we stress that the study of non-lethal parasites during restocking programmes provides valuable information on host contact rates and on factors affecting disease susceptibility.

(c) TRYPANOTOLERANCE

14237. **Guilliams, M., Oldenhove, G., Noel, W., Herin, M., Brys, L., Loi, P., Flamand, V., Moser, M., De Baetselier, P. & Beschin, A., 2007.** African trypanosomiasis: naturally occurring regulatory T cells favor trypanotolerance by limiting pathology associated with sustained type 1 inflammation. *Journal of Immunology,* 179 (5): 2748-2757.

Department of Molecular and Cellular Interactions, Vlaams Instituut voor Biotechnologie, Vrije Universiteit Brussel, 1050 Brussels, Belgium.

Tolerance to African trypanosomes requires the production of IFN-gamma in the early stage of infection that triggers the development of classically activated macrophages controlling parasite growth. However, once the first peak of parasitaemia has been controlled, down-regulation of the type 1 immune response has been described. In this study, we have evaluated whether regulatory T cells (Tregs) contribute to the limitation of the immune response occurring during *Trypanosoma congolense* infection and hereby influence the outcome of the disease in trypanotolerant C57BL/6 host. Our data show that Foxp3+ Tregs originating from the naturally occurring Treg pool expanded in the spleen and the liver of infected mice. These cells produced IL-10 and limited the production of IFN-gamma by CD4+ and CD8+ effector T cells. Tregs also down-regulated classical activation of macrophages resulting in reduced TNF-alpha production. The Treg-mediated suppression of the type 1 inflammatory immune response did not hamper parasite clearance, but was beneficial for the host survival by limiting the tissue damages, including liver injury. Collectively, these data suggest a cardinal role for naturally occurring Tregs in the development of a trypanotolerant phenotype during African trypanosomiasis.

14238**. Nganga, J.; Imbuga, M.; & Iraqi, F.A., 2007.** Comparative genome analysis of trypanotolerance QTL. *African Journal of Biotechnology,* 6: 967-970.

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Certain breeds of domestic ruminants show remarkable resistance to the effects of African trypanosomosis. Unlike susceptible animals, trypanotolerant animals control parasitaemia and do not show severe anaemia or production loss. Identification of trypanotolerance genes in cattle is hampered by cost and breeding time. Marked differences between inbred strains of mice in their response to *T. congolense* infection can be exploited in the analysis of the genetic basis of the infection. Murine trypanotolerance QTLs have been identified on chromosome 17, 5 and 1, and designated as *Tir*1, 2 and 3, respectively. *Tir*1 and 2 have been fine mapped to a confidence interval of 1 cM. In order to find the mouse homologous region on the bovine genome, nucleotide sequence across 95 percent CI of *Tir*2 and 3 were used in the selection of candidate genes. Homologous sequences were used in the definition of synteny relationships and subsequent identification of the shared disease response genes. The homologous genes within the human genome were then identified and aligned to the bovine radiation hybrid map in order to identify the mouse/bovine homologous regions. This revealed homology between murine and bovine QTL on *Tir*3 while the region on *Tir*2 is linked to innate immune response.

(d) TREATMENT

[See also 30: nos. 14209, 14210, 14214, 14239]

14239**. Affognon H., Waibel H., & Randolph T., 2006.** Productivity assessment of trypanocide drugs among small scale livestock keepers in Mali and Burkina Faso. *University of Hannover, Faculty of Economics and Management, Development and Agricultural Economics Working Paper No. 5.*

International Livestock Research Institute, PO Box 30709, 00100 Nairobi, Kenya. [t.randolph@cgiar.org].

Assessing the productivity of trypanocide drug use among livestock keepers in Africa can be conducted using the damage abatement approach. This paper presents the estimates of trypanocide use productivity derived from exponential functional specification of damage control function of trypanocides. Results suggest that in spite of drug resistance livestock keepers in Burkina Faso and Mali under-use trypanocides and they could increase the profitability of cattle production if they increase the amount of trypanocide used.

14240. **Gillingwater, K., Buscher, P. & Brun, R., 2007**. Establishment of a panel of reference *Trypanosoma evansi* and *Trypanosoma equiperdum* strains for drug screening. *Veterinary Parasitology,* **148** (2): 114-121.

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The animal pathogenic protozoan, *Trypanosoma evansi*, leads to a wasting disease in equines, cattle and camels, commonly known as Surra. It is extensively distributed geographically with a wide range of mammalian hosts and causes great economical loss. *Trypanosoma equiperdum* causes a venereal disease called Dourine in horses and donkeys.

Chemotherapy appears to be the most effective form of control for *T. evansi*, whereas infections caused by *T. equiperdum* are considered incurable. Due to emerging drug resistance, efficient control of *T. evansi* is severely threatened, emphasising the urgent need to find new alternative drugs. A drug profile for a panel of *T. evansi* and *T. equiperdum* strains has been established for the four standard drugs currently used in treatment. The ^{3}H-hypoxanthine incorporation assay was used to obtain 50 percent inhibitory concentration (IC(50)) values for each standard drug against the various strains. The results indicate the presence (and in some cases, the emergence) of drug resistance in several strains. This panel of characterised strains with known drug sensitivities and resistances will be of great value for the screening of new active compounds, in comparison with the four standard drugs currently available.

14241. **Grace, D., Randolph, T., Diall, O., & Clausen, P.-H., 2007.** Training farmers in rational drug use improves their management of cattle trypanosomosis: a cluster randomised trial in south Mali. *Preventive Veterinary Medicine*. **In press; corrected proof**.

International Livestock Research Institute, PO Box 30709, 00100 Nairobi, Kenya. [d.grace@cgiar.org].

We carried out a stratified, cluster-randomised, controlled trial in south Mali in 2004 to evaluate the impact of providing information on the diagnosis and treatment of bovine trypanosomosis by farmers. We recruited cattle farmers (444) in 46 villages and used stratified, restricted-randomisation to assign villages to either the test or control group. Farmers in the test group received an information leaflet designed to address gaps in farmer knowledge likely to lead to inadequate treatment; their knowledge was assessed before the intervention, and at 2 weeks and 5 months after the intervention. We assessed the quality of farmer treatments by measuring clinical outcomes in cattle 2 weeks after selection and treatment. As an indicator of herd health, we assessed the mean haematocrit of the village herd before, and 5 months after, the intervention. To account for clustering, we analysed data using generalised estimating equations. Improvements in farmer knowledge of trypanosomosis diagnosis and treatment in the group receiving information were 23 percent and 14 percent greater at 2 weeks and 5 months, respectively. In the test group, 84 percent of farmer treatments were successful, compared to 73 percent in the control group. Giving rational drug-use information to farmers improved their knowledge and management of trypanosomosis as well as clinical outcomes in cattle they treated and had no discernible negative impacts.

14242. **Schad, G. J., Allanson, A., Mackay, S. P., Cannavan, A. & Tettey, J. N., 2007**. Development and validation of an improved HPLC method for the control of potentially counterfeit isometamidium products. *Journal of Pharmaceutical and Biomedical Analysis*. **In press; corrected proof.**

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Isometamidium, a mixture of related substances of which 8-(3-m-amidinophenyl-2-triazeno)-3-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (M&B4180A) is the principal active component, is the only chemical agent available for prophylaxis of veterinary trypanosomiasis. A method for the simultaneous quantitation of the major constituents M&B4180A, 3-(3-m-amidinophenyl-2-triazeno)-8-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (M&B38897), 7-(m-amidinophenyldiazo)-3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (M&B4250) and 3,8-di(3-m-amidinophenyltriazeno)-5-ethyl-6-phenylphenanthridinium chloride dihydrochloride (M&B4596) is described. The related substances are resolved on a Gemini C18 column (150mmx4.6mm, 5mμm) using a mobile phase composed of a mixture of acetonitrile and 50mM ammonium formate buffer pH 2.8 (25:75v/v) at a flow rate of 1ml/min with UV detection at 320nm. The method is compatible with electrospray ionisation mass spectrometry and provides a tool for the control of substandard and counterfeit commercial preparations of isometamidium.

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

[See also 30: no. 14174]

14243. **Thekisoe, O. M., Kuboki, N., Nambota, A., Fujisaki, K., Sugimoto, C., Igarashi, I., Yasuda, J. & Inoue, N., 2007**. Species-specific loop-mediated isothermal amplification (LAMP) for diagnosis of trypanosomosis. *Acta Tropica,* **102** (3): 182-189.

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080 8555, Japan.

In this study, we developed loop-mediated isothermal amplification (LAMP) for the specific detection of both animal and human trypanosomosis using primer sets that are designed from 5.8S rRNA-internal transcribed spacer 2 (ITS2) gene for *Trypanosoma brucei gambiense*, 18S rRNA for both *T. congolense* and *T. cruzi*, and VSG RoTat 1.2 for *T. evansi*. These LAMP primer sets are highly sensitive and are capable of detecting down to 1 fg trypanosomal DNA, which is equivalent to approximately 0.01 trypanosomes. LAMP is a rapid and simple technique since it can be carried out in 1 h and requires only a simple heating device for incubation. Therefore, LAMP has great potential of being used for diagnosis of trypanosomosis in the laboratory and the field, especially in countries that lack sufficient resources needed for application of molecular diagnostic techniques.

(b) PATHOLOGY AND IMMUMOLOGY

[See also 30: no. 14237]

14244. **Barkhuizen, M., Magez, S., Atkinson, R. A. & Brombacher, F., 2007**. Interleukin-12p70-dependent interferon- gamma production is crucial for resistance in African trypanosomiasis. *Journal of Infectious Diseases,* **196** (8): 1253-1260.

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African trypanosomiasis encompasses diseases caused by pathogenic trypanosomes, infecting both humans and animals. In the present article, we dissected the possible role of members of the interleukin (IL)-12 family during infection with *Trypanosoma brucei brucei* and *Trypanosoma evansi* in mice. IL-12p35(-/-), IL-12p40(-/-), and IL-12p35(-/-)/p40(-/-) mice were susceptible to both pathogens, as was demonstrated by the increased mortality among these mice, compared with wild-type C57BL/6 mice. The different IL-12p70(-/-) mouse strains showed similar mortality kinetics, suggesting that IL-12p70--but not the IL-12p80 homodimer or IL-23--plays a crucial role in survival. Although there were similar plasma levels of immunoglobulin (Ig) M and IgG2a in IL-12-deficient mice and wild-type mice, interferon (IFN)- gamma production, especially during early infection, was severely impaired in all IL-12p70(-/-) mouse strains, demonstrating an IL-12p70-dependent mechanism for IFN- gamma production. Because IFN- gamma receptor-deficient mice (IFN-gamma R(-/-)) were also highly susceptible to both *Trypanosoma* species, IL-12p70-dependent IFN- gamma production seems to be the important mechanism involved in resistance against both pathogens.

14245. **Budovsky, A., Sneir, R., Bazarsky, E. & El-On, J., 2007**. Alpha 2 macroglobulin activity in rats infected with *Typanosoma lewisi* and treated with cyclophosphamide and its effect on the malignancy of the disease. *Journal of Vector Borne Diseases,* **44** (2): 128-136.

Department of Microbiology and Immunology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel. [budovsky@bgu.ac.il].

Trypanosoma lewisi is a common, flagellated parasite of the rat. Our previous study showed that rabbits injected with serum collected from rats infected with *Trypanosoma lewisi* and treated with cyclophosphamide (CyI) produced high levels of antibodies against a new protein in the CyI rat serum. In the present study, this protein was characterised as alpha2 macroglobulin (alpha2M) and the kinetics of its production and its influence on the malignancy of the disease were determined. In rats infected with *T. lewisi*, alpha2M was first demonstrated and peaked on the second day post-infection (972 µg/ml) and then reduced gradually, reaching a level of 32 µg/ml on the eighth day post-infection. However, in the CyI rats the level of alpha2M was gradually increased as the disease progressed, reaching a level of 890 µg/ml on the eighth day post-infection. Injection of both crude and purified alpha2M

into rats infected with *T. lewisi* led to increased parasitaemia. The present study suggests that increased levels of alpha2M in the CyI rats contribute to the malignancy of the disease.

14246. **Kanaji, S., Tanaka, Y., Sakata, Y., Takeshita, K., Arima, K., Ohta, S., Hansell, E. J., Caffrey, C., Mottram, J. C., Lowther, J., Donnelly, S., Stack, C., Kadowaki, T., Yamamoto, K., McKerrow, J. H., Dalton, J. P., Coombs, G. H. & Izuhara, K., 2007.** Squamous cell carcinoma antigen 1 is an inhibitor of parasite-derived cysteine proteases. *FEBS Letters,* 581 (22): 4260-4264.

Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, 5-1-1 Nabeshima, Saga 849-8501, Japan.

The physiological significance of the squamous cell carcinoma antigens 1 (SCCA1) and SCCA2, members of the ovalbumin serpin family, remains unresolved. In this study, we examined whether SCCA1 or SCCA2 inhibits protozoa- or helminth-derived cysteine proteases. SCCA1, but not SCCA2, potently inhibited the cysteine protease activities of CPB2.8 from *Leishmania mexicana*, cruzain from *Trypanosoma cruzi*, rhodesain from *Trypanosoma brucei rhodesiense*, and cathepsin L2 from *Fasciola hepatica*. The inhibitory activities of SCCA1 were due to its resistance to cleavage by the cysteine proteases. The findings indicate that induction of cysteine protease inhibitors might be a novel defense mechanism against parasite development.

14247. **Magez, S., Radwanska, M., Drennan, M., Fick, L., Baral, T. N., Allie, N., Jacobs, M., Nedospasov, S., Brombacher, F., Ryffel, B. & De Baetselier, P., 2007.** Tumor necrosis factor (TNF) receptor-1 (TNFp55) signal transduction and macrophage-derived soluble TNF are crucial for nitric oxide-mediated *Trypanosoma congolense* parasite killing. *Journal of Infectious Diseases,* 196 (6): 954-962.

Department of Molecular and Cellular Recognition, Flanders Institute for Biotechnology, Brussels, Belgium. [stemagez@vub.ac.be]

Control of *Trypanosoma congolense* infections requires an early cell-mediated immune response. To unravel the role of tumour necrosis factor (TNF) in this process, 6 different *T. congolense* strains were used in 6 different gene-deficient mouse models that included TNF(-/-), TNF receptor-1 (TNFp55)(-/-), and TNF receptor-2 (TNFp75)(-/-) mice, 2 cell type-specific TNF(-/-) mice, as well as TNF-knock-in mice that expressed only membrane-bound TNF. Our results indicate that soluble TNF produced by macrophages/neutrophils and TNFp55 signalling are essential and sufficient to control parasitaemia. The downstream mechanism in the control of *T. congolense* infection depends on inducible nitric oxide synthase activation in the liver. Such a role for nitric oxide is corroborated *ex vivo*, because the inhibitor N(G)-monomethyl-l-arginine blocks the trypanolytic activity of the adherent liver cell population, whereas exogenous interferon- gamma that stimulates nitric oxide production enhances parasite killing. In conclusion, the control of *T. congolense* infection depends on macrophage/neutrophil-derived soluble TNF and intact TNFp55 signalling, which induces trypanolytic nitric oxide.

