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TSETSE AND TRYPANOSOMIASIS INFORMATION

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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.

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
AW-IPM	area-wide insect pest management
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
IPVM	integrated pest and vector 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
MoAb	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
PCMU	project coordination and management unit
PCR	polymerase chain reaction
PCV	packed cell volume
p.i.	post-infection
ppb	parts per billion (10^9)
PPLPI	pro-poor livestock policy initiative
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
TPU	tsetse production unit
TTI	tsetse and trypanosomiasis information bulletin
UV	ultra-violet
VAT	variable antigen type
VSG	variant surface glycoprotein
WBC	white blood cell
WMS	web mapping service

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
BMZ	German Federal Ministry for Economic Cooperation and Development
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 control 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 Forum on Agricultural Research
GTZ	Deutsche Gesellschaft für Technische Zusammenarbeit
IAEA	International Atomic Energy Agency
IBAR	Interafrican Bureau for Animal Resources
ICCT	Institute for the Control of Trypanosomiasis
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
IGAD	Inter-Governmental Authority on Development
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-TRC	Kenya Agricultural Research Institute - Trypanosomiasis Research Centre
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
PROCORDEL	Programme de Recherche et Développement
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 Trypanosomiasis Control Council
UTRO	Uganda Trypanosomiasis Research Organisation
WHO	World Health Organization

CONTENTS

SECTION A – NEWS

PROGRAMME AGAINST AFRICAN TRYPANOSOMIASIS: REPORT OF THE 12TH MEETING OF THE PROGRAMME COMMITTEE

Foreword

The twelfth meeting of the Programme against African Trypanosomiasis (PAAT) Programme Committee (PC) was convened at Prince Leopold Institute of Tropical Medicine (ITM), Antwerp, Belgium, 8-9 May 2008. The meeting focused on (i) achievements of PAAT mandated organizations (i.e. Food and Agriculture Organization of the United Nations (FAO), African Union / Inter-African Bureau for Animal Resources of the Organization for African Unity (AU-IBAR), International Atomic Energy Agency of the United Nations (IAEA), World Health Organization of the United Nations (WHO) and AU - Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), (ii) implementation of the African Development Bank (AfDB)-PATTEC supported tsetse and trypanosomiasis (T&T) interventions in six sub-Saharan countries (Burkina Faso, Ghana, Mali in West Africa and Ethiopia, Kenya, Uganda in East Africa).

Mr Raffaele Mattioli, convenor of the meeting, introduced Mr Stanny Geerts who, on behalf of the director of ITM, Mr Bruno Gryseels, welcomed the participants to Antwerp and opened the meeting. Mr A.A. Ilemobade, PAAT Chairperson, joined Mr Geerts to welcome the participants. Mr Ilemobade mentioned the main issues of the meeting, including the progress of the ongoing AfDB-supported projects against T&T, the PAAT Information System (PAAT-IS) and the issue of networking, the new developments and challenges ahead for the International Trypanotolerance Centre (ITC) in The Gambia and the role of PAAT in the context of food security.

On this last subject, the PAAT Chairperson stressed the concern that surrounds the issue of food security throughout the world today. Various causes including climate change have brought rising food costs and food shortages in many countries, especially poor African countries, thus aggravating the poverty situation. The presence of T&T has been a long-standing cause of food insecurity, which African Heads of State and Governments acknowledged in their resolution of 2001. With climate change, the situation is becoming increasingly grave. It is the goal of PAAT and of all PAAT stakeholders to ensure that this is minimized by sensible and concerted action. Mr Ilemobade finally emphasized how efforts are being made to maximize the impact of PAAT activities on the Millennium Development Goals (MDGs).

Apologies were received from Ms. Pamela Olet from Kenya and Mr Charles Mahama from Ghana who could not attend the meeting.

The meeting was chaired by Mr A.A. Ilemobade. FAO provided secretarial assistance. Representatives of the private sector were present in order to facilitate solution of issues related to field operations.

Minutes of the previous meeting

The report and recommendations of the 11th PAAT-PC meeting were taken as read and, after further deliberation, adopted.

Outcomes of the 12th PAAT-PC Meeting

Representatives of FAO, IAEA, WHO and AU-IBAR reported on progress, priorities and planned activities.

FAO/PAAT – R.C. Mattioli

FAO/PAAT activities and progress in the implementation of recommendations since the 11th PAAT-PC meeting were presented.

As regards coordination of the AfDB-funded projects, FAO/PAAT participated in the "Regional meeting of National Coordinators", convened by IAEA, July 2007. The meeting acknowledged the role of PAAT and its Information System (PAAT-IS) in creating, harmonizing and sharing technical and scientific knowledge within the community of people concerned with T&T. More details on the latest developments of the PAAT-IS are given below.

FAO/PAAT announced the signing of a Memorandum of Understanding (MoU) between FAO and the International Federation for Animal Health (IFAH) on Quality Control/Quality Assurance (QC/QA) of trypanocides. FAO committed itself to enlarge the MoU to anthelmintics, antibiotics, insecticides and acaricides. FAO approached the World Organization for Animal Health (OIE) and the Union Economique et Monétaire Ouest Africaine (UEMOA) to stimulate interest in this FAO-IFAH initiative and partnership.

In the field of capacity building, training has been provided to staff of the Southern Rift Valley Tsetse Eradication Project in Ethiopia (STEP) within the framework of the Ethiopian Government IAEA/FAO joint project GCP/ETH/072/UNJ (funded by the United Nations Trust Fund for Human Security (UNTFHS)/Japanese Government). Furthermore, in March 2008 experts met at the Joint FAO/IAEA Division to elaborate a detailed programme for a Geographic Information Systems (GIS) training course for tsetse control personnel. The foreseen period to hold the course is the first quarter of 2009. Lastly, the FAO/IAEA Joint Division continues to provide regular training in tsetse mass rearing and matters related to the Sterile Insect Technique (SIT).

Progress report from AU-IBAR – Ahmed el Sawalhy

Mr Ahmed el Sawalhy reminded participants of the mandate of the AU-IBAR, whose activities focus on the component of animal resources with a view towards freeing Africa from hunger and poverty by 2015. The International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) is IBAR's statutory organ that focuses on African trypanosomiasis. IBAR's representative reported on the outcomes of the 32nd Executive Committee and the 29th Conference of ISCTRC that were held in Angola, Luanda, on 30 September and 1-5 October respectively. 105 papers were accepted and included in the

programme of the conference, which was attended by over 200 participants coming from 27 out of 37 T&T affected countries, as well as from non-African countries. Fifteen international institutions were also represented.

The Executive Committee appointed six new regional country members and included one representative of the AfDB for the first time. The main issues discussed were the institutionalization and legal status of ISCTRC, implementation of a consultancy report on the strengthening of the Council and the possibility of raising funds from membership and sponsorship of the private sector.

The Committee also resolved to address the difficulties faced by ITC in the Gambia by strengthening it as a regional institution. Proposed future activities of the ISCTRC Secretariat include (i) announcement of the date of the 33rd Executive Committee meeting to be held immediately after the PAAT- PAG meeting, (ii) promotion of events for the 60th anniversary of ISCTRC to be held in Addis Ababa in 2009, (iii) re-introduction of training of middle level manpower for T&T control, (iv) initiation of a workshop for research institutions and field workers to review current control tools and identify gaps in knowledge, and (v) development of a medium and long term strategic plan for ISCTRC following the review by the PAAT Chairperson.

Progress report from IAEA – U. Feldmann

The Agency contributes to international efforts against T&T with three major mechanisms: (i) assistance to "normative" activities, (ii) research and methods development; and (iii) technical cooperation. Guidelines aimed at standardizing and harmonizing methodologies and procedures were presented. (i) standard operating procedures for mass-rearing of tsetse flies, (ii) FAO/IAEA guidelines for the collection of entomological baseline data for tsetse area-wide integrated pest management (AW-IPM), (iii) guidelines to assessing the feasibility of creating T&T-free zones, and (iv) guidelines for declaring areas free of tsetse flies and tsetse-transmitted animal trypanosomiasis.

In-house research is carried out at the FAO/IAEA Laboratory in Seibersdorf, focusing on (i) automated sexing of late-stage tsetse pupae (near infra-red scanning), (ii) alternatives to use of gamma rays for blood diet decontamination (UV irradiation), (iii) alternatives to the use of gamma irradiation for reproductive sterilization of male tsetse for use in SIT operations (a prototype X-ray machine is under testing to develop standards), and (iv) semi-automated holding and feeding of tsetse during mass production. Research is also carried out through Coordinated Research Projects (CRPs). Three CRPs relevant to the T&T problem are currently in progress: (i) improved and harmonized quality control for expanded tsetse production, sterilization and field application, (ii) improving SIT for tsetse flies though research on their symbionts and pathogens, and (iii) applying GIS and population genetics for managing livestock insect pests.

At present, the Joint FAO/IAEA Division is active in one regional Technical Cooperation Project (TCP) and 7 national TCPs, namely in Ethiopia, Botswana, Burkina Faso, Kenya, Mali, Senegal, South Africa, Uganda, United Republic of Tanzania. Within

these TCPs, FAO/IAEA focuses on the SIT package and strictly adheres to a phased, conditional approach.

Progress report from WHO – P. Simarro

WHO reported on human African trypanosomiasis (HAT) surveillance and control programmes. WHO provides support to affected countries in relation to diagnosis and treatment, logistic support and capacity building. In 2007, 260 staff were trained on diagnosis and treatment; over 2 million Card Agglutination Test for Trypanosomiasis (CATT) reagents and accessories were distributed in collaboration with ITM, as well as 2 000 m-AECT tests (mini-anion-exchange centrifugation technique) for diagnosis. Approximately 100 000 vials of drugs for treatment were distributed from warehouses to patients. Fourteen countries received support for outreach activities. WHO stressed that the number of new cases of HAT reported has continued to decrease also in the last years, reaching the lower value of the last ten years.

In collaboration with other partners, WHO has set up a project to clarify the status of trypanosomiasis in Swaziland. HAT is listed in WHO records to be endemic in Swaziland but no cases have been reported for decades. Preliminary results show that the entomological data collection has detected *Glossina austeni* in the Northern East part of the country near the Mozambique border.

In collaboration with the AfDB-funded project in Ghana, WHO carried out an HAT survey in the Upper West Region. Within this project 32 health staff were trained, including clinicians and laboratory technicians. Technical assistance was provided by two HAT experts, and logistic support has been given through the provision of diagnostic reagents, equipment, vehicles, fuel, etc. Forty villages were studied and over 10 000 people tested. No cases were detected.

In the framework of PAAT, WHO and FAO are also active in the mapping of HAT. Field data collated by WHO from HAT national control programmes, Non-Governmental Organizations (NGOs) and historical files are being harmonized and entered in a geographical database with a view to updating disease distribution maps, and estimating populations at risk and burden of the disease. More information on this activity is given below.

Developments of the PAAT Information System – G. Cecchi

Activities, studies and publications of the PAAT-IS were presented by Mr Cecchi. The new PAAT-IS structure and functionalities were developed with the support of the International Fund for Agricultural Development (IFAD) and they were presented to the international community concerned with T&T at the 29th Meeting of ISCTRC that was held in Angola. The communication given at the meeting resulted in a paper entitled “Creating, harmonizing and sharing the information: the role of the PAAT and its IS”, that will be published in the meeting’s proceedings.

A study on the relationship between vegetation and tsetse fly at different spatial scales is to be published shortly in the PAAT Technical and Scientific (T&S) Series with the title "Standardizing land cover mapping for tsetse and trypanosomiasis decision making". The main outcomes of this study are also described in the paper "Land cover and tsetse fly distributions in sub-Saharan Africa" that has been accepted for publication by Medical and Veterinary Entomology. Standardization and sharing of geographical data and metadata that is carried out within PAAT-IS are described in "The role of FAO GeoNetwork in a multinational development programme: the case of the PAAT"', that has been published by the journal of the Open Source Geospatial foundation (OSGeo).

A new issue of the PAAT T&S Series will be devoted to geospatial analysis. The paper, tentatively entitled "GIS datasets and methods for an environmental approach to African trypanosomiasis" will include a review of state-of-the-art geospatial datasets that are available in the public domain, as well as a few case studies. This publication aims at promoting the use of GIS datasets and techniques for improved decision making. PAAT-IS is also contributing to the joint Livestock Policy Initiative (LPI)/PAAT study "Mapping the benefits of tsetse and trypanosomiasis removal in the IGAD region". In particular, PAAT-IS is assembling a map of livestock oriented production systems (LPS) in the IGAD region by means of the livelihood data generated in the framework of the Household Economy Approach. More information on this activity is given below.

Mr. Cecchi and Mr. Paone jointly presented rationale, methodology and preliminary results of the WHO/FAO collaboration to map the distribution of human African trypanosomiasis in sub-Saharan Africa. Over the last ten years WHO has collated a large amount of spatially-explicit epidemiological data, whose accuracy enables envisaging the production of a harmonized, unified database (DB) of human trypanosomiasis in sub-Saharan Africa. This DB will also form the basis for the Atlas of HAT. The methodology for geo-referencing HAT data takes advantage of public domain databases of named locations, which are combined with epidemiological reports to pin down the exact position of survey villages. If available in the reports, 5 coordinates acquired with GPS (Global Positioning System) devices are checked and imported in the database.

So far, approximately 23 000 HAT cases have been analyzed and entered in the database. Cases refer to 4 200 different geographical entities, out of which approximately 3 000 have been geo-positioned at village level. Data that have been analyzed so far come from ten countries (Angola, Cameroon, Central Africa Republic, Chad, Congo, Democratic Republic of the Congo, Equatorial Guinea, Gabon, Sudan and Uganda) and span from 1985 to 2007.

The DB of HAT will greatly enhance our knowledge of the global distribution of HAT, as well as allowing the updating of previous estimates of population at risk and burden of the disease. It will also provide crucial information to better target interventions with a view to eliminating HAT as a public health problem.

Progress report of the IGAD/LPI-PAAT study: mapping the benefits of tsetse and trypanosomiasis in the Eastern African region – A. Shaw

The purpose of the IGAD LPI is to strengthen the capacity in IGAD countries (Djibouti, Eritrea, Ethiopia, Kenya, Somalia, Sudan, and Uganda), and other regional organizations and stakeholders to formulate and implement livestock sector and related policies that sustainably reduce food insecurity and poverty. The present study draws on a concept developed and tested for West Africa and it provides a GIS-based tool for decision-making and prioritization in T&T control. By means of financial maps this tool adds an economic dimension to GIS-assisted decision making. The model is based on (i) a cattle production systems map, (ii) a 20-year herd and output projection, and (iii) cattle spatial expansion and/or modifications of production systems. The main model output is a map of financial benefits over 20 years.

At the present stage, considerable progress has been made in defining and mapping LPS. Valuable information concerning the spatial distribution of pastoral, agropastoral and mixed-farming systems in the IGAD region has been collected by different institutions in the framework of the Household Economy Approach. Livelihood maps are available at country-level for Djibouti, Kenya, Somalia and Uganda, as well as for some regions of Eritrea, Ethiopia and Sudan.

Harmonization of these datasets will ultimately result in a regional map of LPS, which will also include information on the use of oxen, commercial and semi-commercial dairying and ranching. This product will be matched against independent maps of production systems which are based on climate and other environmental datasets with a view to gaining insight into the relationship between livelihood options and environmental factors. The next steps in this study will concentrate on (i) completing the map of LPS and the remaining baseline herd models, (ii) investigating the extent to which cattle production systems would change in the future (e.g. through movements into new areas, intensification, etc.), (iii) combining cattle population, LPS and tsetse maps, (iv) calculating losses per head of cattle over 20 years in each production system, and (v) producing the financial maps.

Presentation of the questionnaire "Regional Designated Centres for Training relevant to addressing the T&T Problem" – U. Feldmann

Regional Designated Centres (RDC) should meet the needs for training relevant to addressing the T&T problem by making optimal use of the limited resources, avoiding duplication and assuring quality and sustainability. The African Regional Co-operative Agreement for Research, Development and Training related to nuclear science and technology (AFRA) established guidelines for identification and impartial review of candidate RDCs. As concerns the problem of training on T&T, a questionnaire aiming at identifying a limited number of RDCs and at generating information for assessing candidate centres was developed. Nine topics are proposed in the questionnaire: project management, epidemiology of livestock diseases, diagnosis of livestock diseases, T&T control, tsetse mass rearing, agricultural and livestock socio-economics, natural resources management, remote sensing and GIS, HAT diagnosis, epidemiology and control.

Assessment of questionnaires and candidate institutions will be based on the objectives of the training programme offered, detailed curriculum, deployment of human

resources, institutional infrastructure and its internal quality assurance system. Feedback to the questionnaire was obtained from FAO and WHO. AU-PATTEC was informed about the initiative.

The next phase of this activity will include: submission of applications to the National Coordinator of AFRA and subsequent transmission to IAEA, technical assessment of applications by a technical working group, pre-selection of RDCs, auditing of pre-selected RDCs and nomination of RDCs, appointment of RDCs. The process is scheduled to be completed by September 2008.

Flowchart on Guidelines for assessing the feasibility of creating tsetse and trypanosomiasis-free zones – U. Feldmann

The T&T problem is complex and the decision about intervening or not intervening on the problem has a broad range of implications for various sectors. Planners, decision makers and implementers are charged with high responsibilities that embrace politics, finance, public health, livestock and agricultural rural development, and sustainability of natural resources. The proposed Guidelines try to provide assistance to address all relevant components, avoid setbacks, decide responsibly on use of resources and generate a basis for approaching donors.

The Guidelines are based on the phased, conditional approach, which is reflected in the flowchart by five different levels of activity: (i) policy and strategy development, long-term commitment, management structures, (ii) baseline data collection, (iii) technical feasibility assessment, (iv) capacity building and pre-operational work, and (v) operational implementation of AW-IPM measures to create a T&T free zone.

Ethiopia: Review and assessment of AfDB funded project: status of implementation in relation to the proposed "phased feasibility flowchart" – T. Alemu

The report concentrated on the status of the STEP project, for which the AfDB loan and grant are complementary to ongoing efforts. The project area meets the 7 criteria for technical feasibility set by PAAT, especially with regard to the area's high agricultural potential and the presence of important natural barriers to reinvasion. AT is by far the most important problem for Ethiopia, but HAT surveillance should be encouraged particularly in the border areas with the Sudan. Within the project area expanded and intensified mixed farming are possible, especially provided that draft oxen be available. The AfDB and UNTFHS funding will allow further support for the land use and land tenure component of the project, especially in the present context of evolving practices. Since the inception of STEP the Ethiopian government was committed to integrate SIT with the support of IAEA. A thorough needs assessment was made to integrate SIT for tsetse eradication. Capacity building was undertaken and infrastructure was developed. A modern insectary was established and the new facility is now ready for mass rearing, as the colony performance has been stable since last year.

Issues that currently deserve attention are the delay in finalizing the feasibility study for the possible use of the Sequential Aerosol Technique (SAT), problems arising with operations in the NechSar National Park, lack of professional and support staff in critical areas, lack of standard insectary operating procedures, enforcement of strict bio-security to

avoid unwanted circumstances on the fly performance and irradiation source. Future plans include continuing tsetse suppression in agreement with AW-IPM concept, enhancing the tsetse colony build-up and mass rearing, starting the baseline data collection in the remaining blocks, enhancing the monitoring, data analysis and reporting.

Uganda: Review and assessment of AfDB funded project on status implementation in relation to the proposed "phased feasibility flow chart" – L. Semakula

Uganda is presently implementing the feasibility phase for the project "Creation of sustainable tsetse and trypanosomiasis free areas in East and West Africa -Uganda component". The ongoing feasibility phase includes the baseline data collection (entomological, parasitological, socio-economic, and environmental), refurbishment and equipping of the tsetse mass rearing facility at Tororo, the establishment of a tsetse seed colony and training of technical staff. Awareness on programme activities is being created through workshops. In addition, the National Steering Committee and the Parliamentary Committee on agriculture have been sensitized on the T&T subject.

A national team was formed to carry out the feasibility assessment; a team of 20 entomologists has been identified to work with the consulting firm which will undertake the baseline data collection. GIS, satellite imagery and tsetse prediction maps are being used to assess the isolation of the target tsetse population and for demarcation of intervention zones. 46 sites for tsetse population genetic studies have been identified with the assistance of IAEA.

A detailed action plan has been developed and work will begin in May 2008. Computers and satellite imagery for this activity were received from the IAEA. As to fund mobilization, additional resources have been received through a TCP from the IAEA, which has also provided funds for training 20 entomologists who will be involved in baseline data collection.

Burkina Faso: Review and assessment of AfDB funded project on status implementation in relation to the proposed "phased feasibility flow chart" – I. Sidibe

The AfDB funded project in Burkina Faso benefits from collaboration with various national institutions that are in charge of land use, land occupation, environmental impact assessment, HAT, information and sensitization of communities. Following the phased, conditional approach it has been decided to initially carry out interventions over an area of approximately 40 000 km^2 of the total intervention area (100 000 km^2). The project area has a high potential for crop production and livestock development. The zone is at the northern limit of the tsetse distribution and it is therefore suitable for suppression and elimination activities, especially during the dry season from October to May. Furthermore, human interventions are developing natural barriers through the expansion of cotton cultivation and pesticide utilisation.

In the first block of the project area, baseline data collection has been carried out for entomological, parasitological, socio-economic, environmental and land use data. A geo-database has been assembled to centralize and store all geo-spatial information that is being used along with satellite images to select sampling sites for the entomological survey. Data

collection recently started also with a view to finding possible natural barriers to reinvasion or potential sites for placing artificial barriers.

A study is assessing the feasibility of applying SAT for tsetse fly suppression for the creation of T&T-free areas in East and West Africa. The outcome of this study should tell whether SAT is to be used in the agro-ecological setting of Burkina Faso. It is possible that traps, targets, and pour-on formulations could suffice to eliminate tsetse from block 1. In view of the possible use of the SIT technique, efforts are being made to improve the capacity of the insectary at the Centre International de Recherche-Développement sur l'Elevage en Zone Subhumide (CIRDES), while a new building is being planned.

As regards HAT, assessment is in progress in the project areas in collaboration with the Institut de recherche pour le développement (IRD) and CIRDES with different support. Other research activities are focusing on population genetics of tsetse, especially in the context of degradation and fragmentation of habitats.

Mali: Review and assessment of AfDB funded project on status implementation in relation to the proposed "phased feasibility flow chart" – A. Djiteye

With the financial support of the AfDB and the Government of Mali, the project aims at eliminating the T&T problem from an area of approximately 37 000 km^2 (17 000 km^2 in the Niger basin and 28 000 km^2 in the Bani basin). The baseline data collection concerns tsetse fly distribution and population dynamics, animal and human trypanosomiasis prevalence, socio-economic studies, environmental survey and monitoring. Sensitization and raising community awareness have been pursued through regional meetings and communal workshops. Farmers' organizations have been involved through the creation of T&T control brigades in approximately 190 villages; an average of five sergeants per village have been trained in traps impregnated with deltamethrin, trap installation and surveillance. Significant reduction of tsetse densities in the intervention areas has been achieved.

Quality Control/Quality Assurance of trypanocidal drugs - F. van Gool

In the last ten years numerous papers were published indicating resistance of trypanosomes to trypanocidal drugs. However, in the vast majority of cases, investigation on the type and brand of the trypanocidal drug that was used revealed that the drug was of poor quality and even in some cases was a completely fake drug.

To tackle the problem of poor quality and fake trypanocidal drugs circulating in the African market a MoU was signed between FAO and IFAH. The aims of the MoU are (i) to develop reliable methods to control the quality of trypanocidal drugs, and (ii) to create two chemical-analytical laboratories in Africa (one in West Africa and one in East Africa) to control drugs circulating in the different countries. One of the provisions of the MoU is that stakeholders involved in the use of trypanocidal drugs can send samples to these independent laboratories for quality control. Also, samples can be sent to the representatives of the FAO and IFAH to be analyzed by the University of Strathclyde (UK) which is the Reference laboratory for the control of Trypanocidal Drugs.

The IFAH representative also encouraged stakeholders to make optimal use of the published literature concerning the quality of trypanocidal drugs.

The tsetse and trypanosomiasis R&D programme and activities, including training opportunities, at ITM – S.Geerts and collaborators

ITM concentrates on three research themes: (i) vector-parasite interaction to understand the factors determining the infection rate of tsetse, (ii) host-parasite interaction to explore factors affecting the impact of infection, and (iii) the vector host/environment interaction to clarify the effect of a changing environment on the epidemiology and impact of AAT. Research is also carried out at ITM on trypanocidal drug resistance, in particular on the development and validation of molecular techniques for the detection of drug resistant trypanosomes. ITM is the FAO reference centre for "Livestock trypanosomiasis: parasite management and diagnosis". ITM's training activities include an MSc in Tropical Animal Health, a web-based MSc in Tropical Veterinary Medicine (managed in collaboration with the University of Pretoria, the Regional Training Programme for the Southern African Development Community (SADC) Region), and various PhD programmes.

The International Trypanotolerance Centre (ITC): new developments and challenges ahead – S. Geerts

In his report, Mr. Geerts stated that ITC was founded in 1982 by an act of the Gambian Parliament and it initially focused on research, multiplication and dissemination of the trypanotolerant N'Dama cattle in Africa. The present focus is on increasing livestock productivity and utilization in the West African region through optimal and sustainable exploitation of the genetic resistance of indigenous breeds of livestock. ITC's partners are the National Agricultural Research Services (NARS) of The Gambia, Senegal, Guinea, Guinea Bissau, Sierra Leone, Liberia, ILRI and CIRDES. ITC assets include the HQ in Banjul, two field stations, laboratories, training facilities, administration, social facilities, a residential area, animal facilities and herds/flocks.

Due to lack of core funding, ITC had faced recurrent problems to pay staff, with the result that most international staff members left and the DG was replaced by an interim management committee. The Executive Committee of Council that was held in March 2008 concluded that restructuring of ITC was necessary and various options for the future were discussed based on four available reports. The option preferred by the Gambian Government was for ITC to become a Gambian livestock research institute while AU-IBAR preferred ITC to become a regional livestock research centre. The possibility of a merger between ITC and either CIRDES or ILRI was explored but it appeared that ILRI was divesting itself of field sites and CIRDES was not interested in a merger in the short term. The Council of ITC, which decides autonomously, wanted to maintain the regional status of ITC, while the Gambian Government, which owns the land and buildings of ITC, preferred ITC to become a national institute. The international community that has made considerable investments in ITC aims at safeguarding the nucleus herds. Therefore a compromise is urgently needed.

The new AfDB-Global Environment Facility (GEF) project "Sustainable management of endemic ruminant livestock in West Africa" (2008-2018) has ITC as executing agency for

the AfDB- funded component of the project. With its $ 42 million budget this provides a unique opportunity for the future of ITC.

Recommendations

The following recommendations were discussed and agreed:

On the recent agreement between FAO and IFAH on the Control of Veterinary Drugs, PAAT welcomes the signing of the Memorandum of Understanding (MoU) between FAO and IFAH on Quality Control/Quality Assurance (QC/QA), especially of trypanocides. The meeting **recommends** that:

- Awareness be raised of the services provided by reference laboratories accredited to conduct QC of trypanocides. To this end, a section of the TTI is to be devoted to the subject.

Action: PAAT, PATTEC, involved countries.

Reinvasion of reclaimed areas: The meeting notes that the issue of reinvasion is still a major concern to all the PATTEC countries. Therefore, the meeting **reiterates recommendations** made in previous PAAT meetings that:

- The risk of reinvasion be comprehensively assessed (e.g. at the time of baseline entomological surveys) and that measures be put in place aimed at minimizing this risk in a sustainable manner.

Action: PATTEC, involved countries.

Acknowledging the importance of the on-going HAT mapping exercise, the meeting **recommends** that:

- Data on HAT occurrence be submitted to WHO in a timely manner.

Action: involved countries.

Tsetse fly in Swaziland. In view of the recent findings in Swaziland, where flies were discovered although thought to be absent, the meeting **recommends** that:

- South Africa and Mozambique should consider involving Swaziland in their regional eradication project.

Action: PATTEC, involved countries.

Cooperation between countries benefiting from AfDB loans and WHO on HAT. Following the example set by the recent collaboration between WHO and the AfDB-funded tsetse elimination project in Ghana, the meeting **recommends** that:

- Countries presently involved in baseline data collection should contact WHO for support on HAT assessment.

Action: involved countries, PATTEC, WHO.

Land cover classification and sharing of GIS data and metadata. In consideration of the standardization activities carried out by PAAT (e.g. in the field of land cover classification, sharing of GIS data and metadata, etc.), the meeting **recommends** that:

- Efforts be made to adopt the international standards promoted by PAAT.

Action: involved countries, PATTEC.

The International Trypanotolerance Centre (ITC), The Gambia. PAAT recognizes the invaluable role that The Gambia has played in hosting and promoting the activities against tsetse-transmitted trypanosomiasis over the past three decades through the establishment of ITC. It appreciates the difficulties ITC has had in recent years in obtaining core funding and the support needed to carry out its mandate. Despite these difficulties, however, ITC continues to be recognised as a regional centre of excellence, with active and productive work with NARS in its core countries: The Gambia, Guinea, Guinea Bissau and Senegal.

In this context, PAAT greatly welcomes the new project "Sustainable management of endemic ruminant livestock in West Africa", which provides for funding and research. The international community has also invested substantial resources in the ITC's selectively bred herds which constitute an irreplaceable international asset that must be conserved so that their unique genetic resources can continue to be made available to the whole region. **PAAT therefore supports recent efforts by AU/ISCTRC and others** and hopes that a satisfactory regional solution can be found which ensures their continued support.

Regional Development Centres (RDCs). A questionnaire was developed by the FAO/IAEA Joint Division, aimed at (i) identifying a limited number of RDCs for training in the field of Tsetse and Trypanosomosis, and (ii) generating information for subsequent assessment of candidate centres. The meeting **recommends** that:

- Feedback and suggestions be provided by all the recipients of the questionnaire.

Action: PAAT, PATTEC, involved countries.

Flowchart on the feasibility of creating tsetse and trypanosomiasis-free zones. The meeting recognizes the usefulness of the flowchart for assessing the feasibility of creating tsetse and trypanosomiasis-free zones, which may consider the use of SIT, when and where environmentally and technically appropriate. The meeting **recommends**:

- To simplify the layout as developed to facilitate the interpretation and utilization of the flowchart.

Action: FAO/IAEA Joint Division.

The meeting recognizes the role of PAAT as a body for technical review and eventual advocacy for T&T project proposals to be submitted to potential donors. The meeting **urges member countries** that:

- Project proposals dealing with T&T and related matters be presented at PAAT-PC and PAG meetings for assistance in technical review andsubsequent support for advocacy.

Action: PATTEC, involved countries.