14248. **Marinho, C. R., Nunez-Apaza, L. N., Martins-Santos, R., Bastos, K. R., Bombeiro, A. L., Bucci, D. Z., Sardinha, L. R., Lima, M. R. & Alvarez, J. M., 2007**. IFN-gamma, but not nitric oxide or specific IgG, is essential for the *in vivo* control of low-virulence Sylvio X10/4 *Trypanosoma cruzi* parasites. *Scandanavian Journal of Immunology,* **66** (2-3): 297-308.

Department of Immunology, Biomedical Sciences Institute, University of Sao Paulo, Sao Paulo, SP, Brazil.

14249. **Masocha, W., Rottenberg, M. E. & Kristensson, K., 2007**. Migration of African trypanosomes across the blood-brain barrier. *Physiology and Behaviour,* **92** (1-2): 110-114.

Karolinska Institutet, Department of Neuroscience, S-171 77 Stockholm, Sweden.

Subspecies of the extracellular parasite, *Trypanosoma brucei*, which are spread by the tsetse fly in sub-Saharan Africa, cause humans Sleeping Sickness. In experimental rodent models the parasite can at a certain stage of disease pass through the blood-brain barrier across or between the endothelial cells and the vessel basement membranes. The laminin composition of the basement membranes determines whether they are permissive to parasite penetration. One cytokine, interferon-gamma, plays an important role in regulating the trypanosome trafficking into the brain. Treatment strategies aim at developing drugs that can impede penetration of trypanosomes into the brain and/or that can eliminate trypanosomes once they are inside the brain parenchyma, but have lower toxicity than the ones presently in use.

14250. **Palmer, G. H. & Brayton, K. A., 2007**. Gene conversion is a convergent strategy for pathogen antigenic variation. *Trends in Parasitology,* **23** (9): 408-413.

Programs in Vector-borne Diseases and Genomics, Washington State University, Pullman, WA 99164-7040, USA. [gpalmer@vetmed.wsu.edu].

Recent studies on three unrelated vector-borne pathogens, *Anaplasma marginale*, *Borrelia hermsii* and *Trypanosoma brucei*, illustrate the central importance of gene conversion as a mechanism for antigenic variation, which results in subsequent evasion of the immune response and persistence in the reservoir host. The combination of genome sequence data and *in vivo* studies tracking variant emergence not only provides insight into the genetic mechanisms for variant generation and hierarchy in variant expression but also highlights gaps in our knowledge regarding variant capacity and usage *in vivo*.

14251. **Stijlemans, B., Baral, T. N., Guilliams, M., Brys, L., Korf, J., Drennan, M., Van Den Abbeele, J., De Baetselier, P. & Magez, S., 2007.** A glycosylphosphatidylinositol-based treatment alleviates trypanosomiasis-associated immunopathology. *Journal of Immunology,* 179 (6): 4003-4014.

Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussels, Pleinlaan 2, Brussels, Belgium. [bstijlem@vub.ac.be].

The GPI-anchored trypanosome variant surface glycoprotein (VSG) triggers macrophages to produce TNF, involved in trypanosomiasis-associated inflammation and the clinical manifestation of sleeping sickness. Aiming at inhibiting immunopathology during experimental *Trypanosoma brucei* infections, a VSG-derived GPI-based treatment approach was developed. To achieve this, mice were exposed to the GPI before an infectious trypanosome challenge. This GPI-based strategy resulted in a significant prolonged survival and a substantial protection against infection-associated weight loss, liver damage, acidosis, and anaemia; the latter was shown to be Ab-independent and correlated with reduced macrophage-mediated RBC clearance. In addition, GPI-based treatment resulted in reduced circulating serum levels of the inflammatory cytokines TNF and IL-6, abrogation of infection-induced LPS hypersensitivity, and an increase in circulating IL-10. At the level of trypanosomiasis-associated macrophage activation, the GPI-based treatment resulted in an impaired secretion of TNF by VSG and LPS pulsed macrophages, a reduced expression of the inflammatory cytokine genes TNF, IL-6, and IL-12, and an increased expression of the anti-inflammatory cytokine gene IL-10. In addition, this change in cytokine pattern upon GPI-based treatment was associated with the expression of alternatively activated macrophage markers. Finally, the GPI-based treatment also reduced the infection-associated pathology in *Trypanosoma congolense* and *Trypanosoma evansi* model systems as well as in tsetse fly challenge experiments, indicating potential field applicability for this intervention strategy.

14252. **Stijlemans, B., Guilliams, M., Raes, G., Beschin, A., Magez, S. & De Baetselier, P., 2007**. African trypanosomosis: from immune escape and immunopathology to immune intervention. *Veterinary Parasitology,* **148** (1): 3-13.

Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium. [bstijlem@vub.ac.be].

African trypanosomes can cause prolonged chronic infections through a mechanism of antigen variation whereby they manipulate the humoral immune system of their hosts. However, besides antigenic variation these extracellular parasites exert other immunoregulatory activities mainly mediated by innate cells in particular macrophage-like (M) cells. In this review, the modulation of M cells through parasite factors and host cytokines as well as their role in parasite control and immunopathology will be examined. The concept of M cell polarization into distinct activation states (M1, M2) that may contribute to trypanosusceptibility or resistance will be discussed. Finally, the possibility to interfere with such activation states hereby providing new therapeutic modalities in the treatment of this infectious disease will be illustrated.

14253. **Vanhollebeke, B., Lecordier, L., Perez-Morga, D., Amiguet-Vercher, A. & Pays, E., 2007**. Human serum lyses *Trypanosoma brucei* by triggering uncontrolled swelling of the parasite lysosome. *Journal of Eukaryotic Microbiology,* **54** (5): 448-451.

Laboratory of Molecular Parasitology, IBMM, Universite Libre de Bruxelles (U.L.B.), 12, rue des Professeurs Jeener et Brachet, B-6041 Gosselies, Belgium.

Trypanosoma brucei brucei infects a wide range of mammals, but is unable to infect humans because this subspecies is lysed by normal human serum (NHS). The phenotype of cellular lysis is debated. For some authors the lysosome undergoes osmotic swelling due to massive influx of chloride ions from the cytoplasmic compartment, but others describe multiple small cytoplasmic vacuoles and general swelling of the cellular body. Using population-level imaging of live immobilized trypanosomes throughout the lysis process, we report that specific swelling of the lysosome is a genuine and major characteristic of NHS-mediated lysis and that this phenotype is independent of the strain of trypanosomes and of NHS aging or damaging. Thus, irrespective of experimental conditions NHS reproducibly induced the swelling of the parasite lysosome.

14254. **Widener, J., Nielsen, M. J., Shiflett, A., Moestrup, S. K. & Hajduk, S., 2007**. Hemoglobin is a co-factor of human trypanosome lytic factor. *PLoS Pathogens,* **3** (9): 1250-1261.

Program in Pathobiology, Brown University, Providence, Rhode Island, USA.

Trypanosome lytic factor (TLF) is a high-density lipoprotein (HDL) subclass providing innate protection to humans against infection by the protozoan parasite *Trypanosoma brucei brucei*. Two primate-specific plasma proteins, haptoglobin-related protein (Hpr) and apolipoprotein L-1 (ApoL-1), have been proposed to kill *T. b. brucei* both singularly or when co-assembled into the same HDL. To better understand the mechanism of *T. b. brucei* killing by TLF, the protein composition of TLF was investigated using a gentle immunoaffinity purification technique that avoids the loss of weakly associated proteins. HDL particles recovered by immunoaffinity absorption, with either anti-Hpr or anti-ApoL-1, were identical in protein composition and specific activity for *T. b. brucei* killing. Here, we show that TLF-bound Hpr strongly binds Hb and that addition of Hb stimulates TLF killing of *T. b. brucei* by increasing the affinity of TLF for its receptor, and by inducing Fenton chemistry within the trypanosome lysosome. These findings suggest that TLF in uninfected humans may be inactive against *T. b. brucei* prior to initiation of infection. We propose that infection of humans by *T. b. brucei* causes haemolysis that triggers the activation of TLF by the formation of Hpr-Hb complexes, leading to enhanced binding, trypanolytic activity, and clearance of parasites.

14255. **Yoshihara, K., Morris, A., Iraqi, F. & Naessens, J., 2007**. Cytokine mRNA profiles in bovine macrophages stimulated with *Trypanosoma congolense*. *Journal of Veterinary Medical Science,* **69** (4): 421-423.

Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan.

It is known that different breeds of cattle display differential susceptibilities to *Trypanosome congolense* infections, and that N'Dama cattle remain more productive after infection than Boran cattle which are more susceptible to *T. congolense*. Macrophages from both breeds were cultured *in vitro* and the expressions of a number of cytokines and iNOS mRNA were analyzed using real time RT-PCR after stimulation with antibody-opsonized trypanosomes. No significant difference was seen between the responses of the two breeds. However, RNA levels of TNF-alpha in the IFN-gamma-primed macrophages were about 100-fold higher than those in the non-primed macrophages. A significant ten-fold decrease was seen for the anti-inflammatory cytokine IL-10. These results indicate that priming of the cells with IFN-gamma cause a serious shift toward an inflammatory response.

(c) CHEMOTHERAPEUTICS

[See also 30: nos. 14233, 14245, 14252]

14256. **Amadasi, A., Bertoldi, M., Contestabile, R., Bettati, S., Cellini, B., di Salvo, M. L., Borri-Voltattorni, C., Bossa, F. & Mozzarelli, A., 2007.** Pyridoxal 5'-phosphate enzymes as targets for therapeutic agents. *Current Medicinal Chemistry,* 14 (12): 1291-1324.

Dipartimento di Biochimica e Biologia Molecolare, Universita di Parma, Italy.

14257. **Atawodi, S. E. & Alafiatayo, A. A., 2007**. Assessment of the phytochemical and antitrypanosomal properties of some extracts of leaves, stem and root bark of *Landolphia* sp., P. Beauv. *Journal of Ethnopharmacology,* **114** (2): 207-211.

Biochemistry Department, Ahmadu Bello University, Zaria, Nigeria.

There is urgent need to source for alternative chemotherapy against trypanosomosis, a disease of major importance in human and economic animals. Therefore, petroleum ether, chloroform, methanol and aqueous extracts sequentially obtained from the leaves, stem and root barks of *Landolphia uniflora* were evaluated for their *in vitro* and *in vivo* antitrypanosomal activities against *Trypanosoma brucei brucei*, as well as their phytochemical constituents. Steroids and triterpenes, resins, tannins, saponins and flavonosides were detected in almost all the extracts, but alkaloid was absent in methanol extracts of the stem, as well as from the chloroform extracts of the root bark. *In vitro*, all extracts of the roots displayed significant antitrypanosomal activity, while only the chloroform extracts of the leaves and stem bark showed activity at both test concentrations (4 and 2mg/ml). However, under *in vivo* condition, the methanol extracts showed the greatest

activity, eliminating parasitaemia within the 10 days treatment period and prolonging survival period at 200 and 300mg/kg body weight intramuscular doses. These results suggest that *Landolphia uniflora* could be useful in the management of trypanosomiasis.

14258. **Bakunova, S. M., Bakunov, S. A., Wenzler, T., Barszcz, T., Werbovetz, K. A., Brun, R., Hall, J. E. & Tidwell, R. R., 2007.** Synthesis and *in vitro* antiprotozoal activity of bisbenzofuran cations. *Journal of Medicinal Chemistry*. **In press; corrected proof.**

Department of Pathology and Laboratory Medicine, School of Medicine, The University of North Carolina, Chapel Hill, North Carolina 27599-7525, USA, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, Ohio 43210, USA and Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, CH-4002 Basel, Switzerland.

14259. **Bapna, A., Federici, L., Venter, H., Velamakanni, S., Luisi, B., Fan, T. P. & van Veen, H. W., 2007**. Two proton translocation pathways in a secondary active multidrug transporter. *Journal of Molecular Microbiology and Biotechnology,* **12** (3-4): 197-209.

Department of Pharmacology, University of Cambridge, Cambridge, UK.

14260. **Bridges, D. J., Gould, M. K., Nerima, B., Maser, P., Burchmore, R. J. S., & Koning, H.P., 2007.** Loss of the high-affinity pentamidine transporter is responsible for high levels of cross-resistance between arsenical and diamidine drugs in African trypanosomes. *Molecular Pharmacology*, **71**: 1098-1108.

Institute of Biomedical and Life Sciences, Division of Infection and Immunity, University of Glasgow, Glasgow G12 8TA, UK. [h.de-koning@bio.gla.ac.uk].

Treatment of many infectious diseases is under threat from drug resistance. Understanding the mechanisms of resistance is as high a priority as the development of new drugs. This study investigated the basis for cross-resistance between the diamidine and melaminophenyl arsenical classes of drugs in African trypanosomes. We induced high levels of pentamidine resistance in a line without the *tb*at1 gene that encodes the P2 transporter previously implicated in drug uptake. We isolated independent clones that displayed very considerable cross-resistance with melarsen oxide, but not phenylarsine oxide and reduced uptake of [^{3}H] pentamidine. In particular, the high-affinity pentamidine transport (HAPT1) activity was absent in the pentamidine-adapted lines, whereas the low affinity pentamidine transport (LAPT1) activity was unchanged. The parental T*b*at1$^{-/-}$ line was sensitive to lysis by melarsen oxide, and this process was inhibited by low concentrations of pentamidine, indicating the involvement of HAPT1. This pentamidine-inhibitable lysis was absent in the adapted line KO-B48. Likewise, uptake of the fluorescent diamidine 4',6-diamidino-2-phenylindole dihydrochloride was much delayed in live KO-B48 cells and insensitive to competition with up to 10 μM pentamidine. No overexpression of the *Trypanosoma brucei brucei* ATP-binding cassette transporter *Tb*MRPA could be detected in KO-B48. A

laboratory line of *Trypanosoma brucei gambiense*, adapted to high levels of resistance for the melaminophenyl arsenical drug melarsamine hydrochloride (Cymelarsan), had similarly lost *Tb*AT1 and HAPT1 activity while retaining LAPT1 activity. It seems therefore that selection for resistance to either pentamidine or arsenical drugs can result in a similar phenotype of reduced drug accumulation, explaining the occurrence of cross-resistance.

14261. **Cammerer, S. B., Jimenez, C., Jones, S., Gros, L., Lorente, S. O., Rodrigues, C., Rodrigues, J. C., Caldera, A., Ruiz Perez, L. M., da Souza, W., Kaiser, M., Brun, R., Urbina, J. A., Gonzalez Pacanowska, D. & Gilbert, I. H., 2007.** Quinuclidine derivatives as potential antiparasitics. *Antimicrobial Agents and Chemotherapy,* **51** (11): 4049-4061.