Standardization of fabrics and other equipment used in tsetse control. Considering the normative role of PAAT and its harmonization function in relation to T&T control techniques, the meeting **recommends**:

- To explore the possibility to standardize and define quality control and assurance methodologies for fabrics and other equipment used for constructing targets, screens, traps, etc.

Action: PAAT.

Socio-economic and environmental impact assessment. The meeting recognizes the importance of socio-economic and environmental issues/impacts related to T&T intervention programmes and acknowledges the work of the International Livestock Research Institute (ILRI) on these aspects. The meeting **recommends** that:

- A limited number of key parameters are identified which can be consistently collected in a cost-effective manner, to be used as indicators of the socio-economic and environmental impact of T&T interventions.

Action: ILRI, PAAT.

Need for flexibility in budget management of AfDB funds by countries implementing T&T interventions. The six countries (Burkina Faso, Ethiopia, Ghana, Kenya, Mali, Uganda) receiving AfDB loans and grants for T&T interventions expressed their concern about a certain lack of flexibility in adaptive budget management. This does not allow a rapid shift in budget resources to respond to changed field situations and unforeseen events. The meeting **recommends**:

- To bring this matter to the attention of the AfDB during the forthcoming-term review of the respective national AfDB T&T intervention projects.

Action: PATTEC, PAAT, involved countries.

Review of PAAT and its structures. Members expressed the need for review of PAAT and its structures after 10 years of operation. This is meant to further strengthen PAAT and ensure its continued relevance in the challenging field of T&T interventions. Members **recommend** that

- Rather than have an external review panel that may be costly, that this be done in-house.

Action: PAAT Secretariat.

Closing

Mr Ilemobade, Chairman of PAAT, heartily thanked all participants for their contributions. Thereafter, he declared the meeting closed. Mr Mattioli reminded members that the next PAAT-PAG meeting will be held in Kampala, Uganda, while the next PAAT-PC meeting is proposed to be held in Bratislava, Slovak Republic.

FAO/IAEA GUIDELINES ON THE COLLECTION OF ENTOMOLOGICAL BASE-LINE DATA FOR TSETSE AREA-WIDE INTEGRATED PEST MANAGEMENT PROGRAMMES. STEPHEN G.A. LEAK, DEJENE EJIGU AND MARC J.B. VREYSEN. 205 PP., ISSN 1810-0708. FAO ANIMAL PRODUCTION AND HEALTH GUIDELINES SERIES, ROME

Several sub-Saharan Member States have expressed the intention to embark on national or regional programmes to create sustainable tsetse-free zones under the umbrella of the PATTEC initiative. It is imperative that these programmes be implemented using an area-wide integrated pest management approach (i.e. targeting an entire population within a circumscribed area) for the results to be sustainable. Most AW-IPM programmes are technically complex and require in-depth knowledge about the ecology and population dynamics of the target insect. In that respect, new guidelines were developed on the collection of entomological base line data within the context of tsetse AW-IPM and published under the FAO Animal Production and Health series. As most Member States are in the second phase of the phased conditional approach (i.e. feasibility study), the publication of this document is timely.

The document is composed of three parts: (1) the first provides an overview of the basic biology and anatomy of the tsetse fly, and is intended for those who are new to the field of tsetse, (2) the second part covers the planning and preparation of a base line survey, and is intended for use by senior entomological staff whom will be involved in the actual development of the survey strategy, and (3) the third part deals with the implementation of a survey and targets technicians and entomologists in the field. The guidelines make reference to modern spatial tools such as GPS, RS and GIS, emphasize the need for good land use and land cover maps for the planning and implementation of a survey, and provide details of a new Access-based data base (Tsetse Intervention Recording and Reporting System) that has been specifically developed for tsetse surveys and monitoring purposes.

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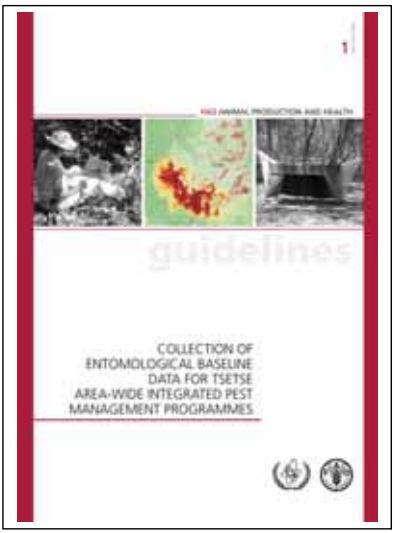

MEMORANDUM OF UNDERSTANDING BETWEEN THE FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS AND THE INTERNATIONAL FEDERATION FOR ANIMAL HEALTH ON COOPERATION IN THE ESTABLISHMENT OF STANDARDS AND PROTOCOLS FOR QUALITY CONTROL OF TRYPANOCIDE DRUGS[1]

PREAMBLE

Whereas the Food and Agriculture Organization of the United Nations, hereinafter referred to as FAO, carries out activities on animal health protection in developing countries, and is one of the parent institutions of the Programme Against African Trypanosomiasis (PAAT) established by FAO Conference Resolution 5/97 of 17 November 1997 and managed by a joint secretariat provided by FAO, the Inter-African Bureau for Animal Resources of the African Union (AU-IBAR), the International Atomic Energy Agency (IAEA) and the World Health Organization (WHO);

Whereas PAAT is an international alliance comprising FAO, WHO, AU-IBAR and IAEA and its main objectives are to support action at country level to advise and assist in the coordination of research, intervention and control activities of Trypanosomiasis;

[1] Adapted from the original by the editor.

Whereas the International Federation for Animal Health, hereinafter referred to as IFAH, is a non-profit federation with liaison status with FAO and representing manufacturers of veterinary medicines, vaccines and other animal health products, in both developed and developing countries;

Whereas IFAH is prepared to provide technical expertise in support of the establishment of standards and protocols for quality control of trypanocidal drugs, while recognizing the independent nature of FAO as an intergovernmental organization of the United Nations system;

Whereas both FAO and IFAH recognize that the proposed cooperation would contribute towards the establishment of a harmonized framework on the trypanocidal drugs and should be welcomed;

Whereas the proposed cooperation was endorsed by the Programme Committee of PAAT, at its Seventh Session in November 2002, and is to be further reviewed by that Committee and its results are to be subject to comprehensive review and endorsement by the Panel of the Programme Against African Trypanosomiasis Advisory Group Coordinators and the PAAT Programme Committee.

FAO and IFAH have agreed as follows:

Article 1
Objectives of the proposed cooperation

1. The overall objective of this Memorandum of Understanding is to formalize the collaboration between FAO and IFAH in the recommendation of internationally and scientifically agreed standards to be presented to the OIE for adoption through their usual procedures and to define protocols for quality control and quality assurance of trypanocidal drugs, in line with the recognition that trypanocides are the most common and cost-effective method to reduce the impact of African animal Trypanosomiasis on livestock agricultural development in sub-Saharan Africa.

2. The specific objectives of this Memorandum of Understanding are:

a. to prepare and recommend standards and protocols for quality control of trypanocidal drugs.
b. to define the requirements of Analytical Quality Assurance (AQA).
c. to establish good laboratory practices for chemical analysis;
d. to make accessible, on an equal basis, to any company and/or stakeholders the generated scientific and technical information; and
e. to transfer the methodology and technology to West and East Africa.

3. FAO and IFAH recognize that as a result of the activities to be carried out under this Memorandum of Understanding:

a. quality control and quality assurance guidelines and principles for chemical analysis and standard operating procedures (SOPs) should be harmonized in compliance with OIE standards;
b. requirements for Analytical Quality Assurance (AQA) should be defined;
c. two laboratories in West Africa and East Africa, respectively, should be identified, and Quality Control/Quality Assurance (QC/QA) standards transferred;
d. establishment of a basis for partnerships, involving the public and private sector in support of the implementation and application of QC/QA standards on a sustainable basis.
e. the outcomes of the joint activity between FAO and IFAH will be brought by FAO to the attention of the appropriate bodies of the Codex Alimentarius Commission.

Article 2
Consultative Committee

1. FAO and IFAH will establish a Consultative Committee which will follow and supervise directly the activities to be carried out under this Memorandum of Understanding.

2. The Consultative Committee will consist of the following members: two representing FAO, one IFAH and one the International Atomic Energy Agency (IAEA), appointed by their respective organizations. The FAO representatives will be technical Officers of the Animal Health Service (AGAH), one from headquarters and the other from the Regional Office for Africa. The Chairmanship and the Secretariat of the Consultative Committee will be assured, respectively, by the Chief, AGAH and the technical Officer of AGAH based at FAO headquarters.

3. The Consultative Committee will monitor and supervise the advancement of the work, with the assistance of the two experts referred to in Article 3, in the development of the experimental design and in the validation of the results. The Consultative Committee may consult external experts as deemed necessary.

4. The Consultative Committee will assist:

(a) in the compilation of scientific reports;

(b) in the communication of the results of the activities to IFAH; and

(c) in the dissemination of the results to the international community through publications in peer reviewed scientific journals.

Article 3
Assignment of experts to laboratories

1. Two experts from public, technically reputed, non-profit institutions, with recognized scientific background in chemical analysis of pharmaceutical products, and provided by IFAH and IAEA, will be assigned to perform the analysis required by the Consultative Committee, under this Memorandum of Understanding, in consultation with the relevant institutes.

2. In liaison with the Consultative Committee, FAO will assist in the identification of the African laboratories to be involved in the proposed cooperation, one in West Africa and the other in East Africa.

3. FAO, through its network of Tsetse and Trypanosomiasis Liaison Officers and Members of the PAAT Advisory Group Coordinators, will assist the experts in the accurate collection of drug samples.

Article 4
Cost-sharing arrangements

1. Contribution of FAO

   (a) FAO will provide through PAAT, within the limits of the budgetary appropriation of the Organization's Regular Programme, adequate environment for scientific concertation and dissemination of the information through its regular statutory meetings and the PAAT-Information System (PAAT-IS) and will promote awareness on Quality Assurance/Quality Control of trypanocides among all concerned stakeholders. FAO will seek to support the presentation of results and technical data by the experts in relevant international meetings, such as the International Scientific Council for Trypanosomiasis Research and Control, ISCTRC, including meetings of its statutory bodies, such as the Panel of the PAAT Advisory Group Coordinators.

   (b) FAO, through PAAT, will act as a neutral body and ensure – through the Consultative Committee and the relevant PAAT structures – that the work is carried out in a correct manner, securing scientific and technical integrity.

2. Contribution of IFAH

   (a) IFAH will contribute, in conditions to be agreed upon within the Consultative Committee, funds to pay for national Liaison Officers for Tsetse and Trypanosomiasis for (i) the sampling, collection and shipment of drug vials encountered in the market of affected African countries and (ii) the chemical analysis by the two collaborating laboratories. These funds will not be channelled through FAO, but will be directly paid to service providers.

(b) IFAH will provide, jointly with FAO, the necessary support for the dissemination of the scientific information on agreed standards and protocols for Quality Assurance/Quality Control of trypanocides to concerned stakeholders.

Article 5
Specific provisions

1. Nothing in this Memorandum of Understanding will be interpreted as conferring upon IFAH or any of the Members of IFAH any exclusive right or any preferential treatment from FAO. In particular, FAO remains free to procure any trypanocide medicines from any producer or supplier which is not a Member of IFAH.

2. In view of the particular status of FAO as a neutral, independent, intergovernmental organization of the United Nations system, IFAH recognizes that any perceived or actual risk of conflict of interest should be avoided. FAO and IFAH will enter into such immediate consultations as may be required with a view to taking corrective action in the event of such perceived or actual risk of conflict of interest. In addition, no visibility will be given to any individual IFAH Member participating in cooperation activities under this Memorandum of Understanding.

Article 6
Programme Against African Trypanosomiasis

All activities carried out under this Memorandum of Understanding will be reviewed by the Programme Against African Trypanosomiasis and subject to such guidance as it may provide through the Consultative Committee.

Article 7
Intellectual property rights

1. Intellectual property rights on documents and materials made available by FAO and IFAH to carry out activities under this Memorandum of Understanding will remain within the originating party.

2. Intellectual property rights on information or techniques developed jointly by IFAH and FAO under this Memorandum of Understanding will be jointly vested in both parties. FAO and IFAH will have full rights to exploit such jointly-owned work, for non-commercial purposes. This shall not preclude the right of originating parties to publish scientific findings, subject to the agreement of the Consultative Committee.

Article 8
Implementation of the Memorandum of Understanding

1. This Memorandum of Understanding will enter into force on the date of signature on behalf of FAO and IFAH. It will remain in force for the period necessary for the attainment

of the objectives defined in Article 1, subject however, to the possibility for FAO or IFAH to give notice of termination of this Memorandum of Understanding to the other party. Such notice of termination will take effect three months following the date of despatch.

2. Twelve months after the entry into force of this Memorandum of Understanding, the representatives of FAO and IFAH within the Consultative Committee will make a first review of its implementation with a view to identifying possible adjustments thereto.

3. This Memorandum of Understanding may be amended by exchange of letters between duly authorized representatives of FAO and IFAH.

4. Nothing in this Memorandum of Understanding or in any document relating thereto shall be construed as constituting a waiver of privileges and immunities of FAO, nor as conferring any privileges and immunities of FAO in IFAH or any person performing services for IFAH.

5. This Memorandum of Understanding is governed by general principles of law, to the exclusion of any national system of law.

Article 9
Settlement of Disputes

1. Any dispute between FAO and IFAH arising out of the interpretation or execution of this Memorandum of Understanding shall be settled by negotiation between the parties. If the dispute is not settled by negotiation between the parties, it shall, at the request of either party, be submitted to one conciliator. Should the parties fail to reach agreement on the name of a sole conciliator, each party shall appoint one conciliator. The conciliation shall be carried out in accordance with the Conciliation Rules of the United Nations Commission on International Trade Law, as at present in force.

2. Any dispute between FAO and IFAH that is unresolved after conciliation shall, at the request of either party be settled by arbitration in accordance with the Arbitration Rules of the United Nations Commission on International Trade Law, as at present in force.

3. The conciliation or arbitration proceedings shall be conducted in English.

4. The parties may request conciliation during the execution of this Memorandum of Understanding and anyway not later than twelve months after the cessation of activities thereunder or the termination of the Memorandum of Understanding. The parties may request arbitration not later than ninety days after the termination of the conciliation proceedings.

On behalf of the Food and Agriculture Organization of the United Nations:	On behalf of the International Federation for Animal Health:
Jim Butler, Officer-in-Charge, Agriculture and Consumer Protection Department	George Gunn President

SECTION B - ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

14801. **Bowater, R. J., Abdelmalik, S. M. & Lilford, R. J., 2009.** The methodological quality of cluster randomised controlled trials for managing tropical parasitic disease: a review of trials published from 1998 to 2007. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **103** (5): 429-436.

Department of Public Health & Epidemiology, School of Medicine, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK. [bowaterj@adf.bham.ac.uk].

The aim of this review was to assess the methodological quality of cluster randomised controlled trials (CRCT) for the management of tropical parasitic disease published between 1998 and 2007. A literature survey was conducted using Medline for CRCTs of interventions aimed at managing any one of the six major tropical parasitic diseases: malaria, leishmaniasis, lymphatic filariasis, onchocerciasis, schistosomiasis and trypanosomiasis (Chagas disease). Information was extracted from the published articles in order that, for each trial, categorical responses could be made to a pre-specified list of 12 questions concerning issues relating to the methodological quality of the trial, including choice of design, generalisability, baseline assessment, blinding, use or non-use of a matched design, and accounting for the intraclass correlation in both design and analysis. The literature survey found 38 CRCTs. Of the 35 CRCTs that reported at least one human outcome, 27 were for interventions in the management of malaria whilst the rest were for managing leishmaniasis (4 trials), lymphatic filariasis (2 trials) and schistosomiasis (2 trials). For every one of the pre-specified questions that concerned an issue associated with methodological quality, the responses were consistent with the practice of trialists in relation to the given issue and indicated poor understanding of the issues involved.