School of Life Sciences, University of Dundee, MSI/WTB/CIR Complex, Dow Street, Dundee DD1 5EH, UK. [i.h.gilbert@dundee.ac.uk].

14262. **George, T. G., Endeshaw, M. M., Morgan, R. E., Mahasenan, K. V., Delfin, D. A., Mukherjee, M. S., Yakovich, A. J., Fotie, J., Li, C. & Werbovetz, K. A., 2007.** Synthesis, biological evaluation, and molecular modelling of 3,5-substituted-N1-phenyl-N4,N4-di-n-butylsulfanilamides as antikinetoplastid antimicrotubule agents. *Bioorganic and Medicinal Chemistry,* **15** (18): 6071-6079.

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA.

14263. **Heby, O., Persson, L. & Rentala, M., 2007**. Targeting the polyamine biosynthetic enzymes: a promising approach to therapy of African sleeping sickness, Chagas' disease, and leishmaniasis. *Amino Acids,* **33** (2): 359-366.

Department of Molecular Biology, Umea University, Umea, Sweden.

Trypanosomatids depend on spermidine for growth and survival. Consequently, enzymes involved in spermidine synthesis and utilization, i.e. arginase, ornithine decarboxylase (ODC), S-adenosylmethionine decarboxylase (AdoMetDC), spermidine synthase, trypanothione synthetase (TryS), and trypanothione reductase (TryR), are promising targets for drug development. The ODC inhibitor alpha-difluoromethylornithine (DFMO) is about to become a first-line drug against human late-stage *gambiense* sleeping sickness. Another ODC inhibitor, 3-aminooxy-1-aminopropane (APA), is considerably more effective than DFMO against *Leishmania* promastigotes and amastigotes multiplying in macrophages. AdoMetDC inhibitors can cure animals infected with isolates from patients with *rhodesiense* sleeping sickness and leishmaniasis, but have not been tested on humans. The antiparasitic effects of inhibitors of polyamine and trypanothione formation, reviewed here, emphasize the relevance of these enzymes as drug targets. By taking advantage of the differences in enzyme structure between parasite and host, it should be possible to design new drugs that can selectively kill the parasites.

14264. **Hoet, S., Pieters, L., Muccioli, G. G., Habib-Jiwan, J. L., Opperdoes, F. R. & Quetin-Leclercq, J., 2007**. Antitrypanosomal activity of triterpenoids and sterols from the leaves of *Strychnos spinosa* and related compounds. *Journal of Natural Products*, **70** (8): 1360-1363.

Laboratoire de Pharmacognosie, Unité d'Analyse Chimique et Physico-Chimique des Médicaments et Pharmacognosie, Université Catholique de Louvain, UCL 72.30-CHAM, Avenue E. Mounier 72, B-1200 Bruxelles, Belgium.

14265. **Huang, T. L., Bacchi, C. J., Kode, N. R., Zhang, Q., Wang, G., Yartlet, N., Rattendi, D., Londono, I., Mazumder, L., Vanden Eynde, J. J., Mayence, A. & Donkor, I. O., 2007.** Trypanocidal activity of piperazine-linked bisbenzamidines and bisbenzamidoxime, an orally active prodrug. *International Journal of Antimicrobial Agents*. **In press; corrected proof.**

College of Pharmacy, Xavier University of Louisiana, 1 Drexel Drive, New Orleans, LA 70125, USA.

A series of 32 piperazine-linked bisbenzamidines (and related analogues) were analysed for their *in vitro* and *in vivo* trypanocidal activity against a drug-sensitive strain of *Trypanosoma brucei brucei* and a drug-resistant strain of *Trypanosoma brucei rhodesiense*. The compounds showed similar potencies against both strains. The most potent compounds were bisbenzamidines substituted at the amidinium nitrogens with a linear pentyl group (8, inhibitory concentration for 50 percent (IC(50))=1.7-3.0nM) or cyclic octyl group (17, IC(50)=2.3-4.6nM). Replacement of the diamidine groups with diamidoxime groups resulted in a prodrug (22) that was effective orally against *T. b. brucei*-infected mice. Three compounds (7, 11 and 15) provided 100 percent cure when administered parenterally. The results indicate that the nature of the substituents at the amidinium nitrogens of bisbenzamidines strongly influence their trypanocidal activity.

14266. **Khrizman, A., Slack, R. D., Remsing, R. C., Little, S., Yardley, V. & Moyna, G., 2007**. Synthesis and *in vitro* protozoocidal evaluation of novel diazabicyclic tropolone derivatives. *Archiv der Pharmazie (Weinheim)*. **In press; corrected proof.**

Department of Chemistry & Biochemistry, University of the Sciences in Philadelphia, Philadelphia, PA, USA.

14267. **Kuettel, S., Zambon, A., Kaiser, M., Brun, R., Scapozza, L. & Perozzo, R., 2007**. Synthesis and evaluation of antiparasitic activities of new 4-[5-(4-phenoxyphenyl)-2H-pyrazol-3-yl]morpholine derivatives. *Journal of Medicinal Chemistry*. **In press; corrected proof.**

Pharmaceutical Biochemistry Group, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland, Dipartimento di Scienze Ambientali, Universita' di

Venezia "Ca'Foscari", Venezia, Italy, and Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland.

14268. **Laxman, S. & Beavo, J. A., 2007**. Cyclic nucleotide signalling mechanisms in trypanosomes: possible targets for therapeutic agents. *Molecular Interventions,* **7** (4): 203-215.

Department of Pharmacology, University of Washington School of Medicine, Seattle, WA 98105, USA. [sunil.laxman@utsouthwestern.edu].

Trypanosome infections cause several major human diseases, including sleeping sickness and Chagas' disease, which affect millions of people in Africa and South America, respectively. Although adenosine 3',5'-monophosphate (cAMP) signalling and regulation have been widely studied in mammalian systems, and these pathways provide targets for the treatment of numerous pathologies, a molecular understanding of cAMP signalling in trypanosomes remains incomplete. Recent studies in these parasites, however, have revealed diverse families of adenylyl cyclase and phosphodiesterase that regulate cAMP concentrations. Importantly, these enzymes differ pharmacologically and biochemically from their mammalian counterparts. In this review, we discuss recent developments, emerging ideas, and gaps in knowledge in this area of research, highlighting aspects of enzymes in the cAMP signalling pathway that may be good targets for antitrypanosomal drug therapy.

14269. **Marcos, V. De., Navarro, A. S., Gomes Dias, S. M., Mello, L. V., da Silva Giotto, M. T., Gavalda, S., Blonski, C., Garratt, R. C. & Rigden, D. J., 2007.** Structural flexibility in *Trypanosoma brucei* enolase revealed by X-ray crystallography and molecular dynamics. *The FEBS Journal,* **274** (19): 5077-5089.

Instituto de Fisica de Sao Carlos, Universidade de Sao Paulo, Sao Carlos SP, Brazil.

Enolase is a validated drug target in *Trypanosoma brucei*. To better characterize its properties and guide drug design efforts, we have determined six new crystal structures of the enzyme, in various ligation states and conformations, and have carried out complementary molecular dynamics simulations. The results show a striking structural diversity of loops near the catalytic site, for which variation can be interpreted as distinct modes of conformational variability that are explored during the molecular dynamics simulations. Our results show that sulphate may, unexpectedly, induce full closure of catalytic site loops whereas, conversely, binding of inhibitor phosphonoacetohydroxamate may leave open a tunnel from the catalytic site to protein surface offering possibilities for drug development. We also present the first complex of enolase with a novel inhibitor 2-fluoro-2-phosphonoacetohydroxamate. The molecular dynamics results further encourage efforts to design irreversible species-specific inhibitors: they reveal that a parasite enzyme-specific lysine may approach the catalytic site more closely than crystal structures suggest and also cast light on the issue of accessibility of parasite enzyme-specific cysteines to chemically modifying reagents. One of the new sulphate structures contains a novel metal-binding site IV within the catalytic site cleft.

14270. **Nwodo, N. J., Brun, R. & Osadebe, P. O., 2007**. *In vitro* and *in vivo* evaluation of the antitrypanosomal activity of fractions of *Holarrhena africana*. *Journal of Ethnopharmacology,* **113** (3): 556-559.

Department of Pharmaceutical Chemistry, University of Nigeria, Nsukka, Nigeria.

The aqueous extract of young leaves of *Holarrhena africana*, a plant used in the Nigerian traditional medicine, exhibited good activity against *Trypanosoma brucei* spp. The extract was fractionated and eight fractions were obtained. One fraction designated as HaF(5) showed *in vitro* activity against *Trypanosoma brucei rhodesiense* with an IC(50) value of 0.785 µg/mg and no overt cytotoxicity against L-6 cells. Fraction HaF(5) was tested *in vivo* at two doses and found to exhibit *in vivo* efficacy in *Trypanosoma brucei brucei* infected mice leading to a complete disappearance of parasitaemia followed by a relapse.

14271. **Papazahariadou, M., Athanasiadis, G. I., Papadopoulos, E., Symeonidou, I., Hatzistilianou, M., Castellani, M. L., Bhattacharya, K., Shanmugham, L. N., Conti, P. & Frydas, S., 2007.** Involvement of NK cells against tumours and parasites. *International Journal of Biological Markers,* **22** (2): 144-153.

Laboratory of Parasitology, Veterinary Faculty, Aristotele University, Thessaloniki, Greece.

14272. **Rodenko, B., van der Burg, A. M., Wanner, M. J., Kaiser, M., Brun, R., Gould, M., de Koning, H. P. & Koomen, G. J., 2007**. 2,N6-disubstituted adenosine analogs with antitrypanosomal and antimalarial activities. *Antimicrobial Agents and Chemotherapy*, **51** (11): 3796-3802.

Institute of Biomedical and Life Sciences, Division of Infection and Immunity, University of Glasgow, Glasgow G12 8TA, United Kingdom. [H.de-Koning@bio.gla.ac.uk].

A library of 2,N(6)-disubstituted adenosine analogues was synthesized and the analogues were tested for their antiprotozoal activities. It was found that 2-methoxy and 2-histamino and N(6)-m-iodobenzyl substitutions generally produced analogues with low levels of antiprotozoal activity. The best antiplasmodial activity was achieved with large aromatic substitutions, such as N(6)-2,2-diphenylethyl and naphthylmethyl, which could indicate a mechanism of action through aromatic stacking with haeme in the digestive vacuole of *Plasmodium* spp. The activities against *Trypanosoma cruzi* trypomastigotes and *Leishmania donovani* amastigotes were generally low; but several analogues, particularly those with cyclopentylamino substitutions, displayed potent activities against *Trypanosoma brucei rhodesiense* and *T. b. brucei* bloodstream forms *in vitro*. The most active were 2-cyclopentylamino-N(6)-cyclopentyladenosine (compound NA42) and 2-cyclopentylamino-N(6)-cyclopentyladenine (compound NA134), with the nucleobase an order of magnitude more potent than the nucleoside, at 26 +/- 4 nM. It was determined that the mode of action of these purines was trypanostatic, with the compounds becoming trypanocidal only at much higher concentrations. Those 2,N(6)-disubstituted purines tested for their effects on purine

transport in *T. b. brucei* displayed at best a moderate affinity for the transporters. It is highly probable that the large hydrophobic substitutions, which bestow high calculated octanol-water coefficient values on the analogues, allow them to diffuse across the membrane. Consistent with this view, the analogues were as effective against a *T. b. brucei* strain lacking the P2 nucleoside transporter as they were against the parental strain. As the analogues were not toxic to human cell lines, the purine analogues are likely to act on a trypanosome-specific target.

14273. **Rodgers, J., Bradley, B. & Kennedy, P. G., 2007**. Combination chemotherapy with a substance P receptor antagonist (aprepitant) and melarsoprol in a mouse model of human African trypanosomiasis. *Parasitology International,* **56** (4): 321-324.

Division of Infection and Immunity, Faculty of Veterinary Medicine, Institute of Comparative Medicine, University of Glasgow Veterinary School, Glasgow, UK.

Drug therapy for late-stage (encephalitic) human African trypanosomiasis (HAT) is currently very unsatisfactory with the most commonly used drug, melarsoprol, having a 5 percent overall mortality. There is evidence in a mouse model of HAT that Substance P (SP) receptor antagonism reduces the neuroinflammatory reaction to CNS trypanosome infection. In this study we investigated the effects of combination chemotherapy with melarsoprol and a humanised SP receptor antagonist aprepitant (EMEND) in this mouse model. The melarsoprol/aprepitant drug combination did not produce any clinical signs of illness in mice with CNS trypanosome infection. This lack of any additional or unexpected CNS toxicity in the mouse model of CNS HAT provides valuable safety data for the future possible use of this drug combination in patients with late-stage HAT.

14274. **Ruda, G. F., Alibu, V. P., Mitsos, C., Bidet, O., Kaiser, M., Brun, R., Barrett, M. P. & Gilbert, I. H., 2007**. Synthesis and biological evaluation of phosphate prodrugs of 4-phospho-D -erythronohydroxamic acid, an inhibitor of 6-phosphogluconate dehydrogenase. *ChemMedChem,* **2** (8): 1169-1180.

Division of Biological Chemistry and Molecular Microbiology, College of Life Sciences, University of Dundee, Sir James Black Centre, Dundee DD1 5EH, UK,

We have previously reported the discovery of potent and selective inhibitors of 6-phosphogluconate dehydrogenase, the third enzyme of the phosphate pentose pathway, from *Trypanosoma brucei*, the causative organism of human African trypanosomiasis. These inhibitors were charged phosphate derivatives with restricted capacity to enter cells. Herein, we report the synthesis of five different classes of prodrugs: phosphoramidate; bis-S-acyl thioethyl esters (bis-SATE); bis-pivaloxymethyl (bis-POM); cyclosaligenyl; and phenyl, S-acyl thioethyl mixed phosphate esters (mix-SATE). Prodrugs were studied for stability and activity against the intact parasites. Most prodrugs caused inhibition of the growth of the parasites. The activity of the prodrugs against the parasites appeared to be related to their stability in aqueous buffer.

14275. **Sanderson, L., Khan, A. & Thomas, S., 2007**. Distribution of suramin, an antitrypanosomal drug, across the blood-brain and blood-cerebrospinal fluid interfaces in wild-type and P-glycoprotein transporter-deficient mice. *Antimicrobial Agents and Chemotherapy,* **51** (9): 3136-3146.

King's College London, Pharmaceutical Sciences Research Division, Guy's Campus, Hodgkin Building, London Bridge, London SE1 1UL, UK.

Although 60 million people are exposed to human African trypanosomiasis, drug companies have not been interested in developing new drugs due to the lack of financial reward. No new drugs will be available for several years. A clearer understanding of the distribution of existing drugs into the brains of sleeping sickness patients is needed if we are to use the treatments that are available more safely and effectively. This proposal addresses this issue by using established animal models. Using *in situ* brain perfusion and isolated incubated choroid plexus techniques, we investigated the distribution of ^{3}H]suramin into the central nervous systems (CNSs) of male BALB/c, FVB (wild-type), and P-glycoprotein-deficient (Mdr1a/Mdr1b-targeted mutation) mice. There was no difference in the [^{3}H]suramin distributions between the three strains of mice. [^{3}H]suramin had a distribution similar to that of the vascular marker, [^{14}C]sucrose, into the regions of the brain parenchyma that have a blood-brain barrier. However, the association of [^{3}H]suramin with the circumventricular organ samples, including the choroid plexus, was higher than that of [^{14}C]sucrose. The association of [^{3}H]suramin with the choroid plexus was also sensitive to phenylarsine oxide, an inhibitor of endocytosis. The distribution of [^{3}H]suramin to the brain was not affected by the presence of other antitrypanosomal drugs or the P-glycoprotein efflux transporter. Overall, the results confirm that [^{3}H]suramin would be unlikely to treat the second or CNS stage of sleeping sickness.

14276. **Santos, A. L., Soares, R. M., Alviano, C. S. & Kneipp, L. F., 2007**. Heterogeneous production of metallo-type peptidases in parasites belonging to the family *Trypanosomatidae*. *European Journal of Protistology*. **In press; corrected proof.**

Departamento de Microbiologia Geral, Instituto de Microbiologia Prof. Paulo de Goes, Universidade Federal do Rio de Janeiro, Cidade Universitaria, Ilha do Fundao, 21941-902 Rio de Janeiro, RJ, Brazil.

14277. **Schlapper, C., Seebacher, W., Kaiser, M., Brun, R., Saf, R. & Weis, R., 2007**. Epimers of bicyclo[2.2.2]octan-2-ol derivatives with antiprotozoal activity. *European Journal of Medicinal Chemistry*. **In press; corrected proof**.

Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens-University, Universitätsplatz 1, A-8010 Graz, Austria.

14278. **Senn, M., Gunzenhauser, S., Brun, R. & Sequin, U., 2007**. Antiprotozoal polyacetylenes from the Tanzanian medicinal plant *Cussonia zimmermannii*. *Journal of Natural Products,* **70** (10): 1565-1569.

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From the petroleum ether extract of the root bark of *Cussonia zimmermannii* four polyacetylenes, 1- 4, were isolated, three of which (1- 3) were active against *Trypanosoma brucei rhodesiense, Trypanosoma cruzi, Plasmodium falciparum*, and *Leishmania donovani*.

14279. **Silva, J. J., Osakabe, A. L., Pavanelli, W. R., Silva, J. S. & Franco, D. W., 2007**. *In vitro* and *in vivo* antiproliferative and trypanocidal activities of ruthenium NO donors. *British Journal of Pharmacology,* **152** (1): 112-121.

Departamento de Quimica e Fisica Molecular, Instituto de Quimica de Sao Carlos-Universidade de Sao Paulo (USP), Sao Carlos, SP, Brazil.

14280. **Sufrin, J. R., Spiess, A. J., Marasco, C. J., Jr., Rattendi, D. & Bacchi, C. J., 2007**. Identification of novel trypanocidal analogs of 5'-(methylthio)-adenosine. *Antimicrobial Agents and Chemotherapy*. **In press; corrected proof**.

Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, NY, 14263; Program of Molecular Pharmacology and Cancer Therapeutics, Roswell Park, Division, State University of New York at Buffalo, Buffalo, NY 14263, USA; Haskins Laboratory and Department of Biological and Health Sciences, Pace University, New York, NY 10038-1502, USA.

14281. **Tayor, M. C., Kaur, H., Blessington, B., Kelly, J. M. & Wilkinson, S. R., 2007.** Validation of spermidine synthase as a drug target in African trypanosomes. *Biochemical Journal*. **In press; corrected proof.**

School of Biological and Chemical Science, Queen Mary, University of London, London E1 4NS, UK [s.r.wilkinson@qmul.ac.uk].

The trypanocidal activity of the ornithine decarboxylase (ODC) inhibitor difluoromethylornithine (DFMO) has validated polyamine biosynthesis as a target for chemotherapy. As DFMO is one of only two drugs used to treat patients with late-stage African trypanosomasis, the requirement for additional drug targets is paramount. Here we report the biochemical properties of *Trypanosoma brucei* spermidine synthase (*Tb*SpSyn), the enzyme immediately down-stream of ODC in this pathway. Recombinant *Tb*SpSyn was purified and shown to catalyse the formation of spermidine from putrescine and decarboxylated S-adenosylmethionine. To determine the functional importance of *Tb*SpSyn in bloodstream form parasites, we used a tetracycline-inducible RNA interference (RNAi) system. Down-regulation of the corresponding mRNA correlated with a decrease in intracellular spermidine and cessation of growth. This phenotype could be complemented by expressing the spermidine synthase gene from *Leishmania major* in cells undergoing RNAi, but could not be rescued by addition of spermidine to the medium due to the lack of a spermidine uptake capacity. These data therefore genetically validate *Tb*SpSyn as a target for drug development and indicate that in the absence of a functional biosynthetic pathway, bloodstream form *T. brucei* cannot scavenge sufficient spermidine from their environment to meet growth requirements.

14282. **Tempone, A. G., Sartorelli, P., Mady, C. & Fernandes, F., 2007**. Natural products to anti-trypanosomal drugs: an overview of new drug prototypes for American Trypanosomiasis. *Cardiovascular and Hematological Agents in Medicinal Chemistry,* **5** (3): 222-235.

Laboratorio de Toxinologia Aplicada, Departamento de Parasitologia, Inst. Adolfo Lutz - Av. Dr. Arnaldo 355, 8 andar, CEP 01246-000, Sao Paulo, Brazil. [atempone@ial.sp.gov.br].

14283. **Trapp, J., Meier, R., Hongwiset, D., Kassack, M. U., Sippl, W. & Jung, M., 2007**. Structure-activity studies on suramin analogues as inhibitors of NAD(+)-dependent histone deacetylases (sirtuins). *ChemMedChem,* **2** (10): 1419-1431.

Institute of Pharmaceutical Sciences, Albert-Ludwigs-Universität Freiburg, Albertstrasse 25, 79104 Freiburg, Germany.

14284. **Zagana, P., Klepetsanis, P., Ioannou, P. V., Loiseau, P. M. & Antimisiaris, S. G., 2007**. Trypanocidal activity of arsonoliposomes: effect of vesicle lipid composition. *Biomedicine and Pharmacotherapy,* **61** (8): 499-504.

Laboratory of Pharmaceutical Technology, Department of Pharmacy, University of Patras, Rio 26500, Greece.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

[See also 30: nos. 14216, 14230, 14240]

14285. **Hamilton, P. B., Adams, E. R., Malele, I. I., & Gibson, W. C., 2007.** A novel, high-throughput technique for species identification reveals a new species of tsetse-transmitted trypanosome related to the *Trypanosoma brucei* subgenus, *Trypanozoon. Infection, Genetics and Evolution.* **In press; corrected proof**.

School of Biosciences, University of Exeter, Exeter EX4 4PS, UK., School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK.,Tsetse and Trypanosomiasis Research Institute, Tanga, PO Box 1026, Tanzania .

We describe a novel method of species identification, fluorescent fragment length barcoding (FFLB), based on length variation in regions of the 18S and 28Sα ribosomal DNA. Fluorescently tagged primers, designed in conserved regions of the 18S and 28Sα ribosomal DNA, were used to amplify fragments with inter-species size variation, and sizes determined

accurately using an automated DNA sequencer. By using multiple regions and different fluorochromes, a barcode unique to each species was generated. The technique was developed for the identification of African tsetse-transmitted trypanosomes and validated using DNA from laboratory isolates representing known species, subspecies and subgroups. To test the methodology, we examined 91 trypanosome samples from infected tsetse fly midguts from Tanzania, most of which had already been identified by species-specific and generic PCR tests. Identifications were mainly in agreement, but the presence of an unknown trypanosome in several samples was revealed by its unique barcode. Phylogenetic analyses based on 18S rDNA and glycosomal glyceraldehyde phosphate dehydrogenase gene sequences confirmed that this trypanosome is a new species and it is within the *Trypanosoma brucei* clade, as a sister group of subgenus *Trypanozoon*. The overall identification rate of trypanosome-infected midgut samples increased from 78 to 96 percent using FFLB instead of currently available PCR tests. This was due to the high sensitivity of FFLB as well as its capacity to identify previously unrecognized species. FFLB also allowed the identification of multiple species in mixed infections. The method enabled high-throughput and accurate species identification and should be applicable to any group of organisms where there is length variation in regions of rDNA.

14286. **Hamilton, P. B., Gibson, W. C. & Stevens, J. R., 2007**. Patterns of co-evolution between trypanosomes and their hosts deduced from ribosomal RNA and protein-coding gene phylogenies. *Molecular Phylogenetics and Evolution,* **44** (1): 15-25.

School of Biosciences, University of Exeter, Exeter, UK.
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Trypanosomes (genus *Trypanosoma*) are widespread blood parasites of vertebrates, usually transmitted by arthropod or leech vectors. Most trypanosomes have lifecycles that alternate between a vertebrate host, where they exist in the bloodstream, and an invertebrate host, where they develop in the alimentary tract. This raises the question of whether one type of host has had greater influence on the evolution of the genus. Working from the generally accepted view that trypanosomes are monophyletic, here we examine relationships between trypanosomes using phylogenies based on the genes for the small subunit ribosomal RNA (SSU rRNA) and the glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH). New analysis of a combined dataset of both these genes provides strong support for many known clades of trypanosomes. It also resolves the deepest split within the genus between the Aquatic clade, which mainly contains trypanosomes of aquatic and amphibious vertebrates, and a clade of trypanosomes from terrestrial vertebrates. There is also strengthened support for two deep clades, one comprising a wide selection of mammalian trypanosomes and a tsetse fly-transmitted reptilian trypanosome, and the other combining two bird trypanosome subclades. Considering the vertebrate and invertebrate hosts of each clade, it is apparent that co-speciation played little role in trypanosome evolution. However most clades are associated with a type of vertebrate or invertebrate host, or both, indicating that 'host fitting' has been the principal mechanism for evolution of trypanosomes.

14287. **Hatama, S., Shibahara, T., Suzuki, M., Kadota, K., Uchida, I. & Kanno, T., 2007**. Isolation of a *Megatrypanum* trypanosome from sika deer (*Cervus nippon yesoensis*) in Japan. *Veterinary Parasitology,* **149** (1-2): 56-64.

Hokkaido Research Station, National Institute of Animal Health, 4 Hitsujigaoka, Toyohira, Sapporo, Hokkaido, Japan. [hatama@affrc.go.jp].

A trypanosome was isolated from a sika deer (*Cervus nippon yesoensis*) in Hokkaido, Japan, during the primary culture of sika deer renal cells. This is the first report of isolation of a *Megatrypanum* trypanosome from Japanese *Cervidae*. The trypanosome, designated TSD1, was propagated and maintained in Eagle's modified essential medium containing 20 percent foetal bovine serum with sika deer renal cells as feeder. The TSD1 trypanosome was morphometrically similar to *Trypanosoma cervi*, which is commonly isolated from American and European deer. PCR analysis with primers for 18S ribosomal DNA and nucleotide sequencing showed that TSD1 is a member of genus *Trypanosoma*, subgenus *Megatrypanum*. Phylogenetically TSD1 is closely related to *T. theileri*, a common trypanosome of cattle, but is distinguishable from *T. theileri* by some morphometrical and biological features.

14288. **Kibona, S. N., Picozzi, K., Matemba, L. & Lubega, G. W., 2007**. Characterisation of the *Trypanosoma brucei rhodesiense* isolates from Tanzania using serum resistance associated gene as molecular marker. *Tanzananian Health Research Bulletin,* **9** (1): 25-31.

National Institute for Medical Research, P.O. Box 482, Tabora, Tanzania. [kibonastbr@yahoo.com].

Serum resistance associated (SRA) gene has been found to confer resistance to the innate trypanolytic factor (TLF) found in normal human serum; thus allowing *Trypanosoma brucei brucei* to survive exposure to normal human serum. This study was carried out to examine the presence of SRA gene and identify the origin of *T. b. rhodesiense* isolates from three districts in Tanzania, namely Kibondo, Kasulu and Urambo. Twenty-six *T. b. rhodesiense* isolates and two references *T. b. rhodesiense* isolates from Kenya were examined for SRA gene using simple polymerase chain reaction technique. The gene was found to be present in all 26 *T. b. rhodesiense* isolates including the two references isolates from Kenya. The SRA gene was confirmed to be specific to *T. b. rhodesiense* since it could not be amplified from all other *Trypanozoon* including *T. b. gambiense*; and gave an amplified fragment of the expected size (3.9kb), confirming that all these isolates were *T. b. rhodesiense* of the northern variant. Although the geographic distributions of *T. b. gambiense* and *T. b. rhodesiense* are clearly localized to west/central Africa and eastern Africa, respectively, natural movement of people and recent influx of large number of refugees into Tanzania from the Democratic Republic of Congo, could have brought *T. b. gambiense* in western Tanzania. The overlap in distribution of both of these pathogenic sub-species could result in erroneous diagnoses since both trypanosome sub-species are morphologically identical, and currently serologic methods have low specificity. Both the susceptible and resistant *T. b. rhodesiense* isolates possessed the SRA gene suggesting that there is no correlation between drug resistance and presence of SRA gene. The use of SRA gene helps to confirm the identity and diversity of some of the isolates resistant to various drugs.

14289. **Koffi, M., Solano, P., Barnabe, C., de Meeus, T., Bucheton, B., Cuny, G. & Jamonneau, V., 2007**. Genetic characterisation of *Trypanosoma brucei* s.l. using microsatellite typing: New perspectives for the molecular epidemiology of human African trypanosomosis. *Infection Genetics and Evolution*. **In press; corrected proof.**

Institut de Recherche pour le Développement, Unité Mixte de Recherche IRD-CIRAD 177, Programme Santé Animale, TA 207/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France; Institut Pierre Richet, Unitéde Recherche "Trypanosomoses", 04 BP 293 Abidjan 04, Côte d'Ivoire; Centre International de Recherche-Développement sur l'Elevage en zones Subhumides (CIRDES), Unit de recherches sur les bases biologiques de la lutte intégrée, 01 BP 454 Bobo-Dioulasso 01, Burkina Faso.