14802. **Courtin, D., Berthier, D., Thevenon, S., Dayo, G. K., Garcia, A. & Bucheton, B., 2008**. Host genetics in African trypanosomiasis. *Infection, Genetics and Evolution,* **8** (3): 229-238.

Radboud University Medical Center, Medical Parasitology, PO Box 9101, 6500 HB Nijmegen, The Netherlands. [d.courtin@gmail.com].

In Africa, the protozoan parasite of the genus *Trypanosoma* causes animal (AAT) and human African trypanosomiasis (HAT). These diseases are responsible for considerable mortality and economic losses, and until now the drugs commonly used have often been very toxic and expensive, with no vaccine available. A range of clinical presentations, from chronic to acute symptoms, is observed in both AAT and HAT. Host, parasite, and environmental factors are likely to be involved in this clinical variability. In AAT, some West African cattle (N'Dama, *Bos taurus*) have the ability to better control the disease development (and therefore to remain productive) than other taurine breeds (Zebu, *Bos indicus*). This phenomenon is called trypanotolerance and seems to have major genetic components. In humans, tolerance/resistance to the disease is suspected, however, this needs confirmation.

This review focuses on recent advances made in the field of host genetics in African trypanosomiasis in animals (mouse and bovine) and humans. The perspectives for the development of new control strategies and their applications as well as a better understanding of the physiopathology of the disease are discussed.

14803. **Courtin, F., Sidibe, I., Rouamba, J., Jamonneau, V., Gouro, A. & Solano, P., 2009**. Population growth and global warming: impacts on tsetse and trypanosomoses in West Africa. *Parasite,* **16** (1): 3-10.

Institut de Recherche pour le Développement, UMR 177 IRD-CIRAD, Centre International de Recherche Développement sur l'Elevage en zone Subhumide, 01 BP 454, Bobo-Dioulasso, Burkina Faso.

Demographic evolution, climatic change and economical development that happened in West Africa during the 20th century had a lot of consequences on human settlement and landscape. These changes have in turn an impact on the pathogenic system of human and animal trypanosomoses. Since last century, the northern tsetse distribution limit has shifted towards the south, probably due to a decrease in rainfall combined with the impact of human pressure. Sleeping sickness (SS) foci have also shifted from the savannah areas (where there is no more SS) to the forest and mangrove areas of West Africa, but animal trypanosomoses are still present in savannah areas. We show a decrease of tsetse of the *morsitans* group as a result of an increase of human densities. On the other hand, tsetse species like *Glossina palpalis* adapt to high human densities and are found in the biggest urban centres of West Africa. There is a need to promote multidisciplinary studies on this demographic-climatic-vector borne disease topic, especially in Africa to be able to define future areas of presence/absence of these diseases in order to help continental plans of control that have recently begun.

14804. **Fenwick, A., 2009**. Host-parasite relations and implications for control. *Advances in Parasitology,* **68**: 247-261.

Division of Epidemiology, Public Health and Primary Care VB1, St. Mary's Campus, Norfolk Place, London W2 1PG

This paper considers the various measures available to control several of the neglected tropical diseases (NTDs). To develop the optimum methods for controlling the parasites that cause these NTDs, knowledge of the life cycles of both the parasites and their vectors is essential. Each NTD requires its own strategy for control based on detailed knowledge of the life cycle. Vector control, chemotherapy, better water supplies and better hygiene are all components that may be appropriate. For some diseases, improved drugs are urgently required, for some the tools are available for elimination, while uniquely guinea worm could be eradicated without any chemotherapeutic drug being used. Several NTDs lend themselves to mass drug administration in which human populations are annually offered safe, effective and usually donated drugs with a view to morbidity control and/or elimination. The drugs could and should be used to improve the quality of millions of lives, prevent suffering, stigma, disfigurement and early death. The role of pharmaceutical companies which have donated their drugs for the treatment of millions of disadvantaged people in the developing world is acknowledged. One result of such drug pressure, however, is that evolutionary

change may result, and it is incumbent on scientists during monitoring and evaluation of control programmes to ensure that such changes are recognised. One other unfortunate development is that a paucity of newly trained vector-borne disease experts may constrain future control efforts.

14805. **Grab, D. J. & Kennedy, P. G., 2008**. Traversal of human and animal trypanosomes across the blood-brain barrier. *Journal of Neurovirology,* **14** (5): 344-351.

Department of Pediatrics, Division of Pediatric Infectious Diseases, Johns Hopkins School of Medicine, Baltimore, Maryland, USA.

The neurological complications of human African trypanosomiasis (HAT) in man caused by the unicellular protozoan parasites *Trypanosoma brucei gambiense* and *T. b. rhodesiense* are a consequence of the penetration of the blood-brain barrier (BBB) by trypanosomes that enter the central nervous system (CNS). Yet the mechanisms by which African trypanosomes cross the true BBB comprised of brain microvascular endothelial cells (BMECs) remain unclear. Human BBB models used to determine how African trypanosomes initially interact *in vitro* with the human BBB proper suggest that parasites cross the human BBB in part by generating Ca^{2+} activation signals in human BMECs through the activity of parasite cysteine proteases. *In vivo* murine models of HAT have suggested additional mechanisms of BBB traversal by trypanosomes, with recent compelling evidence for the important role of interferon-gamma in facilitating this process. A clear understanding of how trypanosomes enter the CNS is critical for both understanding the neuropathogenesis of HAT and in developing more effective drug therapies for late-stage disease.

14806. **John, K., Kazwala, R. & Mfinanga, G. S., 2008**. Knowledge of causes, clinical features and diagnosis of common zoonoses among medical practitioners in Tanzania. *BMC Infectious Diseases,* **8**: 162.

National Institute for Medical Research, Muhimbili Medical Research Centre, Dar es Salaam, Tanzania. [jkundas@yahoo.com].

Many factors have been mentioned as contributing to under-diagnosis and under-reporting of zoonotic diseases particularly in the sub-Sahara African region. These include poor disease surveillance coverage, poor diagnostic capacity, the geographical distribution of those most affected and lack of clear strategies to address the plight of zoonotic diseases. The current study investigates the knowledge of medical practitioners of zoonotic diseases as a potential contributing factor to their under-diagnosis and hence under-reporting. The study was designed as a cross-sectional survey. A semi-structured open-ended questionnaire was administered to medical practitioners to establish the knowledge of anthrax, rabies, brucellosis, trypanosomiasis, echinococcosis and bovine tuberculosis in selected health facilities within urban and rural settings in Tanzania between April and May 2005. Frequency data were analyzed using likelihood ratio chi-square in Minitab version 14 to compare practitioners' knowledge of transmission, clinical features and diagnosis of the zoonoses in the two settings. For each analysis, likelihood ratio chi-square p-value of less than 0.05 was considered to be significant. Fisher's exact test was used where expected results were less than five. The results showed that medical practitioners in rural health facilities had poor knowledge of transmission of sleeping sickness and clinical features of anthrax and rabies in

humans compared with their urban counterparts. In both areas the practitioners had poor knowledge of how echinococcosis is transmitted to humans, clinical features of echinococcosis in humans, and diagnosis of bovine tuberculosis in humans. It is concluded that knowledge of medical practitioners of zoonotic diseases could be a contributing factor to their under-diagnosis and under-reporting in Tanzania. Refresher courses on zoonotic diseases should be conducted particularly for practitioners in rural areas. More emphasis should be put on zoonotic diseases in teaching curricula of medical practitioners' training institutions in Tanzania to improve the diagnosis, reporting and control of zoonotic diseases. Veterinary and medical collaboration should be strengthened to enable more effective control of zoonotic diseases in Tanzania.

14807. **Muskavitch, M. A., Barteneva, N. & Gubbels, M. J., 2008**. Chemogenomics and parasitology: small molecules and cell-based assays to study infectious processes. *Combinatorial Chemistry and High Throughput Screening,* **11** (8): 624-646.

Department of Biology, Boston College, 140 Commonwealth Avenue, Chestnut Hill, MA 02467, USA.

Infectious diseases caused by protozoan parasites - malaria, sleeping sickness, leishmaniasis, Chagas disease, toxoplasmosis--remain chronic problems for humanity. We lack vaccines and have limited drug options effective against protozoa. Research into anti-protozoan drugs has accelerated with improved *in vitro* cultivation methods, enhanced genetic accessibility, the completed genome sequences for key protozoa, and increased prominence of protozoan diseases on the agendas of well-resourced public figures and foundations. Concurrent advances in high-throughput screening (HTS) technologies and availability of diverse small molecule libraries offer the promise of accelerated discovery of new drug targets and new drugs that will reduce disease burdens imposed on humanity by parasitic protozoa. We provide a status report on HTS technologies in hand and cell-based assays under development for biological investigations and drug discovery directed toward the three best-characterized parasitic protozoa: *Trypanosoma brucei*, *Plasmodium falciparum*, and *Toxoplasma gondii*. We emphasize cell growth assays and new insights into parasite cell biology speeding development of better cell-based assays, useful in primary screens for anti-protozoan drug leads and secondary screens to decipher mechanisms of action of leads identified in growth assays. Small molecules that interfere with specific aspects of protozoan biology, identified in such screens, will be valuable tools for dissecting parasite cell biology and developing anti-protozoan drugs. We discuss potential impacts on drug development of new consortia among academic, corporate, and public partners committed to discovery of new, effective anti-protozoan drugs.

14808. **Osorio, E. J., Robledo, S. M. & Bastida, J., 2008**. Alkaloids with antiprotozoal activity. The *Alkaloids. Chemistry and Biology,* **66**: 113-190.

Grupo de Investigación en Sustancias Bioactivas, Facultad de Quimíca-Farmacéutica, Universidad de Antioquia, A. A. 1226, Medellin, Colombia. [josorio48@yahoo.com].

No abstract available.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

14809. **Kleynhans, E. & Terblanche, J. S., 2009**. The evolution of water balance in *Glossina* (Diptera: *Glossinidae)*: correlations with climate. *Biological Letters,* **5** (1): 93-96.

Department of Conservation Ecology and Entomology, Faculty of AgricSciences, Stellenbosch University, Private Bag X1, Matieland 7602, Republic of South Africa.

The water balance of tsetse flies (Diptera: *Glossinidae*) has significant implications for understanding biogeography and climate change responses in these African disease vectors. Although moisture is important for tsetse population dynamics, evolutionary responses of *Glossina* water balance to climate have been relatively poorly explored and earlier studies may have been confounded by several factors. Here, using a physiological and GIS climate database, we investigate potential interspecific relationships between traits of water balance and climate. We do so in conventional and phylogenetically independent approaches for both adults and pupae. Results showed that water loss rates (WLR) were significantly positively related to precipitation in pupae even after phylogenetic adjustment. Adults showed no physiology-climate correlations. Ancestral trait reconstruction suggests that a reduction in WLR and increased size probably evolved from an intermediate ancestral state and may have facilitated survival in dry environments. The results of this study therefore suggest an important role for water balance physiology of pupae in determining interspecific variation and lend support to conclusions reached by early studies of tsetse physiology.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

14810. **Abd-Alla, A. M., Cousserans, F., Parker, A. G., Jridi, C., Bergoin, M. & Robinson, A. S., 2009**. Quantitative PCR analysis of the salivary gland hypertrophy virus (GpSGHV) in a laboratory colony of *Glossina pallidipes*. *Virus Research,* **139** (1): 48-53.

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

Many species of tsetse flies can be infected by a virus that causes salivary gland hypertrophy (SGH) and virus isolated from *Glossina pallidipes* (GpSGHV) has recently been sequenced. Flies having SGH have a reduced fecundity and fertility. To better understand the impact of this virus in a laboratory colony of *G. pallidipes*, where the majority of flies are

infected but asymptomatic, and to follow the development of SGH in symptomatic flies in relation to virus copy number, a quantitative PCR (qPCR) method was developed. The qPCR analyses revealed that in asymptomatic flies virus copy number averaged 1.68E+5, 2.05E+5 and 1.07E+7$\log_{10}$ in DNA from an excised leg, salivary glands and a whole fly, respectively. In symptomatic flies the virus copy number in the same organs averaged 1.34E+7, 1.42E+10 and 1.5E+9, respectively. Despite these statistically significant differences ($p<0.0001$) in virus copy number between asymptomatic and symptomatic flies, there was no correlation between age and virus copy number for either sets in adult flies. A clear correlation between virus copy number in pupae and their mothers was observed. Reverse transcription quantitative PCR (RT-qPCR) of the viral messenger RNA encoding ODV-E66, an envelope protein, revealed a clear correlation between virus copy number and the level of gene expression with values of 2.77$\log_{10}$ in asymptomatic males and 6.10$\log_{10}$ in symptomatic males. Taken together these results confirm the close relationship between virus copy number and SGH syndrome. They demonstrate the vertical transmission of GpSGHV from mother to progeny, and suggest that the development of SGH may be correlated to the virus copy number acquired by the larva during its intra-uterine development.

14811. **Cecchi, G., Paone, M., Franco, J. R., Fevre, E. M., Diarra, A., Ruiz, J. A., Mattioli, R. C. & Simarro, P. P., 2009**. Towards the atlas of human African trypanosomiasis. *International Journal of Health Geographics,* **8**: 15.

Food and Agriculture Organization of the United Nations, Animal Production and Health Division, Rome, Italy. [giuliano.cecchi@fao.org].

Updated, accurate and comprehensive information on the distribution of human African trypanosomiasis (HAT), also known as sleeping sickness, is critically important to plan and monitor control activities. We describe input data, methodology, preliminary results and future prospects of the HAT Atlas initiative, which will allow major improvements in the understanding of the spatial distribution of the disease. Up-to-date as well as historical data collected by national sleeping sickness control programmes, non-governmental organizations and research institutes have been collated over many years by the HAT Control and Surveillance Programme of the World Health Organization. This body of information, unpublished for the most part, is now being screened, harmonized, and analysed by means of database management systems and geographical information systems (GIS). The number of new HAT cases and the number of people screened within a defined geographical entity were chosen as the key variables to map disease distribution in sub-Saharan Africa. At the time of writing, over 600 epidemiological reports and files from seventeen countries were collated and included in the data repository. The reports contain information on approximately 20 000 HAT cases, associated to over 7 000 different geographical entities. The oldest epidemiological records considered so far date back to 1985, the most recent having been gathered in 2008. Data from Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea and Gabon from the year 2000 onwards were fully processed and the preliminary regional map of HAT distribution is presented. In conclusion, the use of GIS tools and geo-referenced, village-level epidemiological data allow the production of maps that substantially improve on the spatial quality of previous cartographic products of similar scope. The significant differences between our preliminary outputs and earlier maps of HAT transmission areas demonstrate the strong need for this systematic approach to mapping sleeping sickness and point to the inaccuracy of any calculation of population at risk based on

previous maps of HAT transmission areas. The Atlas of HAT will lay the basis for novel, evidence-based methodologies to estimate the population at risk and the burden of disease, ultimately leading to more efficient targeting of interventions. Also, the Atlas will help streamline future field data collection in those parts of Africa that still require it.