The pathogenic agent of human African trypanosomiasis (HAT) is a trypanosome belonging to the species *Trypanosoma brucei* s.l. Molecular methods developed for typing *T. brucei* s.l. stocks are for the most part not polymorphic enough to study genetic diversity within *T. brucei gambiense* (*T. b. gambiense*) group 1, the main agent of HAT in West and Central Africa. Furthermore, these methods require high quantities of parasite material and consequently are hampered by a selection bias of the isolation and cultivation techniques. In this study, we evaluated the potential value of microsatellite markers (eight loci) in the genetic characterisation of *T. brucei* s.l. compared to the multi-locus enzyme electrophoresis reference technique. Stocks isolated in Ivory Coast and reference stocks were used for this purpose. Microsatellite markers were shown to be polymorphic enough to evidence the existence of genetic diversity within *T. b. gambiense* group 1 and to show the existence of mixed infections. Furthermore, they were able to amplify trypanosome DNA directly from field samples without the usual culturing stages. While the ability of microsatellite markers to detect mixed infections in such field samples is currently being discussed, they appear to be useful to study the parasite population's geographical structure and may provide new insight into their reproductive mode, a topic that is still under debate. Thus, use of microsatellite markers will contribute to the study of the influence of parasite genetics in the diversity of responses to HAT and may contribute to the improvement of HAT molecular diagnosis.

14290. **Maia Da Silva, F., Junqueira, A. C., Campaner, M., Rodrigues, A. C., Crisante, G., Ramirez, L. E., Caballero, Z. C., Monteiro, F. A., Coura, J. R., Anez, N. & Teixeira, M. M., 2007.** Comparative phylogeography of *Trypanosoma rangeli* and *Rhodnius* (Hemiptera: *Reduviidae*) supports a long coexistence of parasite lineages and their sympatric vectors. *Molecular Ecology,* 16 (16): 3361-3373.

Departamento de Parasitologia, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, Sao Paulo, SP, 05508-900, Brazil.

To make reliable interpretations about evolutionary relationships between *Trypanosoma rangeli* lineages and their insect vectors (triatomine bugs of the genus *Rhodnius*) and, thus, about the determinant factors of lineage segregation within *T. rangeli*, we compared phylogenies of parasite isolates and vector species. Sixty-one *T. rangeli* isolates from invertebrate and vertebrate hosts were initially evaluated in terms of polymorphism of

the spliced-leader gene (SL). Further analysis based on SL and SSUrRNA sequences from 33 selected isolates, representative of the overall phylogenetic diversity and geographical range of *T. rangeli*, supported four phylogenetic lineages within this species. By comparing the phylogeny of *Rhodnius* species with that inferred for *T. rangeli* isolates and through analysis of the geographical range of the isolates, we showed that there is a very significant overlap in the distribution of *Rhodnius* species and *T. rangeli* lineages. Congruence between phylogeographical analysis of both *T. rangeli* lineages and complexes of *Rhodnius* species is consistent with the hypothesis of a long coexistence of parasites and their vectors, with lineage divergence associated with sympatric species of *Rhodnius* apparently without association with particular vertebrate hosts. Separation of *T. rangeli* isolates from vectors of distinct complexes living in sympatry favours the absence of gene flow between the lineages and suggests evolution of *T. rangeli* lineages in independent transmission cycles, probably associated to specific *Rhodnius* spp. ecotopes. A polymerase chain reaction assay based on SL intergenic sequences was developed for simultaneous identification and lineage genotyping of *T. rangeli* in epidemiological surveys.

14291. **Maina, N., Maina, K. J., Maser, P. & Brun, R., 2007**. Genotypic and phenotypic characterization of *Trypanosoma brucei gambiense* isolates from Ibba, South Sudan, an area of high melarsoprol treatment failure rate. *Acta Tropica,* **104** (2-3): 84-90.

Trypanosomiasis Research Institute (TRC), P.O. Box 362, Kikuyu, Kenya; Swiss Tropical Institute (STI), P.O. Box, CH-4002 Basel, Switzerland.

Resistance of trypanosomes to melarsoprol is ascribed to reduced uptake of the drug via the P2 nucleoside transporter. The aim of this study was to look for evidence of drug resistance in *Trypanosoma brucei gambiense* isolates from sleeping sickness patients in Ibba, South Sudan, an area of high melarsoprol failure rate. Eighteen *T. b. gambiense* stocks were phenotypically and only 10 strains genotypically characterized. *In vitro*, all isolates were sensitive to melarsoprol, melarsen oxide, and diminazene. Infected mice were cured with a 4 day treatment of 2.5mg/kg bwt melarsoprol, confirming that the isolates were sensitive. The gene that codes for the P2 transporter, *Tb*ATI, was amplified by PCR and sequenced. The sequences were almost identical to the *Tb*AT1(sensitive) reference, except for one point mutation, C1384T resulting in the amino acid change proline-462 to serine. None of the described *Tb*AT1(resistant)-type mutations were detected. In a *T. b. gambiense* sleeping sickness focus where melarsoprol had to be abandoned due to the high incidence of treatment failures, no evidence for drug resistant trypanosomes or for *Tb*AT1(resistant)-type alleles of the P2 transporter could be found. These findings indicate that factors other than drug resistance contribute to melarsoprol treatment failures.

14292. **Maslov, D. A. & Simpson, L., 2007**. Strategies of kinetoplastid cryptogene discovery and analysis. *Methods in Enzymology,* **424**: 127-139.

Department of Biology, University of California, Riverside, California, USA.

The experimental approach to revealing the genetic information hidden in kinetoplastid cryptogenes and expressed through the posttranscriptional mRNA processing of U-

insertion/deletion editing proceeds in reverse to the informational flow of the RNA editing process itself. While the editing integrates the informational content of maxicircle-encoded cryptogenes with that of minicircle-encoded gRNAs to produce functional edited mRNAs, the cryptogene analysis utilizes a comparison of the mature mRNA sequence with the cryptogene sequence to deduce the locations of edited sites and editing patterns, and a comparison of that mRNA sequence with the minicircle (or minicircle equivalent) sequences to identify the corresponding guide RNAs. Although a "direct" approach (prediction of a fully edited sequence pattern based on the analysis of cryptogene and minicircle sequences) seems to be theoretically possible, it proved to be not practically feasible. The major steps of the procedures utilized to decipher editing in a broad range of kinetoplastid species are presented in this chapter.

14293. **Morrison, L. J., McCormack, G., Sweeney, L., Likeufack, A. C., Truc, P., Turner, C. M., Tait, A. & MacLeod, A., 2007**. Use of multiple displacement amplification to increase the detection and genotyping of *Trypanosoma* species samples immobilized on FTA filters. *American Journal of Tropical Medicine and Hygiene,* **76** (6): 1132-1137.

Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow, UK. [lm78y@udcf.gla.ac.uk].

Whole genome amplification methods are a recently developed tool for amplifying DNA from limited template. We report its application in trypanosome infections, characterized by low parasitaemias. Multiple displacement amplification (MDA) amplifies DNA with a simple *in vitro* step and was evaluated on mouse blood samples on FTA filter cards with known numbers of *Trypanosoma brucei* parasites. The data showed a 20-fold increase in the number of PCRs possible per sample, using primers diagnostic for the multicopy ribosomal ITS region or 177-bp repeats, and a 20-fold increase in sensitivity over nested PCR against a single-copy microsatellite. Using MDA for microsatellite genotyping caused allele dropout at low DNA concentrations, which was overcome by pooling multiple MDA reactions. The validity of using MDA was established with samples from Human African Trypanosomiasis patients. The use of MDA allows maximal use of finite DNA samples and may prove a valuable tool in studies where multiple reactions are necessary, such as population genetic analyses.

14294. **Picozzi, K., Carrington, M. & Welburn, S. C., 2007**. A multiplex PCR that discriminates between *Trypanosoma brucei brucei* and zoonotic *T. b. rhodesiense*. *Experimental Parasitology***. In press; corrected proof.**

Centre for Infectious Diseases, College of Medicine and Veterinary Medicine, Royal (Dick) School of Veterinary Science, The University of Edinburgh, Edinburgh EH25 9RG, UK.

Two subspecies of *Trypanosoma brucei* s.l. co-exist within the animal populations of Eastern Africa; *T. b. brucei* a parasite which only infects livestock and wildlife and *T. b. rhodesiense* a zoonotic parasite which infects domestic livestock, wildlife, and which in humans, results in the disease known as Human African Trypanosomiasis (HAT) or sleeping

sickness. In order to assess the risk posed to humans from HAT it is necessary to identify animals harbouring potentially human infective parasites. The multiplex PCR method described here permits differentiation of human and non-human infective parasites *T. b. rhodesiense* and *T. b. brucei* based on the presence or absence of the SRA gene (specific for East African *T. b. rhodesiense*), inclusion of GPI-PLC as an internal control indicates whether sufficient genomic material is present for detection of a single copy *T. brucei* gene in the PCR reaction.

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14295. **Allen, C. L., Liao, D., Chung, W. L. & Field, M. C., 2007**. Dileucine signal-dependent and AP-1-independent targeting of a lysosomal glycoprotein in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology,* **156** (2): 175-190.

The Molteno Building, Department of Pathology, Tennis Court Road, University of Cambridge, Cambridge CB2 1QP, UK.

14296. **Aphasizhev, R. & Aphasizheva, I., 2007**. RNA editing uridylyltransferases of trypanosomatids. *Methods in Enzymology,* **424**: 55-73.

Department of Microbiology and Molecular Genetics, University of California, Irvine, California, USA.

Terminal RNA uridylyltransferases (TUTases) catalyze the transfer of UMP residues to the 3' hydroxyl group of RNA. These enzymes belong to the DNA polymerase beta superfamily, which also includes poly(A) polymerases, CCA-adding enzymes, and other nucleotidyltransferases. Studies of uridylyl insertion/deletion RNA editing in mitochondria of trypanosomatids provided the first examples of biological functions for TUTases: posttranscriptional uridylylation of guide RNAs by RNA editing TUTase 1 (RET1) and U-insertion mRNA editing by RNA editing TUTase 2 (RET2). The editing TUTases are unified by the presence of conserved catalytic and nucleotide base recognition domains, yet differ substantially in auxiliary function-specific domains, quaternary structure, RNA substrate specificity, and processivity. This chapter describes isolation of TUTases and their complexes from trypanosomatids, methods used for analysis of interactions involving RET1 and RET2, purification of recombinant proteins, and enzyme kinetic assays.

14297. **Baines, A. & Gull, K., 2007**. WCB is a C2 domain protein defining the plasma membrane - sub-pellicular microtubule corset of kinetoplastid parasites. *Protist*. **In press; corrected proof.**

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK.

14298. **Barlow, J. N. & Steyaert, J., 2007**. Examination of the mechanism and energetic contribution of leaving group activation in the purine-specific nucleoside hydrolase from *Trypanosoma vivax*. *Biochimica et Biophysica Acta*. **In press; corrected proof.**

Department of Molecular and Cellular Interactions, VIB, Free University of Brussels, Pleinlaan 2, 1050 Brussel, Belgium.

14399. **Benz, C. & Clayton, C. E., 2007**. The F-box protein CFB2 is required for cytokinesis of bloodstream-form *Trypanosoma brucei*. *Molecular Biochemistry and Parasitology,* **156** (2): 217-224.

Zentrum fur Molekulare Biologie der Universität Heidelberg (ZMBH), Im Neuenheimer Feld 282, 69120 Heidelberg, Germany.

14300. **Bouzaidi-Tiali, N., Aeby, E., Charriere, F., Pusnik, M. & Schneider, A., 2007**. Elongation factor 1a mediates the specificity of mitochondrial tRNA import in *T. brucei. Embo Journal,* **26** (20): 4302-4312.

Department of Biology/Cell and Developmental Biology, University of Fribourg, Chemin du Musée 10, Fribourg, Switzerland.

14301. **Burreson, E. M. & Karlsbakk, E., 2007**. Multiplication of *Trypanosoma pacifica* (*Euglenozoa*: Kinetoplastea) in English sole, *Parophrys vetulus*, from Oregon coastal waters. *Journal of Parasitology,* **93** (4): 932-933.

Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062, USA. [gene@vims.edu].

14302. **Burton, P., McBride, D. J., Wilkes, J. M., Barry, J. D. & McCulloch, R., 2007**. Ku heterodimer-independent end joining in *Trypanosoma brucei* cell extracts relies upon sequence microhomology. *Eukaryotic Cell,* **6** (10): 1773-1781.

The Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow Biomedical Research Centre, 120 University Place, Glasgow, G12 8TA, UK. [rmc9z@udcf.gla.ac.uk].

14303. **Callejas, S. & Melville, S., 2007**. Comparative genomics and drug discovery in trypanosomatids. *SEB Experimental Biology Series,* **58**: 1-24.

University of Cambridge, UK.

No abstract available.

14304. **Carnes, J. & Stuart, K. D., 2007**. Uridine insertion/deletion editing activities. *Methods in Enzymology,* **424**: 25-54.

Seattle Biomedical Research Institute, Seattle, Washington, USA.

The uridine nucleotide insertion and deletion editing of trypanosomatid mitochondrial mRNAs is catalyzed by a macromolecular complex, the editosome. Many investigations of RNA editing involve some assessment of editosome activity either *in vitro* or *in vivo*. Assays to detect insertion or deletion editing activity on RNAs *in vitro* have been particularly useful, and can include the initial endonucleolytic step (full-round) or bypass it (precleaved). Additional assays to examine individual catalytic steps have also proved useful to dissect particular steps in editing. Detection of RNA editing activity *in vivo* has been significantly advanced by the application of real-time PCR technology, which can simultaneously assay several edited and pre-edited targets. Here we describe these assays to assess editing both *in vitro* (full-round insertion and deletion; precleaved insertion and deletion; individual TUTase, ligase, or helicase activity) and *in vivo* (real-time PCR).

14305. **Carnes, J., Trotter, J. R., Peltan, A., Fleck, M. & Stuart, K., 2007**. RNA editing in *Trypanosoma brucei* requires three different editosomes. *Molecular and Cell Biology*. **In press; corrected proof.**

Seattle Biomedical Research Institute, Seattle, WA 98109, USA; Department of Pathobiology, University of Washington, Seattle, WA 98195, USA; Immunology and Infection Unit, Department of Biology, University of York, Heslington, York, YO10 5YW, UK.

Trypanosoma brucei has three distinct approximately 20S editosomes which catalyze RNA editing by the insertion and deletion of uridylates. Editosomes with the KREN1 or KREN2 RNase III type endonucleases specifically cleave deletion and insertion editing site substrates, respectively. We report here that editosomes with KREPB2, which also has an RNase III motif, specifically cleave cytochrome oxidase II (COII) pre-mRNA insertion editing site substrates *in vitro*. Conditional repression and mutation studies also show that KREPB2 is an editing endonuclease specifically required for COII mRNA editing *in vivo*. Furthermore, KREPB2 expression is essential for the growth and survival of bloodstream forms. Thus, editing in *T. brucei* requires at least three compositionally and functionally distinct approximately 20S editosomes, two of which distinguish between different insertion editing sites. This unexpected finding reveals an additional level of complexity in the RNA editing process and suggests a mechanism for how the selection of sites for editing *in vivo* is controlled.

14306. **Cassola, A., De Gaudenzi, J. G. & Frasch, A. C., 2007**. Recruitment of mRNAs to cytoplasmic ribonucleoprotein granules in trypanosomes. *Molecular Microbiology,* **65** (3): 655-670.

Instituto de Investigaciones Biotecnologicas-Instituto Tecnologico Chascomus, UNSAM-CONICET, 1650 San Martin, Provincia de Buenos Aires, Argentina.

Trypanosomes are outstanding examples of the importance of mRNA metabolism in the regulation of gene expression, as these unicellular eukaryotes mostly control protein synthesis by post-transcriptional mechanisms. Here, we show that mRNA metabolism in these organisms involves recruitment of mRNAs and proteins to microscopically visible ribonucleoprotein granules in the cytoplasm. These structures engage transcripts that are being translated and protect mRNAs from degradation. Analysis of the protein composition of trypanosomal mRNA granules indicated that they contain orthologous proteins to those present in P bodies and stress granules from metazoan organisms. Formation of mRNA granules was observed after carbon-source deprivation of parasites in axenic culture. More important, mRNA granules are formed naturally in trypanosomes present in the intestinal tract of the insect vector. We suggest that trypanosomes make use of mRNA granules for transient transcript protection as a strategy to cope with periods of starvation that they have to face during their complex life cycles.

14307. **Comini, M. A., Krauth-Siegel, R. L. & Flohe, L., 2007**. Depletion of the thioredoxin homologue tryparedoxin impairs antioxidative defence in African trypanosomes. *The Biochemical Journal,* **402** (1): 43-49.

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In trypanosomes, the thioredoxin-type protein TXN (tryparedoxin) is a multi-purpose oxidoreductase that is involved in the detoxification of hydroperoxides, the synthesis of DNA precursors and the replication of the kinetoplastid DNA. African trypanosomes possess two isoforms that are localized in the cytosol and in the mitochondrion of the parasites respectively. Here we report on the biological significance of the cTXN (cytosolic TXN) of *Trypanosoma brucei* for hydroperoxide detoxification. Depending on the growth phase, the concentration of the protein is 3-7-fold higher in the parasite form infecting mammals (50-100 µM) than in the form hosted by the tsetse fly (7-34 µM). Depletion of the mRNA in bloodstream trypanosomes by RNA interference revealed the indispensability of the protein. Proliferation and viability of cultured trypanosomes were impaired when TXN was lowered to 1 mµM for more than 48 h. Although the levels of glutathione, glutathionylspermidine and trypanothione were increased 2-3.5-fold, the sensitivity against exogenously generated H2O2 was significantly enhanced. The results prove the essential role of the cTXN and its pivotal function in the parasite defence against oxidative stress.

14308. **Cristodero, M. & Clayton, C. E., 2007**. Trypanosome MTR4 is involved in rRNA processing. *Nucleic Acids Research*. **In press; corrected proof.**

Zentrum fur Molekulare Biologie der Universität Heidelberg (ZMBH), Im Neuenheimer Feld 282, 69120 Heidelberg, Germany.

14309. **Cruz-Reyes, J., 2007**. RNA-protein interactions in assembled editing complexes in trypanosomes. *Methods in Enzymology,* **424**: 107-125.

Department of Biochemistry & Biophysics, Texas A&M University, College Station, Texas, USA.

Multisubunit RNA editing complexes recognize thousands of pre-mRNA sites in the single mitochondrion of trypanosomes. Specific determinants at each editing site must trigger the complexes to catalyze a complete cycle of either uridylate insertion or deletion. While a wealth of information on the protein composition and catalytic activities of these complexes is currently available, the precise mechanisms that govern substrate recognition and editing site specificity remain unknown. This chapter describes basic assays to visualize direct photocrosslinking interactions between purified editing complexes and targeted deletion and insertion sites in model substrates for full-round editing. It also illustrates how variations of these assays can be applied to examine the specificity of the editing enzyme/substrate association, and to dissect structural or biochemical requirements of both the substrates and enzyme complex.

14310. **Dreesen, O. & Cross, G. A., 2007**. Telomere length in *Trypanosoma brucei*. *Experimental Parasitology*. **In press; corrected proof.**

Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399, USA.

Trypanosoma brucei thwarts the host immune response by replacing its variant surface glycoprotein (VSG). The actively transcribed VSG is located in one of approximately 20 telomeric expression sites (ES). Antigenic variation can occur by transcriptional switching, reciprocal translocations, or duplicative gene conversion events among ES or with the large repertoire of telomeric and non-telomeric VSG. In recently isolated strains, duplicative gene conversion occurs at a high frequency and predominates, but the switching frequency decreases dramatically upon laboratory-adaptation. Uniquely, *T. brucei* telomeres grow-apparently indefinitely-at a steady rate of 6-12 base pairs (bp) per population doubling (PD), but the telomere adjacent to an active ES undergoes frequent truncations. Using two-dimensional gel electrophoresis, we demonstrate that all of the chromosome classes of fast-switching and minimally propagated *T. brucei* have shorter telomeres than extensively propagated Lister 427 clones, suggesting a link between laboratory adaptation, telomere growth, and VSG switching rates.

14311. **Fang, J., Rohloff, P., Miranda, K. & Docampo, R., 2007**. Ablation of a small transmembrane protein of *Trypanosoma brucei* (TbVTC1) involved in the synthesis of polyphosphate alters acidocalcisome biogenesis and function, and leads to a cytokinesis defect. *The Biochemical Journal,* **407** (2): 161-170.

Center for Tropical and Emerging Global Disease and Department of Cellular Biology, University of Georgia, Athens, GA 30602, USA.

14312. **Felu, C., Pasture, J., Pays, E. & Perez-Morga, D., 2007**. Diagnostic potential of a conserved genomic rearrangement in the *Trypanosoma brucei gambiense*-specific TGSGP locus. *American Journal of Tropical Medicine and Hygiene,* **76** (5): 922-929.

Laboratory of Molecular Parasitology, Institut de Biologie et Médecine Moleculaires, Université Libre de Bruxelles, Gosselies, Belgium.

We have previously identified TGSGP as a gene specific to the *Trypanosoma brucei gambiense* subspecies. TGSGP is a truncated VSG-like telomeric gene transcribed by RNA polymerase II. The TGSGP protein localizes to the flagellar pocket, and exhibits features compatible with a role as surface receptor. Here we show that TGSGP is physically linked to a truncation of a gene homologous to yeast AUT1 (APG3), a gene involved in internal vesicular formation. Further analysis indicated that *T. b. gambiense* is heterozygous for AUT1 (AUT1/aut1), with each allele located on independent chromosome II homologues. In 18 *T. b. gambiense* isolates from distinct geographical origins and different hosts, this genomic rearrangement was conserved. The size of the intergenic region between TGSGP and truncated aut1 varied among isolates but was similar in isolates of the same geographical area, and this observation may be used in epidemiology to trace the geographical origin of *T. b. gambiense* isolates.

14313. **Fisher, P., Hedeler, C., Wolstencroft, K., Hulme, H., Noyes, H., Kemp, S., Stevens, R. & Brass, A., 2007**. A systematic strategy for large-scale analysis of genotype phenotype correlations: identification of candidate genes involved in African trypanosomiasis. *Nucleic Acids Research,* **35** (16): 5625-5633.

School of Computer Science, Kilburn Building, University of Manchester, Oxford Road, Manchester, UK. [pfisher@cs.manchester.ac.uk].

It is increasingly common to combine microarray and Quantitative Trait Loci data to aid the search for candidate genes responsible for phenotypic variation. Workflows provide a means of systematically processing these large datasets and also represent a framework for the re-use and the explicit declaration of experimental methods. In this article, we highlight the issues facing the manual analysis of microarray and QTL data for the discovery of candidate genes underlying complex phenotypes. We show how automated approaches provide a systematic means to investigate genotype-phenotype correlations. This methodology was applied to a case of resistance to African trypanosomiasis in the mouse. Pathways represented in the results identified Daxx as one of the candidate genes within the Tir1 QTL region. Subsequent re-sequencing in Daxx identified a deletion of an amino acid, identified in susceptible mouse strains, in the Daxx-p53 protein-binding region. This supports recent experimental evidence that apoptosis could be playing a role in the trypanosomiasis resistance phenotype. Workflows developed in this investigation, including a guide to loading and executing them with example data, are available at http://workflows.mygrid.org.uk/repository/myGrid/PaulFisher/.

14314. **Garcia, L. T., Leite, N. R., Alfonzo, J. D. & Thiemann, O. H., 2007**. Effects of *Trypanosoma brucei* tryptophanyl-tRNA synthetases silencing by RNA interference. *Mem Inst Oswaldo Cruz.* **In press; corrected proof**.

Instituto de Fisica de Sao Carlos, Universidade de Sao Paulo, Sao Carlos, SP, 13560-590, Brazil.

The kinetoplast genetic code deviates from the universal code in that 90 percent of mitochondrial tryptophans are specified by UGA instead of UGG codons. A single nucleus-encoded tRNA Trp(CCA) is used by both nuclear and mitochondria genes, since all

kinetoplast tRNAs are imported into the mitochondria from the cytoplasm. To allow decoding of the mitochondrial UGA codons as tryptophan, the tRNA Trp(CCA) anticodon is changed to UCA by an editing event. Two tryptophanyl tRNA synthetases (TrpRSs) have been identified in *Trypanosoma brucei*: *Tb*TrpRS1 and *Tb*TrpRS2 which localize to the cytoplasm and mitochondria respectively. We used inducible RNA interference (RNAi) to assess the role of *Tb*TrpRSs. Our data validate previous observations of TrpRS as potential drug design targets and investigate the RNAi effect on the mitochondria of the parasite.

14315. **Grandgenett, P. M., Otsu, K., Wilson, H. R., Wilson, M. E. & Donelson, J. E., 2007**. A function for a specific zinc metalloprotease of African trypanosomes. *PLoS Pathogens,* **3** (10): e150

Genetics Program, University of Iowa, Iowa City, Iowa, USA, Department of Biochemistry, University of Iowa, Iowa City, Iowa, USA, Department of Internal Medicine, University of Iowa, Iowa City, Iowa, USA, Department of Microbiology, University of Iowa, Iowa City, Iowa, USA, Veterans Affairs Medical Center, Iowa City, Iowa, USA. [john-donelson@uiowa.edu].

The *Trypanosoma brucei* genome encodes three groups of zinc metalloproteases, each of which contains approximately 30 percent amino acid identity with the major surface protease (MSP, also called GP63) of *Leishmania*. One of these proteases, *Tb*MSP-B, is encoded by four nearly identical, tandem genes transcribed in both bloodstream and procyclic trypanosomes. Earlier work showed that RNA interference against *Tb*MSP-B prevents release of a recombinant variant surface glycoprotein (VSG) from procyclic trypanosomes. Here, we used gene deletions to show that *Tb*MSP-B and a phospholipase C (GPI-PLC) act in concert to remove native VSG during differentiation of bloodstream trypanosomes to procyclic form. When the four tandem *Tb*MSP-B genes were deleted from both chromosomal alleles, bloodstream B (-/-) trypanosomes could still differentiate to procyclic form, but VSG was removed more slowly and in a non-truncated form compared to differentiation of wild-type organisms. Similarly, when both alleles of the single-copy GPI-PLC gene were deleted, bloodstream PLC (-/-) cells could still differentiate. However, when all the genes for both *Tb*MSP-B and GPI-PLC were deleted from the diploid genome, the bloodstream B (-/-) PLC (-/-) trypanosomes did not proliferate in the differentiation medium, and 60 percent of the VSG remained on the cell surface. Inhibitors of cysteine proteases did not affect this result. These findings demonstrate that removal of 60 percent of the VSG during differentiation from bloodstream to procyclic form is due to the synergistic activities of GPI-PLC and *Tb*MSP-B.

14316. **Griffiths, S., Portman, N., Taylor, P. R., Gordon, S., Ginger, M. L. & Gull, K., 2007**. RNA interference mutant induction *in vivo* demonstrates the essential nature of trypanosome flagellar function during mammalian infection. *Eukaryotic Cell,* **6** (7): 1248-1250.

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK.

We demonstrate that trypanosomes compromised in flagellar function are rapidly cleared from infected mice. Analysis of the PFR2 bloodstream RNA interference mutant revealed that defective cell motility occurred prior to cytokinesis failure. This validation provides a paradigm for the flagellum as a target for future assays and interventions against this human pathogen.

14317. **Hammarton, T. C., Kramer, S., Tetley, L., Boshart, M. & Mottram, J. C., 2007**. *Trypanosoma brucei* polo-like kinase is essential for basal body duplication, kDNA segregation and cytokinesis. *Molecular Microbiology,* **65** (5): 1229-1248.

Infection and Immunity, Wellcome Centre for Molecular Parasitology, University of Glasgow, Biomedical Research Centre, 120 University Place, Glasgow G12 8TA, UK. [t.hammarton@bio.gla.ac.uk].

Polo-like kinases (PLKs) are conserved eukaryotic cell cycle regulators, which play multiple roles, particularly during mitosis. The function of *Trypanosoma brucei* PLK was investigated in procyclic and bloodstream-form parasites. In procyclic trypanosomes, RNA interference (RNAi) of PLK, or overexpression of TY1-epitope-tagged PLK (PLKty), but not overexpression of a kinase-dead variant, resulted in the accumulation of cells that had divided their nucleus but not their kinetoplast (2N1K cells). Analysis of basal bodies and flagella in these cells suggested the defect in kinetoplast division arose because of an inhibition of basal body duplication, which occurred when PLK expression levels were altered. Additionally, a defect in kDNA replication was observed in the 2N1K cells. However, the 2N1K cells obtained by each approach were not equivalent. Following PLK depletion, the single kinetoplast was predominantly located between the two divided nuclei, while in cells overexpressing PLKty, the kinetoplast was mainly found at the posterior end of the cell, suggesting a role for PLK kinase activity in basal body and kinetoplast migration. PLK RNAi in bloodstream trypanosomes also delayed kinetoplast division, and was further observed to inhibit furrow ingression during cytokinesis. Notably, no additional roles were detected for trypanosome PLK in mitosis, setting this protein kinase apart from its counterparts in other eukaryotes.