14812. **Garcia-Maruniak, A., Abd-Alla, A. M., Salem, T. Z., Parker, A. G., Lietze, V. U., van Oers, M. M., Maruniak, J. E., Kim, W., Burand, J. P., Cousserans, F., Robinson, A. S., Vlak, J. M., Bergoin, M. & Boucias, D. G., 2009.** Two viruses that cause salivary gland hypertrophy in *Glossina pallidipes* and *Musca domestica* are related and form a distinct phylogenetic clade. *Journal of General Virology,* **90** (Pt 2): 334-346.

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Glossina pallidipes and *Musca domestica* salivary gland hypertrophy viruses (GpSGHV and MdSGHV) replicate in the nucleus of salivary gland cells causing distinct tissue hypertrophy and reduction of host fertility. They share general characteristics with the non-occluded insect nudiviruses, such as being insect-pathogenic, having enveloped, rod-shaped virions, and large circular double-stranded DNA genomes. MdSGHV measures 65x550 nm and contains a 124 279 bp genome (approximately 44 mol percent G+C content) that codes for 108 putative open reading frames (ORFs). GpSGHV, measuring 50x1 000 nm, contains a 190 032 bp genome (28 mol percent G+C content) with 160 putative ORFs. Comparative genomic analysis demonstrates that 37 MdSGHV ORFs have homology to 42 GpSGHV ORFs, as some MdSGHV ORFs have homology to two different GpSGHV ORFs. Nine genes with known functions (dnapol, ts, pif-1, pif-2, pif-3, mmp, p74, odv-e66 and helicase-2), a homologue of the conserved baculovirus gene Ac81 and at least 13 virion proteins are present in both SGHVs. The amino acid identity ranged from 19 to 39 percent among ORFs. An (A/T/G)TAAG motif, similar to the baculovirus late promoter motif, was enriched 100 bp upstream of the ORF transcription initiation sites of both viruses. Six and seven putative microRNA sequences were found in MdSGHV and GpSGHV genomes, respectively. There was genome co-linearity between the two SGHVs, but not between the SGHVs and the nudiviruses. Phylogenetic analysis of conserved genes clustered both SGHVs in a single clade separated from the nudiviruses and baculoviruses. Although MdSGHV and GpSGHV are different viruses, their pathology, host range and genome composition indicate that they are related.

14813. **Guerrini, L., Bord, J. P., Ducheyne, E. & Bouyer, J., 2008**. Fragmentation analysis for prediction of suitable habitat for vectors: example of riverine tsetse flies in Burkina Faso. *Journal of Medical Entomology,* **45** (6): 1180-1186.

CIRAD, UPR Epidémiologie, Montpellier, F-34398, France.
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Tsetse flies are the cyclic vectors of sleeping sickness and African animal trypanosomosis. The possibility to classify the natural habitat of riverine tsetse species is explored in the Mouhoun River basin, Burkina Faso: the objectives were to discriminate the riverine forests community types and their fragmentation levels by using Landsat 7 enhanced

thematic mapper images, to map tsetse densities. *Glossina palpalis gambiensis* Vanderplank 1949 (Diptera: *Glossinidae*) and *G. tachinoides* Westwood, 1850 are the vectors of trypanosomoses in this area. After a supervised classification, the community types were discriminated using the water area in 400-m-wide polygons around the river. A fragmentation analysis of the swamp forest unit, cross-tabulated with the community types, lead to identification of the final landscapes where tsetse apparent densities (ADT) were implemented using a training data set of 608 trap locations. The predicted ADT were then compared with an independent validation data set of 78 trap locations. The correlation between the model predictions and the validation data set was high, validating this approach ($p < 0.001$). The riverine forest community type and fragmentation level are critical factors for riverine tsetse species, which should be taken into consideration to map their suitable habitat.

14814. **Koffi, M., De Meeus, T., Bucheton, B., Solano, P., Camara, M., Kaba, D., Cuny, G., Ayala, F. J. & Jamonneau, V., 2009**. Population genetics of *Trypanosoma brucei gambiense,* the agent of sleeping sickness in Western Africa. *Proceedings of the National Academy of Sciences U S A,* **106** (1): 209-214.

Institut de Recherche pour le Développement, Unité Mixte de Recherche IRD-CIRAD 177, Campus International de Baillarguet, 34398 Montpellier, Cedex 5, France. [math_kof@yahoo.fr].

Human African trypanosomiasis, or sleeping sickness caused by *Trypanosoma brucei gambiense*, occurs in Western and Central Africa. *T. brucei s.l.* displays a huge diversity of adaptations and host specificities, and questions about its reproductive mode, dispersal abilities, and effective size remain under debate. We have investigated genetic variation at 8 microsatellite loci of *T. b. gambiense* strains isolated from human African trypanosomiasis patients in the Ivory Coast and Guinea, with the aim of knowing how genetic information was partitioned within and between individuals in both temporal and spatial scales. The results indicate that (i) migration of *T. b. gambiense* group 1 strains does not occur at the scale of West Africa, and that even at a finer scale (e.g., within Guinea) migration is restricted; (ii) effective population sizes of trypanosomes, as reflected by infected hosts, are probably higher than what the epidemiological surveys suggest; and (iii) *T. b. gambiense* group 1 is most likely a strictly clonally reproducing organism.

14815. **Solano, P., Ravel, S., Bouyer, J., Camara, M., Kagbadouno, M. S., Dyer, N., Gardes, L., Herault, D., Donnelly, M. J. & De Meeus, T., 2009.** The population structure of *Glossina palpalis gambiensis* from island and continental locations in coastal Guinea. *PLoS Neglected Tropical Diseases,* **3** (3), 392. **e-Publication ahead of print.**

CIRDES/IRD UMR 177 IRD-CIRAD, Bobo-Dioulasso, Burkina Faso.

We undertook a population genetics analysis of the tsetse fly *Glossina palpalis gambiensis*, a major vector of sleeping sickness in West Africa, using microsatellite and mitochondrial DNA markers. Our aims were to estimate effective population size and the degree of isolation between coastal sites on the mainland of Guinea and Loos Islands. The sampling locations encompassed Dubreka, the area with the highest human African

trypanosomosis (HAT) prevalence in West Africa, mangrove and savannah sites on the mainland, and two islands, Fotoba and Kassa, within the Loos archipelago. These data are discussed with respect to the feasibility and sustainability of control strategies in those sites currently experiencing, or at risk of, sleeping sickness. We found very low migration rates between sites except between those sampled around the Dubreka area that seems to contain a widely dispersed and panmictic population. In the Kassa island samples, various effective population size estimates all converged on surprisingly small values that suggest either a recent bottleneck, and/or other biological or ecological factors such as strong variance in the reproductive success of individuals. Whatever their origin, the small effective population sizes suggest high levels of inbreeding in tsetse flies within the island samples in marked contrast to the large diffuse deme in Dubreka zones. We discuss how these genetic results suggest that different tsetse control strategies should be applied on the mainland and islands.

14816. **Walshe, D. P., Lehane, S. M., Lehane, M. J. & Haines, L. R., 2009**. Prolonged gene knockdown in the tsetse fly *Glossina* by feeding double stranded RNA. *Insect Molecular Biology,* **18** (1): 11-19.

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Reverse genetic studies based on RNA interference (RNAi) have revolutionized analysis of gene function in most insects. However the necessity of injecting double stranded RNA (dsRNA) inevitably compromises many investigations particularly those on immunity. Additionally, injection of tsetse flies often causes significant mortality. We demonstrate, at transcript and protein level, that delivering dsRNA in the blood meal to *Glossina morsitans morsitans* is as effective as injection in knockdown of the immunoresponsive midgut-expressed gene, tsetseEP. However, feeding dsRNA fails to knockdown the fat body expressed transferrin gene, 2A192, previously shown to be silenced by dsRNA injection. Mortality rates of the dsRNA fed flies were significantly reduced compared to injected flies 14 days after treatment (fed: 10.1 percent+/- 1.8 percent; injected: 37.9 percent +/- 3.6 percent (mean +/- s.e.m). This is the first demonstration in Diptera of gene knockdown by feeding and the first example of knockdown in a blood-sucking insect by including dsRNA in the bloodmeal.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

14817. **Bouyer, J., Stachurski, F., Gouro, A. S. & Lancelot, R., 2009**. Control of bovine trypanosomosis by restricted application of insecticides to cattle using footbaths. *Veterinary Parasitology,* **161** (3-4): 187-193.

UMR Contrôle des maladies animales exotiques et émergentes, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (Cirad), Campus International de Baillarguet, 34398 Montpellier Cedex 5, France; Centre International de Recherche-Développement de l'Elevage en Zone Sub-humide (Cirdes), 01 BP 454, Bobo Dioulasso, Burkina Faso.

African animal trypanosomoses are the main parasitological constraints to livestock production in many sub-Saharan African countries infested with tsetse flies. A prospective survey was implemented in Dafinso (Burkina Faso) to assess the effect of deltamethrin 0.005 percent (Vectocid (ND), CEVA Santé Animale) impregnation of cattle on trypanosomes transmission in cattle. Two herds were involved in the survey. They were watered at two different waterpoints located on the same river harbouring a Guinean riparian forest infested with two different species of tsetse flies (Diptera: *Glossinidae*), *Glossina palpalis gambiensis* Vanderplank and *G. tachinoides* Westwood. Animals belonging to one of the herds were impregnated with deltamethrin applied with a footbath whereas the other herd was used as control. The overall incidence of cattle trypanosomoses was reduced ($p=0.01$) from 0.76 (control group) to 0.11 (footbath-treated group). A positive effect of the footbath treatment on packed-cell volume was observed ($p<0.001$). The conditions requested to use a footbath to prevent cattle trypanosomoses are discussed.

14818. **Sindato, C., Kimbita, E. N. & Kibona, S. N., 2008.** Factors influencing individual and community participation in the control of tsetse flies and human African trypanosomiasis in Urambo District, Tanzania. *Tanzanian Journal of Health Research*, **10** (1): 20-27.

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

This study was carried out to assess the knowledge and level of individual and community participation in the control of human African trypanosomiasis in Urambo District, western Tanzania. Semi structured questionnaires were used to collect information from individuals at household level. Retrospective data of HAT was sought from the medical officers-in-charge of health facilities. The results indicate that, 191 (90.5 percent, n = 211) individuals knew tsetse flies and 187 (88.6 percent, n = 211) knew HAT. All nine key informants reported that, the communities were aware of HAT while seven key informants reported that, the communities were aware of health risks associated with tsetse bites in human. There was poor knowledge about the role played by animals in the transmission of HAT (26.7 percent, n = 187). Majority of those who knew HAT (n = 187) were willing to contribute labour (70.1 percent) and money (64.2 percent) to tsetse and HAT control whereas amongst those who knew tsetse flies, 66.5 percent and 60.7 percent were willing to contribute labour and money, respectively. Amongst those who knew any HAT control technique (n = 108), 78.7 percent and 82.4 percent were willing to contribute money and labour respectively. A total of 454 cases of HAT were reported in the area from 1999 to 2006. It is concluded that, the factors influencing individual and community participation include the knowledge of tsetse, HAT and control measures.

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

[See also **32**:14803, 14811]

14819. **Bartlett-Healy, K., Crans, W. & Gaugler, R., 2008**. Vertebrate hosts and phylogenetic relationships of amphibian trypanosomes from a potential

invertebrate vector, *Culex territans* Walker (Diptera: *Culicidae*). *Journal of Parasitology,* **1**: **e-Publication ahead of print October 13.**

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The bloodmeals of field-collected female *Culex territans* (Diptera: *Culicidae*) were concurrently assayed for the presence of trypanosomes and for vertebrate host identification. We amplified vertebrate DNA in 42 of 119 females, and made positive identification to the host species level in 29 of those samples. Of the 119 field-collected *C. territans* females, 24 were infected with trypanosomes. Phylogenetic analysis placed the trypanosomes in the amphibian portion of the aquatic clade of the *Trypanosomatidae.* These trypanosomes were isolated from *C. territans* females that had fed on the frog species, *Rana clamitans, R. catesbeiana, R. virgatipes,* and *Rana* sp. Results support a potential new lineage of Dipteran transmitted amphibian trypanosomes may occur within the aquatic clade. The frequency in which female *C. territans* acquire trypanosomes, through diverse feeding habits, indicates a new relationship between amphibian trypanosomes and mosquitoes that has not been previously examined. Combining *Trypanosoma* species, invertebrate, and vertebrate hosts to existing phylogenies can elucidate trypanosome and host relationships.

14820. **Cortez, A. P., Rodrigues, A. C., Garcia, H. A., Neves, L., Batista, J. S., Bengaly, Z., Paiva, F. & Teixeira, M. M., 2009**. Cathepsin L-like genes of *Trypanosoma vivax* from Africa and South America-characterization, relationships and diagnostic implications. *Molecular and Cellular Probes,* **23** (1): 44-51.

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We characterized sequences from genes encoding cathepsin L-like (CatL-like) cysteine proteases from African and South American isolates of *Trypanosoma vivax* and *T. vivax*-like organisms, and evaluated their suitability as genetic markers for population structure analysis and diagnosis. Phylogenetic analysis of sequences corresponding to CatL-like catalytic domains revealed substantial polymorphism, and clades of sequences (*Tvi*CatL1-9) were separated by large genetic distances. *Tvi*CatL1-4 sequences were from cattle isolates from West Africa (Nigeria and Burkina Faso) and South America (Brazil and Venezuela), which belonged to the same *T. vivax* genotype. *T. vivax*-like genotypes from East Africa showed divergent sequences, including *Tvi*CatL5-7 for isolates from Mozambique and *Tvi*CatL8-9 for an isolate from Kenya. Phylogenetic analysis of CatL-like gene data supported the relationships among trypanosome species reflected in the phylogenies based on the analysis of small subunit (SSU) of ribosomal RNA gene sequence data. The discovery of different CatL-like sequences for each genotype, defined previously by ribosomal DNA data, indicate that these sequences provide useful targets for epidemiological and population genetic studies. Regions in CatL-like sequences shared by all *T. vivax* genotypes but not by other trypanosomes allowed the establishment of a specific and sensitive diagnostic PCR for epidemiological studies in South America and Africa.

14821. **Grebaut, P., Bena, J. M., Manzambi, E. Z., Mansinsa, P., Khande, V., Ollivier, G., Cuny, G. & Simo, G., 2009**. Characterization of sleeping sickness

transmission sites in rural and periurban areas of Kinshasa (Democratic Republic of Congo). *Vector Borne Zoonotic Diseases.* **e-Publication ahead of print March 9.**

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To characterize the potential transmission sites of sleeping sickness in Kinshasa, two entomologic surveys were carried out during the dry and the rainy seasons in rural and periurban areas of Kinshasa in 2005. About 610 pyramidal traps were set up, and 897 *Glossina fuscipes quanzensis* were captured. Environmental and biologic factors were reported, and relationships between these factors were evaluated using logistic regression and multiple correspondence analysis. The biologic factors (the presence of tsetse flies, human blood meals, and teneral flies) were progressively accumulated at each capture site to permit the characterization of the sleeping sickness transmission risk. The dry season was found to be a more favourable period for the disease transmission than the rainy season. Moreover, the landscapes characterized by the presence of argillaceous soils and raised ground cover with forest residues and rivers were identified as types of environments with greater risk of sleeping sickness transmission. Pig breeding appeared as an important factor increasing the disease transmission. If vector control is continuously performed along rivers segments at high risk, the transmission of sleeping sickness in rural and periurban areas of Kinshasa will considerably decrease.