14318. **Hartmann, C., Benz, C., Brems, S., Ellis, L., Luu, V. D., Stewart, M., D'Orso, I., Busold, C., Fellenberg, K., Frasch, A. C., Carrington, M., Hoheisel, J. & Clayton, C. E., 2007.** The small trypanosome RNA-binding proteins *Tb*UBP1 and *Tb*UBP2 influence expression of F-box protein mRNAs in bloodstream trypanosomes. *Eukaryotic Cell*. **In press; corrected proof.**

Zentrum fur Molekulare Biologie der Universität Heidelberg (ZMBH), Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany; Division of Functional Genome Analysis, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 506, 69120 Heidelberg, Germany; Department of Biochemistry, 80 Tennis Court Rd., Cambridge CB2 1GA, UK; Instituto de Investigaciones Biotecnologicas, Universidad Nacional de General San MartIn, INTI-Av Gral Paz 5445, Edificio 24, 1650 San Martin, Buenos Aires, Argentina.

In the African trypanosome *Trypanosoma brucei* nearly all control of gene expression is post-transcriptional; sequences in the 3'-untranslated regions of mRNAs determine steady-state mRNA levels by regulation of RNA turnover. Here we investigate the roles of two related proteins, *Tb*UBP1 and *Tb*UBP2, containing a single RNA recognition motif, in trypanosome gene expression. *Tb*UBP1 and *Tb*UBP2 are in the cytoplasm and nucleus, comprise about 0.1 percent of the total protein, and are not associated with polysomes or RNA degradation enzymes. Over-expression of *Tb*UBP2 up-regulated the levels of several mRNAs potentially involved in cell division, including the CFB1 mRNA which encodes a protein with a cyclin F-box domain. CFB1 regulation was mediated by the 3'-untranslated region and involved stabilisation of the mRNA. Depletion of *Tb*UBP2 and *Tb*UBP1 inhibited growth and down-regulated expression of the cyclin F box protein gene CFB2; trans splicing was unaffected. Results of pull-down assays indicated that all tested mRNAs were bound to *Tb*UBP2 or *Tb*UBP1, with some preference for CFB1. We suggest that *Tb*UBP1 and *Tb*UBP2 may be relatively non-specific RNA binding proteins, and that specific effects of over-expression or depletion could depend on competition between various different proteins for RNA binding.

14319. **Hellman, K. M., Ciganda, M., Brown, S. V., Li, J., Ruyechan, W. & Williams, N., 2007**. Two Trypanosome-specific proteins are essential factors for 5S rRNA abundance and ribosomal assembly in *Trypanosoma brucei*. *Eukaryotic Cell,* **6** (10): 1766-1772.

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We have previously identified and characterized two novel nuclear RNA binding proteins, p34 and p37, which have been shown to bind 5S rRNA in *Trypanosoma brucei.* These two proteins are nearly identical, with one major difference, an 18-amino-acid insert in the N-terminal region of p37, as well as three minor single-amino-acid differences. Homologues to p34 and p37 have been found only in other trypanosomatids, suggesting that these proteins are unique to this ancient family. We have employed RNA interference (RNAi) studies in order to gain further insight into the interaction between p34 and p37 with 5S rRNA in *T. brucei*. In our p34/p37 RNAi cells, decreased expression of the p34 and p37 proteins led to morphological alterations, including loss of cell shape and vacuolation, as well as to growth arrest and ultimately to cell death. Disruption of a higher-molecular-weight complex containing 5S rRNA occurs as well as a dramatic decrease in 5S rRNA levels, suggesting that p34 and p37 serve to stabilize 5S rRNA. In addition, an accumulation of 60S ribosomal subunits was observed, accompanied by a significant decrease in overall protein synthesis within p34/p37 RNAi cells. Thus, the loss of the trypanosomatid-specific proteins p34 and p37 correlates with a diminution in 5S rRNA levels as well as a decrease in ribosome activity and an alteration in ribosome biogenesis.

14320. **Hellman, K., Prohaska, K. & Williams, N., 2007**. *T. brucei* RNA binding proteins, p34 and p37, mediate NOPP44/46 cellular localization via the exportin 1 nuclear export pathway. *Eukaryotic Cell.* **In press; corrected proof.**

Department of Microbiology and Immunology & Witebsky Center for Microbial Pathogenesis and Immunology, 253 Biomedical Research Building, University at Buffalo, Buffalo, NY 14214, USA.

14321. **Hutchinson, O. C., Picozzi, K., Jones, N. G., Mott, H., Sharma, R., Welburn, S. C. & Carrington, M., 2007**. Variant surface glycoprotein gene repertoires in *Trypanosoma brucei* have diverged to become strain-specific. *BMC Genomics,* **8**: 234.

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In a mammalian host, the cell surface of African trypanosomes is protected by a monolayer of a single variant surface glycoprotein (VSG). The VSG is central to antigenic variation; one VSG gene is expressed at any one time and there is a low frequency stochastic switch to expression of a different VSG gene. The genome of *Trypanosoma brucei* contains a repertoire of > 1000 VSG sequences. The degree of conservation of the genomic VSG repertoire in different strains has not been investigated in detail. Eighteen expressed VSGs from Ugandan isolates were compared with homologues (> 40 percent sequence identity) in the two available *T. brucei* genome sequences. Fourteen homologues were present in the genome of *Trypanosoma brucei brucei* TREU927 from Kenya and fourteen in the genome of *T. b. gambiense* Dal972 from Cote d'Ivoire. The Ugandan VSGs averaged 71 percent and 73 percent identity to homologues in *T. b. brucei* and *T. b. gambiense* respectively. The sequence divergence between homologous VSGs from the three different strains was not random but was more prevalent in the parts of the VSG believed to interact with the host immune system on the living trypanosome. It is probable that the VSG repertoires in the different isolates contain many common VSG genes. The location of divergence between VSGs is consistent with selection for strain-specific VSG repertoires, possibly to allow superinfection of an animal by a second strain. A consequence of strain-specific VSG repertoires is that any vaccine based on large numbers of VSGs from a single strain will only provide partial protection against other strains.

14322. **Kieft, R., Brand, V., Ekanayake, D. K., Sweeney, K., DiPaolo, C., Reznikoff, W. S. & Sabatini, R., 2007**. JBP2, a SWI2/SNF2-like protein, regulates *de novo* telomeric DNA glycosylation in bloodstream form *Trypanosoma brucei*. *Molecular Biochemistry and Parasitology,* **156** (1): 24-31.

Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA.

Synthesis of the modified thymine base, beta-d-glucosyl-hydroxymethyluracil or J, within telomeric DNA of *Trypanosoma brucei* correlates with the bloodstream form specific epigenetic silencing of telomeric variant surface glycoprotein genes involved in antigenic variation. In order to analyze the function of base J in the regulation of antigenic variation, we are characterizing the regulatory mechanism of J biosynthesis. We have recently proposed a model in which chromatin remodeling by a SWI2/SNF2-like protein (JBP2) regulates the developmental and *de novo* site-specific localization of J synthesis within bloodstream form trypanosome DNA. Consistent with this model, we now show that JBP2 (-/-) bloodstream

form trypanosomes contain five-fold less base J and are unable to stimulate *de novo* J synthesis in newly generated telomeric arrays.

14323. **Li, Z., Gourguechon, S. & Wang, C. C., 2007**. Tousled-like kinase in a microbial eukaryote regulates spindle assembly and S-phase progression by interacting with Aurora kinase and chromatin assembly factors. *Journal of Cell Science,* **120** (21): 3883-3894.

Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94158-2280, USA.

14324. **Lu, S., Suzuki, T., Iizuka, N., Ohshima, S., Yabu, Y., Suzuki, M., Wen, L. & Ohta, N., 2007**. *Trypanosoma brucei* vacuolar protein sorting 41 (VPS41) is required for intracellular iron utilization and maintenance of normal cellular morphology. *Parasitology,* **134** (11): 1639-1647.

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Procyclic forms of *Trypanosoma brucei brucei* remain and propagate in the midgut of tsetse fly where iron is rich. Additional iron is also required for their growth in *in vitro* culture. However, little is known about the genes involved in iron metabolism and the mechanism of iron utilization in procyclic-form cells. Therefore, we surveyed the genes involved in iron metabolism in the *T. b. brucei* genome sequence database. We found a potential homologue of vacuole protein sorting 41 (VPS41), a gene that is required for high-affinity iron transport in *Saccharomyces cerevisiae* and cloned the full-length gene (*Tb*VPS41). Complementation analysis of *Tb*VPS41 in deltaScvps41 yeast cells showed that *Tb*VPS41 could partially suppress the inability of DeltaScvps41 yeast cells to grow on low-iron medium, but it could not suppress the fragmented vacuole phenotype. Further RNA interference (RNAi)-mediated gene knock-down in procyclic-form cells resulted in a significant reduction of growth in low-iron medium; however, no change in growth was observed in normal culture medium. Transmission electron microscopy showed that RNAi caused *T. b. brucei* cells to have larger numbers of small intracellular vesicles, similar to the fragmented vacuoles observed in deltaScvps41 yeast cells. The present study demonstrates that *Tb*VPS41 plays an important role in the intracellular iron utilization system as well as in the maintenance of normal cellular morphology.

14325. **Luscher, A., Onal, P., Schweingruber, A. M. & Maser, P., 2007**. Adenosine kinase of *Trypanosoma brucei* and its role in susceptibility to adenosine antimetabolites. *Antimicrobial Agents and Chemotherapy,* **51** (11): 3895-3901.

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Trypanosoma brucei cannot synthesize purines *de novo* and relies on purine salvage from its hosts to build nucleic acids. With adenosine being a preferred purine source of bloodstream-form trypanosomes, adenosine kinase (AK; EC 2.7.1.20) is likely to be a key

player in purine salvage. Adenosine kinase is also of high pharmacological interest, since for many adenosine antimetabolites, phosphorylation is a prerequisite for activity. Here, we cloned and functionally characterized adenosine kinase from *T. brucei* (*Tb*AK). *Tb*AK is a tandem gene, expressed in both procyclic- and bloodstream-form trypanosomes, whose product localized to the cytosol of the parasites. The RNA interference-mediated silencing of *Tb*AK suggested that the gene is nonessential under standard growth conditions. Inhibition or downregulation of *Tb*AK rendered the trypanosomes resistant to cordycepin (3'-deoxyadenosine), demonstrating a role for *Tb*AK in the activation of adenosine antimetabolites. The expression of *Tb*AK in *Saccharomyces cerevisiae* complemented a null mutation in the adenosine kinase gene ado1. The concomitant expression of *Tb*AK with the *T. brucei* adenosine transporter gene *Tb*AT1 allowed *S. cerevisiae* ado1 ade2 double mutants to grow on adenosine as the sole purine source and, at the same time, sensitized them to adenosine antimetabolites. The coexpression of *Tb*AK and *Tb*AT1 in *S. cerevisiae* ado1 ade2 double mutants proved to be a convenient tool for testing nucleoside analogues for uptake and activation by *T. brucei* adenosine salvage enzymes.

14326. **Mandava, V., Fernandez, J. P., Deng, H., Janzen, C. J., Hake, S. B. & Cross, G. A., 2007**. Histone modifications in *Trypanosoma brucei*. *Molecular Biochemistry and Parasitology,* **156** (1): 41-50.

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Several biological processes in *Trypanosoma brucei* are affected by chromatin structure, including gene expression, cell cycle regulation, and life-cycle stage differentiation. In *Saccharomyces cerevisiae* and other organisms, chromatin structure is dependent upon posttranslational modifications of histones, which have been mapped in detail. The tails of the four core histones of *T. brucei* are highly diverged from those of mammals and yeasts, so sites of potential modification cannot be reliably inferred, and no cross-species antibodies are available to map the modifications. We therefore undertook an extensive survey to identify posttranslational modifications by Edman degradation and mass spectrometry. Edman analysis showed that the N-terminal alanine of H2A, H2B, and H4 could be monomethylated. We found that the histone H4 N-terminus is heavily modified, while, in contrast to other organisms, the histone H2A and H2B N-termini have relatively few modifications. Histone H3 appears to have a number of modifications at the N-terminus, but we were unable to assign many of these to a specific amino acid. Therefore, we focused our efforts on uncovering modification states of H4. We discuss the potential relevance of these modifications.

14327. **Marcello, L. & Barry, J. D., 2007**. Analysis of the VSG gene silent archive in *Trypanosoma brucei* reveals that mosaic gene expression is prominent in antigenic variation and is favoured by archive substructure. *Genome Research,* **17** (9): 1344-1352.

Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow Biomedical Research Centre, Glasgow G12 8TA, UK.

Trypanosoma brucei evades host acquired immunity through differential activation of its large archive of silent variant surface glycoprotein (VSG) genes, most of which are pseudogenes in subtelomeric arrays. We have analyzed 940 VSGs, representing one half to two thirds of the arrays. Sequence types A and B of the VSG N-terminal domains were confirmed, while type C was found to be a constituent of type A. Two new C-terminal domain types were found. Nearly all combinations of domain types occurred, with some bias to particular combinations. One-third of encoded N-terminal domains, but only 13 percent of C-terminal domains, are intact, indicating a particular need for silent VSGs to gain a functional C-terminal domain to be expressed. About 60 percent of VSGs are unique, the rest occurring in subfamilies of two to four close homologues (>50 percent - 52 percent peptide identity). We found a subset of VSG-related genes, differing from VSGs in genomic environment and expression patterns, and predict they have distinct function. Almost all (92 percent) full-length array VSGs have the partially conserved flanks associated with the duplication mechanism that activates silent genes, and these sequences have also contributed to archive evolution, mediating most of the conversions of segments, containing >/=1 VSG, within and between arrays. During infection, intact array genes became activated by duplication after two weeks, and mosaic VSGs assembled from pseudogenes became expressed by week three and predominated by week four. The small subfamily structure of the archive appears to be fundamental in providing the interacting donors for mosaic formation.

14328. **Moraes, M. C., Jesus, T. C., Hashimoto, N. N., Dey, M., Schwartz, K. J., Alves, V. S., Avila, C. C., Bangs, J. D., Dever, T. E., Schenkman, S. & Castilho, B. A., 2007.** A novel membrane-bound eIF2(alpha) kinase in the flagellar pocket of *Trypanosoma brucei. Eukaryotic Cell*. **In press; corrected proof.**

Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Sao Paulo, Sao Paulo, SP, Brazil, Laboratory of Gene Regulation and Development, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, U.S.A. and Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA.

Translational control mediated by phosphorylation of the alpha subunit of the eukaryotic initiation factor 2 (eIF2alpha) is central to stress-induced programmes of gene expression. Trypanosomatids, important human pathogens, display differentiation processes elicited by contact with the distinct physiological milieu found in their insect vectors and mammalian hosts, likely representing stress situations. *Trypanosoma brucei*, the agent of African trypanosomiasis, encodes three potential eIF2alpha kinases (*Tb*eIF2K1-K3). We show here that *Tb*eIF2K2 is a transmembrane glycoprotein expressed both in procyclic and bloodstream forms. The catalytic domain of *Tb*eIF2K2 phosphorylates yeast and mammalian eIF2alpha at Ser51. It also phosphorylates the highly unusual form of eIF2alpha found in trypanosomatids specifically at residue Thr169, that corresponds to Ser51 in other eukaryotes. *T. brucei* eIF2alpha, however, is not a substrate for GCN2 or PKR *in vitro.* The putative regulatory domain of *Tb*eIF2K2 does not share any sequence similarity with known eIF2alpha kinases. In both procyclic and bloodstream forms *Tb*eIF2K2 is mainly localized in the membrane of the flagellar pocket, an organelle that is the exclusive site of exo- and

endocytosis in these parasites. It can also be detected in endocytic compartments but not in lysosomes, suggesting it is recycled between endosomes and the flagellar pocket. *Tb*eIF2K2 location suggests a relevance in sensing protein or nutrient transport in *T. brucei*, an organism that relies heavily on post transcriptional regulatory mechanisms to control gene expression in different environmental conditions. This is the first membrane-associated eIF2alpha kinase described in unicellular eukaryotes.

14329. **Natesan, S. K., Peacock, L., Matthews, K., Gibson, W. & Field, M. C., 2007**. Activation of endocytosis as an adaptation to the mammalian host by trypanosomes. *Eukaryotic Cell*. **In press; corrected proof.**

The Molteno Building, Department of Pathology, Tennis Court Road, University of Cambridge, Cambridge CB2 1QP, UK; School of Biological Sciences, University of Bristol, Bristol, BS8 1UG, UK; and Institute of Infection and Immunology Research, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK.

Immune evasion in African trypanosomes is principally mediated by antigenic variation, but rapid internalization of surface-bound immune factors may contribute to survival. Endocytosis is upregulated approximately ten-fold in bloodstream compared to procyclic forms, and surface coat remodeling accompanies transition between these life stages. Here we examined expression of endocytosis markers in tsetse stages *in vivo* and monitored modulation during transition from bloodstream to procyclic forms *in vitro*. Among bloodstream stages non-proliferative stumpy forms have endocytic activity similar to rapidly dividing slender forms, while differentiation of stumpy to procyclic forms is accompanied by rapid down-regulation of Rab11 and clathrin, suggesting modulation of endocytic and recycling systems accompanies this differentiation event. Significantly, rapid down-regulation of endocytic markers occurs upon entering the insect midgut and expression of Rab11 and clathrin remain low throughout subsequent development, which suggests that high endocytic activity is not required for remodeling the parasite surface or survival within the fly. However, salivary gland metacyclic forms dramatically increase expression of clathrin and Rab11, indicating that emergence of mammalian infective forms is coupled to re-acquisition of a high activity endocytic/recycling system. These data suggest that high endocytosis in *T. brucei* is an adaptation required for viability in the mammalian host.

14330. **Pelletier, M., Read, L. K. & Aphasizhev, R., 2007**. Isolation of RNA binding proteins involved in insertion/deletion editing. *Methods in Enzymology,* **424**: 75-105.

Department of Microbiology and Immunology, SUNY Buffalo School of Medicine, Buffalo, New York, USA.

RNA editing is a collective term referring to a plethora of reactions that ultimately lead to changes in RNA nucleotide sequences apart from splicing, 5' capping, or 3' end processing. In the mitochondria of trypanosomatids, insertion and deletion of uridines must occur, often on a massive scale, in order to generate functional messenger RNAs. The current state of knowledge perceives the editing machinery as a dynamic system, in which heterogeneous

protein complexes undergo multiple transient RNA-protein interactions in the course of gRNA processing, gRNA-mRNA recognition, and the cascade of nucleolytic and phosphoryl transfer reactions that ultimately change the mRNA sequence. Identification of RNA binding proteins that interact with the mitochondrial RNAs, core editing complex, or contribute to mRNA stability is of critical importance to our understanding of the editing process. This chapter describes purification and characterization of three RNA binding proteins from kinetoplastid mitochondria that have been genetically demonstrated to affect RNA editing.

14331. **Price, H. P., Stark, M., Smith, B. & Smith, D. F., 2007**. TbARF1 influences lysosomal function but not endocytosis in procyclic stage *Trypanosoma brucei*. *Molecular Biochemistry and Parasitology,* **155** (2): 123-127.

Immunology and Infection Unit, Department of Biology, University of York, Heslington, York YO10 5YW, UK.

14332. **Rogers, K., Gao, G. & Simpson, L., 2007**. Uridylate-specific 3' 5'-exoribonucleases involved in uridylate-deletion RNA editing in trypanosomatid mitochondria. *Journal of Biological Chemistry,* **282** (40): 29073-29080.

Department of Microbiology, Immunology, and Molecular Genetics, David Geffen School of Medicine at UCLA, Los Angeles, California 90095, USA.

14333 **Romagnoli, R., Baraldi, P. G., Carrion, M. D., Cara, C. L., Preti, D., Cruz-Lopez, O., Tabrizi, M. A., Moorman, A. R., Gessi, S., Fogli, E., Sacchetto, V. & Borea, P. A., 2007.** From tyrosine to glycine: synthesis and biological activity of potent antagonists of the purinergic P2X7 receptor. *Journal of Medicinal Chemistry,* 50 (15): 3706-3715.

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The characterization of the native and recombinant P2X7 receptor continues to be hindered by the lack of specific and subtype-selective antagonists with a "druglike" profile. However, a tyrosine derivative named KN-62 exhibits selective P2X7 receptor-blocking properties. As a molecular simplification of KN-62, the present study was designed to evaluate the functional antagonistic properties of a novel series of glycine derivatives characterized by the presence of different phenyl-substituted piperazine moieties. Antagonistic activity of these glycine derivatives was tested on HEK293 cells transfected with the human P2X7 receptor. The most potent P2X7 receptor antagonist identified in this study (compound 4g) contains an o-fluorine substituent on the phenylpiperazine moiety and had an IC50 of 12.1 nM. The biological responses investigated were ATP-dependent Ca2+ influx across the plasma membrane and ethidium bromide uptake.

14334. **Santos, C. C., Coombs, G. H., Lima, A. P. & Mottram, J. C., 2007**. Role of the *Trypanosoma brucei* natural cysteine peptidase inhibitor ICP in differentiation and virulence. *Molecular Microbiology*. **In press; corrected proof.**

Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Bloco G, C.C.S., Cidade Universitaria, Rio de Janeiro, RJ, 21949-900, Brazil.

ICP is a chagasin-family natural tight binding inhibitor of Clan CA, family C1 cysteine peptidases (CPs). We investigated the role of ICP in *Trypanosoma brucei* by generating bloodstream form ICP-deficient mutants (Deltaicp). A threefold increase in CP activity was detected in lysates of Deltaicp, which was restored to the levels in wild type parasites by re-expression of the gene in the null mutant. Deltaicp displayed slower growth in culture and increased resistance to a trypanocidal synthetic CP inhibitor. More efficient exchange of the variant surface glycoprotein (VSG) to procyclin during differentiation from bloodstream to procyclic form was observed in Deltaicp, a phenotype that was reversed in the presence of synthetic CP inhibitors. Furthermore, we showed that degradation of anti-VSG IgG is abolished when parasites are pretreated with synthetic CP inhibitors, and that parasites lacking ICP degrade IgG more efficiently than wild type. In addition, Deltaicp reached higher parasitaemia than wild type parasites in infected mice, suggesting that ICP modulates parasite infectivity. Taken together, these data suggest that CPs of *T. brucei* bloodstream form play a role in surface coat exchange during differentiation, in the degradation of internalized IgG and in parasite infectivity, and that their function is regulated by ICP.

14335. **Sharma, R., Peacock, L., Gluenz, E., Gull, K., Gibson, W. & Carrington, M., 2007**. Asymmetric cell division as a route to reduction in cell length and change in cell morphology in Trypanosomes. *Protist*. **In press; corrected proof.**

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African trypanosomes go through at least five developmental stages during their life cycle. The different cellular forms are classified using morphology, including the order of the nucleus, flagellum and kinetoplast along the anterior-posterior axis of the cell, the predominant cell surface molecules and the location within the host. Here, an asymmetrical cell division cycle that is an integral part of the *Trypanosoma brucei* life cycle has been characterised in further detail through the use of cell cycle stage specific markers. The cell cycle leading to the asymmetric division includes an exquisitely synchronised mitosis and exchange in relative location of organelles along the anterior-posterior axis of the cell. These events are coupled to a change in cell surface architecture. During the asymmetric division, the behaviour of the new flagellum is consistent with a role in determining the location of the plane of cell division, a function previously characterised in procyclic cells. Thus, the asymmetric cell division cycle provides a mechanism for a change in cell morphology and also an explanation for how a reduction in cell length can occur in a cell shaped by a stable microtubule array.

14336. **Simo, G., Cuny, G., Demonchy, R. & Herder, S., 2007**. *Trypanosoma brucei gambiense*: Study of population genetic structure of Central African stocks using amplified fragment length polymorphism (AFLP). *Experimental Parasitology*. **In press; corrected proof.**

Medical Research Centre, Institute of Medical Research and Medicinal Plant Studies (IMPM/MINRESI), P.O. Box 6163, Yaounde, Cameroon.

To understand the maintenance and resurgence of historical Human African Trypanosomiasis (HAT) foci, AFLP was used to genotype 100 Central African *Trypanosoma brucei* s.l. stocks. This technique confirmed the high genetic stability of *T. b. gambiense* group 1 stocks and the micro genetic variability within Central African *T. b. gambiense* stocks. It revealed several *T. b. gambiense* genotypes and allowed the identification of minor and major genotypes in HAT foci. The coexistence of these genotypes in the same focus suggests that clustering of stocks according to HAT focus does not provide the true genetic picture of trypanosomes circulating within the disease focus because the minor genotypes are generally underestimated. The presence of minor and major genotypes in HAT foci may explain the persistence and the resurgence of Central African sleeping sickness foci.

14337. **Turnock, D. C., Izquierdo, L. & Ferguson, M. A., 2007**. The *de novo* synthesis of GDP-fucose is essential for flagellar adhesion and cell growth in *Trypanosoma brucei*. *Journal of Biological Chemistry,* **282** (39): 28853-28863.

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The protozoan parasite *Trypanosoma brucei* causes human African sleeping sickness in sub-Saharan Africa. The parasite makes several essential glycoproteins, which has led to the investigation of the sugar nucleotides and glycosyltransferases required to synthesize these structures. Fucose is a common sugar in glycoconjugates from many organisms; however, the sugar nucleotide donor GDP-fucose was only recently detected in *T. brucei*, and the importance of fucose metabolism in this organism is not known. In this paper, we identified the genes encoding functional GDP-fucose biosynthesis enzymes in *T. brucei* and created conditional null mutants of *Tb*GMD, the gene encoding the first enzyme in the pathway from GDP-mannose to GDP-fucose, in both bloodstream form and procyclic form parasites. Under nonpermissive conditions, both life cycle forms of the parasite became depleted in GDP-fucose and suffered growth arrest, demonstrating that fucose metabolism is essential to both life cycle stages. In procyclic form parasites, flagellar detachment from the cell body was also observed under nonpermissive conditions, suggesting that fucose plays a significant role in flagellar adhesion. Fluorescence microscopy of epitope-tagged *Tb*GMD revealed that this enzyme is localized in glycosomes, despite the absence of PTS-1 or PTS-2 target sequences.

14338. **Uzcategui, N. L., Carmona-Gutierrez, D., Denninger, V., Schoenfeld, C., Lang, F., Figarella, K. & Duszenko, M., 2007**. Antiproliferative effect of dihydroxyacetone on *Trypanosoma brucei* bloodstream forms: Cell cycle progression, subcellular alterations, and cell death. *Antimicrobial Agents and Chemotherapy,* **51** (11): 3960-3968.

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We evaluated the effects of dihydroxyacetone (DHA) on *Trypanosoma brucei* bloodstream forms. DHA is considered an energy source for many different cell types. *T. brucei* takes up DHA readily due to the presence of aquaglyceroporins. However, the parasite is unable to use it as a carbon source because of the absence of DHA kinase (DHAK). We could not find a homologue of the relevant gene in the genomic database of *T. brucei* and have been unable to detect DHAK activity in cell lysates of the parasite, and the parasite died quickly if DHA was the sole energy source in the medium. In addition, during trypanosome cultivation, DHA induced growth inhibition with a 50 percent inhibitory concentration of about 1 mM, a concentration that is completely innocuous to mammals. DHA caused cell cycle arrest in the G(2)/M phase of up to 70 percent at a concentration of 2 mM. Also, DHA-treated parasites showed profound ultrastructural alterations, including an increase of vesicular structures within the cytosol and the presence of multivesicular bodies, myelin-like structures, and autophagy-like vacuoles, as well as a marked disorder of the characteristic mitochondrion structure. Based on the toxicity of DHA for trypanosomes compared with mammals, we consider DHA a starting point for a rational design of new trypanocidal drugs.

14339. **van Hellemond, J. J., Hoek, A., Schreur, P. W., Chupin, V., Ozdirekcan, S., Geysen, D., van Grinsven, K. W., Koets, A. P., Van den Bossche, P., Geerts, S. & Tielens, A. G., 2007.** Energy metabolism of bloodstream form *Trypanosoma theileri*. *Eukaryotic Cell,* **6** (9): 1693-1696.

Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80176, 3508 TD Utrecht, The Netherlands.

Bloodstream form *Trypanosoma theileri* degrades glucose to acetate (47 percent) and succinate (45 percent) and, therefore, does not solely rely on glycolysis for ATP production. This trypanosomatid does not use amino acids for energy metabolism. These results refute the prevailing hypothesis that substrate availability determines the type of energy metabolism of trypanosomatids.

14340. **Vaughan, S., Kohl, L., Ngai, I., Wheeler, R. J. & Gull, K., 2007**. A repetitive protein essential for the flagellum attachment zone filament structure and function in *Trypanosoma brucei*. *Protist*. **In press; corrected proof**.

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The flagellum is attached along the length of the cell body in the protozoan parasite *Trypanosoma brucei* and is a defining morphological feature of this parasite. The flagellum attachment zone (FAZ) is a complex structure and has been characterised morphologically as comprising a FAZ filament structure and the specialised microtubule quartet (MtQ) plus the specialised areas of flagellum: plasma membrane attachment. Unfortunately, we have no information as to the molecular identity of the FAZ filament components. Here, by screening an expression library with the monoclonal antibody L3B2 which identifies the FAZ filament we identify a novel repeat containing protein FAZ1. It is kinetoplastid-specific and provides the first molecular component of the FAZ filament. Knockdown of FAZ1 by RNA interference (RNAi) results in the assembly of a compromised FAZ and defects in flagellum

attachment and cytokinesis in procyclic trypanosomes. The complexity of FAZ structure and assembly is revealed by the use of other monoclonal antibody markers illustrating that FAZ1 is only one protein of a complex structure. The cytokinesis defects provide further evidence for the role of an attached flagellum in cellular morphogenesis in these trypanosomes.

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