14822. **Opara, M. N. & Fagbemi, B. O., 2008**. Haematological and plasma biochemistry of the adult wild African grasscutter (*Thryonomys swinderianus*). *Annals of the New York Academy of Sciences,* **1149**: 394-397.

Tropical Animal Health and Production, Department of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria. [oparamax@yahoo.com].

Haematological and plasma biochemical values of wild grasscutters were evaluated to determine their potential to transmit zoonotic pathogens. Three 5-mL blood samples were collected from each of 1 000 grasscutters caught in the wild for haematology, biochemical, and parasitological tests. Haematological and biochemical values were compared with those from captive-reared grasscutters. There are significantly ($p < 0.05$) higher lymphocyte, eosinophil, and basophil values for wild grasscutters compared to those that are captive reared. Parasitological examination revealed a 15 percent prevalence of blood protozoa in the wild grasscutters. Blood pathogens encountered were *Trypanosoma* sp. (66.7 percent) and *Plasmodium* sp. (33.3 percent), with 20.7 percent mixed infection. Sex does not significantly ($p > 0.05$) affect blood protozoa infection, while season does. We therefore concluded that wild grasscutters serve as efficient reservoir hosts for agents of African trypanosomiasis and malaria in the tropical humid rainforest region of Nigeria.

14823. **Rayaisse, J. B., Courtin, F., Akoundjin, M., Cesar, J. & Solano, P., 2009**. Influence of anthropisation on local vegetation and tsetse abundance in southern Burkina Faso. *Parasite,* **16** (1): 21-28.

Centre International de Recherche-Développement sur l'Elevage en zone Subhumide, 01 BP 454 Bobo Dioulasso 01, Burkina Faso.

Entomological and phyto-sociological surveys were undertaken in Folonzo, southern Burkina Faso, along the Comoe river. The purpose of this survey was to compare densities and diversity of tsetse species in a protected versus a non protected area, by the mean of transects going from the river bank to the savannah. A detailed phytological description was made in all the trapping sites. The entomological data were also compared to what was obtained in 1980 in the same trapping sites. The phytogeographical study showed great vegetation homogeneity between transects, particularly in the forest gallery, while savannah showed more heterogeneity. Four tsetse species were caught in the area, with 74 percent *G. tachinoides*, 20 percent *G. m. submorsitans*, 4 percent *G. p. gambiensis* and 2 percent *G. medicorum*. There was a significant difference in tsetse densities between the protected and the non-protected area, with in average, four times more tsetse in the protected one. This difference was particularly high for *G. m. submorsitans* with a ratio of 1:9. This decrease was attributed to the reduction in wildlife density in the non protected area, and can be applied to the situation of the whole country where this tsetse species is of decreasing importance. It is one of the consequences of the increase in human densities, this latter causing much less visible changes in phytological species composition. From the comparison between old (1980) and new data collected on the river bank, we see a general trend of decrease in density, which affects less *G. palpalis gambiensis.*

14824. **Rouamba, J., Jamonneau, V., Sidibe, I., Solano, P. & Courtin, F., 2009**. Impact of the dynamics of human settlement on tsetse and trypanosomosis distribution in the Mouhoun river basin (Burkina Faso). *Parasite,* **16** (1): 11-19.

Centre Muraz, 01 BP 390, Bobo-Dioulasso, Burkina Faso.

In Burkina Faso, the Mouhoun river basin (formerly "Black Volta") constitutes a historical focus of human (HAT) and animal (AAT) African trypanosomoses, both transmitted by tsetse flies. Nowadays, HAT seems to have disappeared from this area, while AAT still causes severe economic losses. In order to explain these different epidemiological situations, we undertook a geographical study based on the analysis of aerial pictures between 1952 and 2007, and field surveys to collect medical, entomological, and veterinary data on trypanosomoses. Our results suggest that in this area, landscapes have been dramatically modified as a consequence of population growth, and in turn have had an impact on the number and distribution of tsetse flies. Combined with the historical medical action on HAT which probably led to the disappearance of *T. b. gambiense*, this environmental degradation and the development of hydrological structures provide explanations for the local disappearance of HAT, and for the maintenance of AAT. It appears necessary to extrapolate these studies to other areas in order to identify the factors explaining the presence/absence of trypanosomoses in the context of human population growth and climatic changes, in order to help to target priority areas for the control of these diseases.

14825. **Rutto, J. J. & Karuga, J. W., 2009**. Temporal and spatial epidemiology of sleeping sickness and use of geographical information system (GIS) in Kenya. *Journal of Vector Borne Diseases,* **46** (1): 18-25.

Kenya Agricultural Research Institute-Trypanosomiasis Research Centre, Kikuyu, Kenya. [jjrutto@yahoo.co.uk].

In Kenya, sleeping sickness (SS) caused by *Trypanosoma brucei rhodesiense* is confined to the Nyanza and Western Provinces tsetse belts. Over the last two decades, the disease has exhibited great spatial variability in its spread and distribution. The objectives of the study were to map the spatial and temporal distribution of SS and determine possible risk factors associated with the disease in western Kenya. Geographical coordinates of villages were obtained using a Global Positioning System (GPS). SS data were analyzed retrospectively and the mapping of villages was done using MapInfo Software. Epidemiological data of villages affected by SS were then correlated to human and cattle population. The results showed that SS has spread northwards affecting the western parts of Busia, Teso, and of Bungoma districts in the late 1990s. Most of the SS cases were reported between March and June. The mainly affected age groups were from 20 to 49 years. SS was highest in areas with low human population density, ranging from 0-340/km^2 and high livestock population, ranging from 5 000 to 10 000 cattle. In conclusion, there was a shift of SS occurrence from the old foci into new foci occurring at low transmission levels and causing occasional epidemic outbreaks. The study concludes that seasons influenced disease incidences with higher numbers of SS cases being recorded during the wet seasons. Gender and age determined the disease occurrence with most productive age groups being at higher risk. Areas with high livestock populations had low human population densities and had higher SS cases.

14826. **Vassella, E., Oberle, M., Urwyler, S., Renggli, C. K., Studer, E., Hemphill, A., Fragoso, C., Butikofer, P., Brun, R. & Roditi, I., 2009**. Major surface glycoproteins of insect forms of *Trypanosoma brucei* are not essential for cyclical transmission by tsetse. *PLoS ONE,* **4** (2): e4493.

Institut für Zellbiologie, Universität Bern, Bern, Switzerland.

Procyclic forms of *Trypanosoma brucei* reside in the midgut of tsetse flies where they are covered by several million copies of glycosylphosphatidylinositol-anchored proteins known as procyclins. It has been proposed that procyclins protect parasites against proteases and/or participate in tropism, directing them from the midgut to the salivary glands. There are four different procyclin genes, each subject to elaborate levels of regulation. To determine if procyclins are essential for survival and transmission of *T. brucei*, all four genes were deleted and parasite fitness was compared *in vitro* and *in vivo*. When co-cultured *in vitro*, the null mutant and wild type trypanosomes (tagged with cyan fluorescent protein) maintained a near-constant equilibrium. In contrast, when flies were infected with the same mixture, the null mutant was rapidly overgrown in the midgut, reflecting a reduction in fitness *in vivo*. Although the null mutant is patently defective in competition with procyclin-positive parasites, on its own it can complete the life cycle and generate infectious metacyclic forms. The procyclic form of *T. brucei* thus differs strikingly from the bloodstream form, which does not tolerate any perturbation of its variant surface glycoprotein coat, and from other parasites such as *Plasmodium berghei*, which requires the circumsporozoite protein for successful transmission to a new host.

14827. **Wyatt, K. B., Campos, P. F., Gilbert, M. T., Kolokotronis, S. O., Hynes, W. H., DeSalle, R., Ball, S. J., Daszak, P., MacPhee, R. D. & Greenwood, A. D., 2008.** Historical mammal extinction on Christmas Island (Indian Ocean) correlates with introduced infectious disease. *PLoS ONE,* **3** (11): e3602.

Biological Sciences Department, Old Dominion University, Norfolk, VA, USA.

It is now widely accepted that novel infectious disease can be a leading cause of serious population decline and even outright extinction in some invertebrate and vertebrate groups (e.g., amphibians). In the case of mammals, however, there are still no well-corroborated instances of such diseases having caused or significantly contributed to the complete collapse of species. A case in point is the extinction of the endemic Christmas Island rat (*Rattus macleari*): although it has been argued that its disappearance ca. AD 1900 may have been partly or wholly caused by a pathogenic trypanosome carried by fleas hosted on recently-introduced black rats (*Rattus rattus*), no decisive evidence for this scenario has ever been adduced. Using ancient DNA methods on samples from museum specimens of these rodents collected during the extinction window (AD 1888-1908), we were able to resolve unambiguously sequence evidence of murid trypanosomes in both endemic and invasive rats. Importantly, endemic rats collected prior to the introduction of black rats were devoid of trypanosome signal. Hybridization between endemic and black rats was also previously hypothesized, but we found no evidence of this in examined specimens, and conclude that hybridization cannot account for the disappearance of the endemic species. This is the first molecular evidence for a pathogen emerging in a naive mammal species immediately prior to its final collapse.

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE

(b) PATHOLOGY AND IMMUNOLOGY

[See also **32**: 14805]

14828. **Blum, J. A., Schmid, C., Burri, C., Hatz, C., Olson, C., Fungula, B., Kazumba, L., Mangoni, P., Mbo, F., Deo, K., Mpanya, A., Dala, A., Franco, J. R., Pohlig, G. & Zellweger, M. J., 2009.** Cardiac alterations in human African trypanosomiasis (*T. b. gambiense*) with respect to the disease stage and antiparasitic treatment. *PLoS Neglected Tropical Diseases,* **3** (2): e 383.

Swiss Tropical Institute, Basel, Switzerland.

In human African trypanosomiasis, neurological symptoms dominate and cardiac involvement has been suggested. Because of increasing resistance to the available drugs for HAT, new compounds are desperately needed. Evaluation of cardiotoxicity is one parameter of drug safety, but without knowledge of the baseline heart involvement in HAT, cardiologic

findings and drug-induced alterations will be difficult to interpret. The aims of the study were to assess the frequency and characteristics of electrocardiographic findings in the first stage of HAT, to compare these findings to those of second stage patients and healthy controls and to assess any potential effects of different therapeutic antiparasitic compounds with respect to ECG changes after treatment. Four hundred and six patients with first stage HAT were recruited in the Democratic Republic of Congo, Angola and Sudan between 2002 and 2007 in a series of clinical trials comparing the efficacy and safety of the experimental treatment DB289 to the standard first stage treatment, pentamidine. These ECGs were compared to the ECGs of healthy volunteers (n = 61) and to those of second stage HAT patients (n = 56). In first and second stage HAT, a prolonged QTc interval, repolarization changes and low voltage were significantly more frequent than in healthy controls. Treatment in first stage was associated with repolarization changes in both the DB289 and the pentamidine group to a similar extent. The QTc interval did not change during treatment. On the basis of these results, cardiac involvement in HAT, as demonstrated by ECG alterations, appears early in the evolution of the disease. The prolongation of the QTc interval comprises a risk of fatal arrhythmias if new drugs with an additional potential of QTc prolongation will be used. During treatment ECG abnormalities such as repolarization changes consistent with peri-myocarditis occur frequently and appear to be associated with the disease stage, but not with a specific drug.

14829. **Checchi, F., Filipe, J. A., Barrett, M. P. & Chandramohan, D., 2008.** The natural progression of *gambiense* sleeping sickness: what is the evidence? *PLoS Neglected Tropical Diseases*, **2** (12): e303.

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK.

Gambiense human African trypanosomiasis (HAT, sleeping sickness) is widely assumed to be 100 percent pathogenic and fatal. However, reports to the contrary exist, and human trypano-tolerance has been postulated. Furthermore, there is uncertainty about the actual duration of both stage 1 and stage 2 infections, particularly with respect to how long a patient remains infectious. Understanding such basic parameters of HAT infection is essential for optimising control strategies based on case detection. We considered the potential existence and relevance of human trypanotolerance, and explored the duration of infectiousness, through a review of published evidence on the natural progression of *gambiense* HAT in the absence of treatment, and biological considerations. Published reports indicate that most *gambiense* HAT cases are fatal if untreated. Self-resolving and asymptomatic chronic infections probably constitute a minority if they do indeed exist. Chronic carriage, however, deserves further study, as it could seed renewed epidemics after control programmes cease.

14830. **Courtioux, B., Pervieux, L., Vatunga, G., Marin, B., Josenando, T., Jauberteau-Marchan, M. O., Bouteille, B. & Bisser, S., 2009.** Increased CXCL-13 levels in human African trypanosomiasis meningo-encephalitis. *Tropical Medicine and International Health*. **e Publication ahead of print, March 2.**

Institut de Neurologie Tropicale, Université de Limoges, Limoges, France.

To determine the role of the B-cell attracting chemokine CXCL-13, which may initiate B-cell trafficking and IgM production in diagnosing HAT meningo-encephalitis, we determined CXCL-13 levels by ELISA on paired sera and CSF of 26 patients from Angola and of 16 controls (six endemic and ten non-endemic). Results were compared to standard stage determination markers and IgM intrathecal synthesis. CXCL-13 levels in patients' sera had a median value of 386.6 pg/ml and increased levels were associated with presence of trypanosomes in the CSF but not with other stage markers. CXCL-13 levels in patients' CSF had a median value of 80.9 pg/ml and increased levels were associated with all standard stage determination markers and IgM intrathecal synthesis. In conclusion, CXCL-13 levels in CSF increased significantly during the course of HAT. Hence the value of CXCL-13 for diagnosis, follow-up or as a marker of disease severity should be tested in a well-defined cohort study.

14831. **Darby, J. D., Huber, M. G., Sieling, W. L. & Spelman, D. W., 2008**. African trypanosomiasis in two short-term Australian travelers to Malawi. *Journal of Travel Medicine,* **15** (5): 375-377.

Department of Infectious Diseases, The Alfred Hospital, Melbourne, Victoria, Australia.[jondarby76@yahoo.com.au].

We report two microbiologically confirmed cases of trypanosomiasis in short-term Australian travelers to Malawi. The initial diagnosis was followed by medical evacuation to South Africa where suramin therapy was commenced. The treatment course was completed on return to Australia, with subsequent follow-up. This diagnosis should be considered in travelers returning from an endemic region.

14832. **Fevre, E. M., Wissmann, B. V., Welburn, S. C. & Lutumba, P., 2008**. The burden of human African trypanosomiasis. *PLoS Neglected Tropical Diseases,* **2** (12): e333.

Centre for Infectious Diseases, University of Edinburgh, Ashworth Laboratories, Edinburgh, UK.

Human African trypanosomiasis (HAT, or sleeping sickness) is a protozoan parasitic infection caused by *Trypanosoma brucei rhodesiense* or *Trypanosoma brucei gambiense*. These are neglected tropical diseases, and *T. b. rhodesiense* HAT is a zoonosis. We review current knowledge on the burden of HAT in sub-Saharan Africa, with an emphasis on the disability-adjusted life year (DALY), data sources, and methodological issues relating to the use of this metric for assessing the burden of this disease. We highlight areas where data are lacking to properly quantify the impact of these diseases, mainly relating to quantifying under-reporting and disability associated with infection, and challenge the HAT research community to tackle the neglect in data gathering to enable better evidence-based assessments of burden using DALYs or other appropriate measures.

14833. **Hope-Rapp, E., Moussa Coulibaly, O., Klement, E., Danis, M., Bricaire, F. & Caumes, E., 2009**. Double trypanosomal chancre revealing West African trypanosomiasis in a Frenchman living in Gabon. *Annales de dermatologie et de vénéréologie,* **136** (4): 341-345.

Service de maladies infectieuses et tropicales, Hôpital Pitié-Salpêtrière, 47-83, Boulevard de l'Hôpital, 75651 Paris cedex 13, France. [milirapp@wanadoo.fr]

Human African trypanosomiasis (sleeping sickness), an endemic disease, is currently reemerging in Africa with an estimated incidence of 45 000 new cases per year. It is caused by *Trypanosoma brucei* subspecies and transmitted by day-biting tsetse flies. We report a case of West African trypanosomiasis due to *Trypanosoma brucei gambiense* involving a Frenchman living in Libreville, Gabon. The patient presented with fever and polyadenopathies as well as two skin ulcerations highly suggestive of trypanosomiasis. Microscopic examination of cutaneous and peripheral blood smears confirmed the diagnosis of haemolymphatic infection with *T. b. gambiense* with trypanosomal chancres. Examination of the cerebrospinal fluid was normal. The patient was successfully treated with pentamidine isethionate. It is concluded that recognition of cutaneous manifestations may allow a rapid diagnosis of African trypanosomiasis that is essential for timely and efficient treatment and survival.

14834. **Lun, Z. R., Reid, S. A., Lai, D. H. & Li, F. J., 2009**. Atypical human trypanosomiasis: a neglected disease or just an unlucky accident? *Trends in Parasitology,* **25** (3): 107-108.

Center for Parasitic Organisms, State Key Laboratory of Biocontrol, School of Life Sciences, and Key Laboratory of Tropical Diseases Control (the Ministry of Education), Sun Yat-Sen (Zhongshan) University, Guangzhou 510275, China.

No abstract available.

14835. **Pays, E. & Vanhollebeke, B., 2008**. The controversial story of the human trypanolytic factor. *Médecine sciences (Paris),* **24** (10): 792-793.

Laboratory of Molecular Parasitology, Université Libre de Bruxelles, Gosselies, Belgium. [epays@ulb.ac.be].

No abstract available

(c) TREATMENT

[See also: 14807]

14836. **Bukachi, S. A., Wandibba, S. & Nyamongo, I. K., 2009**. The treatment pathways followed by cases of human African trypanosomiasis in western Kenya and eastern Uganda. *Annals of Tropical Medicine and Parasitology,* **103** (3): 211-220.

Institute of Anthropology, Gender and African Studies, University of Nairobi, P.O. Box, 30197-00100, Nairobi, Kenya.

Although early diagnosis and treatment are key factors in the effective control of human African trypanosomiasis (HAT), many cases of the disease delay taking appropriate

action, leading to untold suffering. As a better understanding of treatment-seeking behaviour should help in identifying the obstacles to early diagnosis and effective treatment, the treatment pathways followed by 203 former HAT cases in western Kenya and eastern Uganda have recently been explored. About 86 percent of the HAT cases had utilized more than two different healthcare options before being correctly diagnosed for HAT, with about 70 percent each using more than three different health facilities. Only about 8 percent of the cases reported that they had been correctly diagnosed the first time they sought treatment. Just over half (51 percent) of the HAT cases had been symptomatic for >2 months before being correctly diagnosed for HAT, and such time lags in diagnosis contributed to 72 percent of the cases receiving their first appropriate treatment only in the late stage of the disease. The likelihood of a correct diagnosis increased with the time the case had been symptomatic. These observations indicate an urgent need to build the diagnostic capacity of the primary healthcare facilities in the study area, so that all HAT cases can be identified and treated in the early stage of the disease.

14837. **d'Alessandro, E., 2009**. Médecins Sans Frontières (MSF) and sleeping sickness control. From bush to international health space. *Bulletin de la Société de Pathologie Exotiqu*e (Paris)*,* **102** (1): 41-48.

CNRS UMR 6578 et Fondation MSF. [eugeniealessandro@hotmail.com]

In this article, we provide a history of the management of HAT by Médecins Sans Frontières since the 1980's. Through this, we highlight medical innovations in the field of diagnosis and treatment. MSF's efforts have been successfully invested in (1) epidemiological and clinical diagnosis, (2) evaluation of available drugs and (3) development of new treatment protocols. After working in isolation, MSF will have to collaborate with other international health organizations. Specific problems for medical practice and research in Southern countries should be the major challenges for medical innovations.

14838. **Mumba Ngoyi, D., Lejon, V., N'Siesi, F. X., Boelaert, M. & Buscher, P., 2009**. Comparison of operational criteria for treatment outcome in *gambiense* human African trypanosomiasis. *Tropical Medicine and International Health,* **14** (4): 438-444.

Institut National de Recherche Biomédicale, Avenue de la Démocratie, Kinshasa, Democratic Republic of the Congo.

The objective of this study was to develop a simple and standard operational decision tool for the diagnosis of relapse after treatment for human African trypanosomiasis (HAT), by evaluating the performance of several criteria currently used by HAT control programs and research projects. We identified 10 different criteria for relapse, based on trypanosome presence and/or white blood cell count in cerebrospinal fluid, and compared their specificity, sensitivity and time to diagnosis on a data set containing 63 relapsed and 247 cured *T. b. gambiense* patients. The results showed that at any time point, the criterion 'Trypanosomes present and/or a cerebrospinal white blood cell count > or =50/µL' allowed accurate and timely detection of HAT relapse, irrespective of disease stage. This criterion was 13-25 percent more sensitive ($p <$ or $= 0.013$) than trypanosome detection alone and was >97 percent specific. Lumbar punctures at the end of treatment and at 3-month post-treatment

provided limited clinical information. It was concluded that adequate detection of relapse was possible with a simple criterion but these findings should be validated in a prospective study before adoption in clinical practice.

14839. **Rodgers, J., 2009**. Human African trypanosomiasis, chemotherapy and CNS disease. *Journal of Neuroimmunology*. **Available on line 9 March.**

Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, Bearsden Road, Glasgow, G61 1QH, UK.

Trypanosomes have been recognised as human pathogens for over a century. Human African trypanosomiasis is endemic in an area sustaining 60 million people and is fatal without chemotherapeutic intervention. Available trypanocidal drugs require parenteral administration and are associated with adverse reactions including the development of a severe post-treatment reactive encephalopathy (PTRE). Following infection the parasites proliferate in the systemic compartment before invading the CNS where a cascade of events results in neuroinflammation. This review summarises the clinical manifestations of the infection and chemotherapeutic regimens as well as the current research findings and hypotheses regarding the neuropathogenesis of the disease.

14840. **Sindato, C., Kibona, S. N., Nkya, G. M., Mbilu, T. J., Manga, C., Kaboya, J. S. & Rawille, F., 2008**. Challenges in the diagnosis and management of sleeping sickness in Tanzania: a case report. *Tanzanian Journal of Health Research,* **10** (3): 177-181.

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

In Tanzania sleeping sickness presents a serious threat to human health with a country-wide average of 400 cases reported annually. Both wild and domestic animals have been found to play a significant role in the epidemiology of sleeping sickness. Serengeti National Park in northern Tanzania, has experienced a number of sleeping sickness epidemics since 1922. The epidemics were associated with abundant game animals in the areas and *Glossina swynnertoni* was incriminated as the main vector. However since 2001 there has been no case of sleeping sickness reported from the park. This case report highlights on the possibility of resurgence and challenges in the diagnosis and management of sleeping sickness in Serengeti. A 38 years old Tanzanian man working in the Serengeti National Park who had experienced various tsetse bites was presented with a febrile condition and history of unsuccessful case management at different health facilities. Blood and cerebrospinal fluid (CSF) samples were examined for the presence of trypanosomes using wet film, Field's stain and concentration techniques. *Trypanosoma brucei rhodesiense* were detected in both the blood and CSF samples. The patient was treated successfully with melarsoprol. The results of this case study highlight the possibility of resurgence of sleeping sickness in the park hence call for the need to create more awareness among the community and clinicians. There is need for early reporting to health facility and strengthening the diagnostic capacity of healthcare facilities in and around national parks endemic for sleeping sickness.

14841. **Tshimungu, K., Kalambayi, B. B., Kiyombo, M., Okenge, L. N. & Mol, P. D., 2008**. Knowledge, behaviours, practices and beliefs regarding human African trypanosomiasis (HAT) among inhabitants of Kinshasa (Democratic Republic of Congo). *Santé,* **18** (3): 141-147.

Laboratory of Medical Microbiology Faculty of Medicine University Hospital University of Liege, Kingdom of Belgium, Department of Epidemiology and Biostatistics Faculty of Medicine University Notre-Dame of Kasai Kananga Democratic Republic of Congo, School of Public Health Faculty of Medicine University of Kinshasa Democratic Republic of Congo, Demography Faculty of Economic Sciences University of Kinshasa Kinshasa Democratic Republic of Congo.

In Kinshasa, an average of less than 50 new cases of human African trypanosomiasis was notified, per year, between 1969 and 1995. The situation of endemic sleeping sickness suddenly worsened in 1996 with 254 new cases identified thanks to passive detection. No study dealing with conceptions relative to sleeping sickness was ever listed to date. The objective of this study was to determine the level of knowledge, behaviours, practices and local beliefs about sleeping sickness among residents of the endemic zone of Kinshasa. The investigation relied on a case/control study. We used a quantitative and qualitative methodology (structured questionnaire and focus on discussion groups). Case-patients were people affected by trypanosomiasis between the 1 January 2004 and the 31 December 2005 and who registered to the National Human African Trypanosomiasis Program (PNLTHA-RDC). Controls were sero negative residents. The case/control ratio was 1/2. A total of 437 case patients and 874 controls were included in the study. Level of knowledge of elementary concepts about trypanosomiasis was low among case-patients (44 percent). The proportion of participants with a low level of education was more important in the group of case-patients (40 percent) than in the control group (25.6 percent). The supernatural origin of trypanosomiasis was evoked such as divine, sorcery and transgression of rules. Many respondents (31.4 percent) call on churches for help when they are not satisfied with the health centre where first therapeutic aid is provided. An important proportion of people who participated to the study (87 percent) were in favour of a passive detection. After testing the degree of statistical significance, several variables appeared to be determining factors for the acquisition of knowledge of human African trypanosomiasis in the city of Kinshasa: education level (elevated: 81 percent, low: 19 percent; $p < 0.0001$), age (>/=20 years old: 89.9 percent, < 20 years old: 10.1 percent; $p < 0.0001$), sex (57.2 percent of patients were male and 42.8 percent were female; $p < 0.001$), birth place (51.4 percent were not native of Kinshasa and 48.6 percent were indigenous or born in Kinshasa; $p < 0.05$) and travel/stay in endemic areas (yes: 56.3 percent, no: 43.7 percent; $p < 0.0001$). The very restrained knowledge of people involves a generalized lack of interest. Their behaviour illustrates their lack of concern by the fight against trypanosomiasis. Beliefs and practices of Kinshasa's inhabitants (coming from their conceptions) also stand in the way of plans meant to fight the disease. It is necessary to improve the knowledge of preventive strategies and to fight social prejudice and false beliefs by informing and educating populations.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

[See also **32**: 14803]

14842. **Cordon-Obras, C., Berzosa, P., Ndong-Mabale, N., Bobuakasi, L., Buatiche, J. N., Ndongo-Asumu, P., Benito, A. & Cano, J., 2009**. *Trypanosoma brucei gambiense* in domestic livestock of Kogo and Mbini foci (Equatorial Guinea). *Tropical Medicine and International Health*. **e Publication ahead of print March 23**.

National Centre of Tropical Medicine, Institute of Health Carlos III, Madrid, Spain.

To evaluate *Trypanosoma brucei gambi*ense infection in peri-domestic livestock from Kogo and Mbini foci (Equatorial Guinea) in order to investigate its possible implication in the sleeping sickness transmission cycle in these hypoendemic foci, samples from 698 domestic animals (goats, sheep and pigs) from trypanosomiasis-endemic localities of Kogo and Mbini foci were tested for animal trypanosomes and *T. b. gambiense* (group I) by species-specific polymerase chain reaction. *Trypanosoma brucei s.l.,* the predominant trypanosome species, was detected in 182 (52.6 percent) samples from Mbini and in 127 (36.1 percent) samples from Kogo. *T. b. gambiense* was only identified in seven (2 percent) of the Mbini samples and one co-infection (with *T. vivax)* was observed. The occurrence of *T. b. gambiense* in peri-domestic livestock in Mbini and its absence in Kogo could explain the epidemiological differences between the two foci and could have significant implications for sleeping sickness control in Equatorial Guinea.

14843. **Enwezor, F. N., Umoh, J. U., Esievo, K. A., Halid, I., Zaria, L. T. & Anere, J. I., 2009**. Survey of bovine trypanosomosis in the Kachia grazing reserve, Kaduna State, Nigeria. *Veterinary Parasitology,* **159** (2): 121-125.

Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), P. M. B. 2077, Kaduna, Kaduna State, Nigeria. [feliciaenwezor@yahoo.com].

This study assessed the prevalence of trypanosomes in cattle at the Kachia grazing reserve (KGR) in March and June 2004 and in February 2005. A total of 1 293 cattle blood samples were collected at random. The samples were analysed using the buffy coat technique and Giemsa thin blood films for parasite detection and identification. The effects of herd pen location to watering and grazing point's distances (using the global positioning system (GPS)) were determined and the mean packed cell volume (PCV) assessed. Overall, the detected prevalence of trypanosomosis was 8.4 percent, much higher than the previous prevalence of 5.3 percent before the present study was conducted. The prevalences in the months of March, June (2004) and February (2005) were 2.3 percent, 11.6 percent and 15.4 percent, respectively. Increased prevalence was associated with proximity of herd pens to watering point's distances (chi^2 for linear trend=4.447, $P<0.05$), but no association of herd pens to

grazing point distances (chi^2=2.186, P>0.05); suggesting that hydrological network played an important part in trypanosomosis transmission. The mean PCV of parasitaemic and aparasitaemic cattle were respectively 25.99+/-1.82 percent and 29.31+/-1.70 percent. The drop in mean PCV was most in 0-1-year age group, 23.47+/-3.10 percent and was statistically significant (P<0.05), suggesting that anaemia was most pronounced in this age group. Factors that may have contributed to the increased prevalence obtained were collapse of control measures and breed susceptibility. Since Zebu cattle were the predominant breeds in the reserve, the study advocates effective use of insecticide impregnated screens (traps and targets) with community participation in mind for sustainability. If government intervenes through PATTEC, ground spraying of insecticides in the reserve is recommended. In addition, chemotherapy and chemoprophylaxis should be systematically used to fight the problem of trypanosomosis in the KGR towards improved livestock production.

14844. **Ezeani, M. C., Okoro, H., Anosa, V. O., Onyenekwe, C. C., Meludu, S. C., Dioka, C. E. & Azikiwe, C. C., 2008**. Immunodiagnosis of bovine trypanosomiasis in Anambra and Imo states, Nigeria, using enzyme-linked immunosorbent assay: zoonotic implications to human health. *Journal of Vector Borne Diseases,* **45** (4): 292-300.

Department of Immunology, College of Health Sciences, University of Ibadan, Oyo State. Nigeria [mikezeani@yahoo.com].

The prevalence of trypanosomiasis was studied in cattle, being a major source of animal protein in Nigeria and thus a very likely means of spread of human African trypanosomosis (HAT). Enzyme-linked immunosorbent assay (ELISA) was used to diagnose bovine trypanosomiasis in 264 samples collected from adult cattle of mixed breeds, age and sex, in Anambra and Imo States, Nigeria. Out of 264 samples analysed, 21 (7.96 percent) were seropositive for *Trypanosoma congolense* while 20 (7.58 percent) were seropositive for *T. vivax* and 8 (3.03 percent) were seropositive for *T. brucei* infections in both the states. Thus, the predominant species was found to be *T. congolense*. Mixed infection of three species, *T. vivax, T. congolense* and *T. brucei* were found to dominate other mixed infections in both the states. ELISA detected the infection of the three species of trypanosomes in the same group of animals. The usefulness of antigen capture ELISA in the diagnosis of human or animal trypanosomiasis was established, and the possibility of the spread of HAT caused by *T. brucei gambiense* and *T. b. rhodesiense* through cattle was expressed.

14845. **Mamabolo, M. V., Ntantiso, L., Latif, A. & Majiwa, P. A., 2009**. Natural infection of cattle and tsetse flies in South Africa with two genotypic groups of *Trypanosoma congolense. Parasitology,* **136** (4): 425-431.

ARC-Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort 0110, South Africa.

The polymerase chain reaction was used to detect trypanosomes in samples collected from cattle, wild animals and tsetse flies in KwaZulu-Natal Province, South Africa. A total of 673 samples from cattle and 266 from tsetse flies in the study area located near the Hluhluwe-Umfolozi game reserve were analysed. Both *Trypanosoma congolense* and *T. vivax* were found as single or mixed infections in cattle and tsetse flies. Moreover, the *T. congolense* in

the infections were found to comprise two genotypic groups: the Savannah-type and the Kilifi-type, which were present either as single or mixed infections in cattle and in tsetse flies.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **32**: 114805]

14846. **Berlin, D., Loeb, E. & Baneth, G., 2009**. Disseminated central nervous system disease caused by *Trypanosoma evansi* in a horse. *Veterinary Parasitology,* **161** (3-4): 316-319.

School of Veterinary Medicine, The Hebrew University, P.O. Box 12, Rehovot 76100, Israel.

Trypanosomiasis caused by *Trypanosoma evansi* ("Surra") is mainly a wasting disease affecting equids, camels and cattle as well as other domestic and wild animal species. In horses, infection may cause severe neurological abnormalities; however, the clinical progression, pathogenesis and molecular ante-mortem detection of this form of the disease have not been described in detail. A mare with progressive ataxia, head tilt, nystagmus and cranial nerve deficits submitted to treatment was diagnosed with central nervous system trypanosomiasis following the detection of a *Trypanosoma* trypomastigote in cerebrospinal fluid cytology. Histopathology following necropsy showed that the brain, spinal cord and kidneys were the main affected tissues with disseminated multifocal non-suppurative meningoencephalitis of the central nervous system and membranoproliferative glomerulonephritis. Serology for *T. evansi* was positive and PCR indicated the presence of parasite DNA in the cerebellum, brain stem, spinal cord and bone marrow but not in other organs and confirmed the identity of causative agent as *T. evansi.* This is the first report of ante-mortem detection of *T. evansi* in the cerebrospinal fluid of a horse and the first description of post-mortem PCR identification of the parasite DNA in the nervous system.

14847. **Mbaya, A. W., Aliyu, M. M. & Ibrahim, U. I., 2009**. The clinico-pathology and mechanisms of trypanosomosis in captive and free-living wild animals: a review. *Veterinary Research Communications*. **e Publication ahead of print, April 2.**

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Reports on the clinico-pathology and mechanisms of trypanosomosis in free-living and captive wild animals showed that clinical disease and outbreaks occur more commonly among captive than free-living wild animals. This is because the free-living wild animals co-exist with the disease until subjected to captivity. In exceptional cases however, drought, starvation and intercurrent diseases often compromised trypanotolerance leading to overt trypanosomosis in free-living wild animals. Meanwhile, in captivity, space restriction, reduced social interactions, change in social herd structure, reduced species-to-species specific behaviours, altered habitat and translocation were the major stressors that precipitated the disease. The cumulative effect of these factors produced severe physiological and somatic stress leading to diminished immune response due to increased blood cortisol output from adrenal cortex. The major symptoms manifested were pyrexia, inapetence,

increased respiration, anaemia, cachexia and death. At necropsy, pulmonary oedema, splenomegaly, hepatomegaly, lympadenopathy and atrophy of body fats were the gross changes encountered. At the ultra-structural level, the tissues manifested degenerative changes, haemorrhages, necrosis and mononuclear cellular infiltrations. The mechanisms of cellular and tissue injuries were primarily associated with physical and metabolic activities of the organisms. From the foregoing, it is evident that stress is the underlying mechanism that compromises trypanotolerance in wild animals leading to severe clinico-pathological effects.

14848. **Saleh, M. A., Al-Salahy, M. B. & Sanousi, S. A., 2009**. Oxidative stress in blood of camels (*Camelus dromedaries*) naturally infected with *Trypanosoma evansi*. *Veterinary Parasitology,* **162** (3-4): 192-199.

Biochemistry Unit, Regional Animal Health Research Laboratory, Animal Health Research Institute, El-Kharga, El-Wadi El-Gadid 725211, Egypt.

Oxidative stress is an imbalance between radical-generating and radical-scavenging activity, resulting in oxidation products and tissue damage. The present study aimed to estimate oxidation and antioxidant status in blood of camels naturally infected with *Trypanosoma evansi*. Blood samples from *T. evansi*-infected and healthy (control) female camels were used to determine the free radical nitric oxide (NO) generation in serum, malondialdehyde production in serum (sMDA) and in erythrocytes (eMDA) as a biomarker of lipid peroxidation, blood methaemoglobin formation (MetHb, a biomarker of haemoglobin oxidation), the antioxidants serum ascorbate and albumin levels, erythrocytic glutathione concentration (GSH), superoxide dismutase (SOD) and catalase (CAT) activities. The infected camels were characterized by macrocytic hypochromic anemia. Trypanosomiasis in camels resulted in significant ($p<0.001$) stimulation of serum NO (78.93 percent), eMDA (110.04 percent), sMDA (67.39 percent) and MetHb (1.5-fold) coupled with significant reduction ($p<0.001$) of albumin (27.6 percent), ascorbate (25.38 percent), GSH (43.36 percent), SOD (32.47 percent) and non-significant increase in CAT (7.06 percent, $p=0.322$) compared to control values. In infected camels, a significant positive correlation of NO with eMDA ($r=0.546$, $p=0.009$) and MetHb ($r=0.490$, $p=0.021$) was detected. By contrast, NO was inversely correlated with RBC ($r=-0.546$, $P=0.009$), PCV ($r=-0.427$, $p=0.048$) and Hb ($r=-0.612$, $p=0.002$). On the other hand, eMDA was inversely correlated with RBC ($r=-0.596$, $P=0.003$), PCV ($r=-0.516$, $p=0.014$) and Hb ($r=-0.613$, $p = 0.002$). In addition, methaemoglobinemia was negatively correlated with RBC ($r=-0.560$, $p=0.007$), PCV ($r=-0.470$, $p=0.027$) and Hb ($r=-0.585$, $p=0.004$). Our results suggest that chronic *T. evansi* infection in camels is associated with a state of oxidative process.

14849. **Villa, A., Gutierrez, C., Gracia, E., Moreno, B., Chacon, G., Sanz, P. V., Buscher, P. & Touratier, L., 2008**. Presence of *Trypanosoma theileri* in Spanish cattle. *Annals of the New York Academy of Sciences,* **1149**: 352-354.

Exopol Laboratory, Zaragoza, Spain.

Trypanosoma theileri (Laveran, 1902) has been diagnosed in many countries and is commonly considered as a nonpathogenic haemoparasite, although some authors have described clinical signs in cattle infected with *T. theileri*. In April and May, 2005, 12 blood samples were received at the Exopol Diagnostic Laboratory (Zaragoza, Spain) from a

Spanish bull-fighting farm located at Seville province. Clinical exploration of the animals revealed fever, progressive weight loss, anaemia, and frequent recumbent position. Microscopic examination showed *Theileria* spp. in all cases (12), and in four of them, *T. theileri* was also observed. The clinical picture observed in the animals could be compatible with *T. theileri* infection. However, the contribution of *T. theileri* to the clinical signs seen at least in four cases is unknown. Further studies are necessary to determine the pathogenicity of *T. theileri* in the different animal species. To our knowledge, this is the first isolation of *T. theileri* in Spain.

14850. **Watier-Grillot, S., 2008**. Outbreak of animal trypanosomiasis (*T. evansi*) in the Aveyron department of France: risk for implantation of an animal disease with zoonotic potential. *Médecine Tropicale (Mars),* **68** (5): 468-470.

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No abstract available.

14851. **Welburn, S., Picozzi, K., Coleman, P. G. & Packer, C., 2008**. Patterns in age-seroprevalence consistent with acquired immunity against *Trypanosoma brucei* in Serengeti lions. *PLoS Neglected Tropical Diseases,* **2** (12): e347.

Centre for Infectious Disease, College of Medicine and Veterinary Medicine, The University of Edinburgh, Edinburgh, UK.

Trypanosomes cause disease in humans and livestock throughout sub-Saharan Africa. Although various species show evidence of clinical tolerance to trypanosomes, until now there has been no evidence of acquired immunity to natural infections. We discovered a distinct peak and decrease in age prevalence of *T. brucei s.l.* infection in wild African lions that is consistent with being driven by an exposure-dependent increase in cross-immunity following infections with the more genetically diverse species, *T. congolense sensu lato*. The causative agent of human sleeping sickness, *T. brucei rhodesiense*, disappears by 6 years of age apparently in response to cross-immunity from other trypanosomes, including the non-pathogenic subspecies, *T. brucei brucei*. These findings may suggest novel pathways for vaccinations against trypanosomiasis despite the notoriously complex antigenic surface proteins in these parasites.

(c) TRYPANOTOLERANCE

[See also **32**: 14802]

14852. **Berthier, D., Chantal, I., Thevenon, S., Sakande, H., Maillard, J. C., Bengaly, Z., Piquemal, D., Marti, J. & Cuny, G., 2008**. Study of bovine trypanotolerance by whole transcriptome analysis. *Annals of the New York Academy of Sciences,* **1149**: 71-76.

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African trypanosomiases are parasitic diseases transmitted by tse-tse flies, considered as the main sanitary obstacle to animal production development in sub-Saharan Africa. However, if trypanosomiases have dramatic consequences on zebu (*Bos indicus*) populations, they have a weaker impact on the western African taurine (*Bos taurus*), which is known to be naturally tolerant to trypanosome infection. Mechanisms governing this trypanotolerant trait are still poorly understood, but today, recent postgenomic biotechnologies, such as the SAGE technique (serial analysis of gene expression) allow us to explore the full transcriptome. Twelve SAGE libraries were constructed from two trypanotolerant animals (N'Dama and Baoulé) and one susceptible species of cattle (the Sudanese zebu) during an experimental *Trypanosoma congolense* infection; 43 458 different tags were obtained at several particular points during the infection (before infection, at the maximum of parasitemia, the maximum of anemia, and at the end of the experiment after value normalization). Bioinformatics analyses highlighted some interesting gene variations with respect to the trypanotolerance status of the animal.

14853. **Dayo, G. K., Thevenon, S., Berthier, D., Moazami-Goudarzi, K., Denis, C., Cuny, G., Eggen, A. & Gautier, M., 2009**. Detection of selection signatures within candidate regions underlying trypanotolerance in outbred cattle populations. *Molecular Ecology,* **18** (8): 1801-1813.

Institut de Recherche pour le Développement, Unité Mixte de Recherche Trypanosomes, TA A-17/A Campus international de Baillarguet, Montpellier cedex 5, France.

Breeding indigenous African taurine cattle tolerant to trypanosomosis is a straightforward approach to control costs generated by this disease. A recent study identified quantitative trait loci (QTL) underlying trypanotolerance traits in experimental crosses between tolerant N'Dama and susceptible Boran zebu cattle. As trypanotolerance is thought to result from local adaptation of indigenous cattle breeds, we propose an alternative and complementary approach to study the genetic architecture of this trait, based on the identification of selection signatures within QTL or candidate genes. A panel of 92 microsatellite markers was genotyped on 509 cattle belonging to four West African trypanotolerant taurine breeds and 10 trypanosusceptible European or African cattle breeds. Some of these markers were located within previously identified QTL regions or candidate genes, while others were chosen in regions assumed to be neutral. A detailed analysis of the genetic structure of these different breeds was carried out to confirm *a priori* grouping of populations based on previous data. Tests based on the comparison of the observed heterozygosities and variances in microsatellite allelic size among trypanotolerant and trypanosusceptible breeds led to the identification of two significantly less variable microsatellite markers. BM4440, one of these two outlier loci, is located within the confidence interval of a previously described QTL underlying a trypanotolerance-related trait. Detection of selection signatures appears to be a straightforward approach for unravelling the molecular determinism of trypanosomosis pathogenesis. We expect that a whole genome approach will help confirm these results and achieve a higher resolving power.

14854. **Geerts, S., Osaer, S., Goossens, B. & Faye, D., 2009**. Trypanotolerance in small ruminants of sub-Saharan Africa. *Trends in Parasitology,* **25** (3): 132-138.

Institute of Tropical Medicine, Animal Health Department, Nationalestraat 155, B-2000 Antwerp, Belgium; International Trypanotolerance Centre, PMB 14, Banjul, The Gambia.

Although a lot of information is currently available on trypanotolerance in cattle, until recently the trypanotolerant nature of small ruminants was not well known. Trypanotolerance in small ruminants is less pronounced than in cattle and should be considered as resilience rather than resistance. West African Dwarf (WAD) goats seem to be less trypanotolerant than Djallonké sheep. However, recent studies have shown that there is an important introgression of genes of trypanosusceptible breeds into WAD goat populations, which possibly explains the loss of trypanotolerance in these animals. Measures need to be taken to safeguard and upgrade the genetic purity of trypanotolerant goat and sheep breeds in Africa.

14855. **Stein, J., Ayalew, W., Rege, J. E., Mulatu, W., Malmfors, B., Dessie, T. & Philipsson, J., 2009**. Livestock keeper perceptions of four indigenous cattle breeds in tsetse infested areas of Ethiopia. *Tropical Animal Health and Production*. **e Publication ahead of print, February 23.**

Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, P.O. Box 7023, 750 07, Uppsala, Sweden, [jennie.stein@hgen.slu.se].

Four cattle breeds indigenous to western and south-western Ethiopia - Abigar, Gurage, Horro and Sheko - were included in a study of the perceptions of smallholder cattle keepers regarding cattle management, production levels and constraints for production. A semi-structured questionnaire was used and 60 cattle keepers from each of the four areas were interviewed. Diseases were reported as the main constraint to cattle production by a majority of livestock keepers in all areas except in the Sheko area, where over-stocking was the main constraint. Among diseases, trypanosomosis was the main livestock disease according to more than half of Gurage, Horro and Sheko keepers, whereas anthrax was most important in the Abigar area. Gurage had highest age at first calving, longest calving interval and also the lowest milk production, whereas Sheko and Abigar had the most favourable characteristics both for milk production (600-700 kg) and fertility (age at first mating 3-3.5 years and above 8 calves/cow). Cattle keepers in the Sheko area reported relatively less problems with cattle diseases compared to the other areas, especially regarding trypanosomosis. Abigar showed a different disease pattern than the other breeds and may also have advantages as regards trypanotolerance.

(d) TREATMENT

14856. **Gutierrez, C., Corbera, J. A., Bayou, K. & van Gool, F., 2008**. Use of cymelarsan in goats chronically infected with *Trypanosoma evansi*. *Annals of the New York Academy of Sciences,* **1149**: 331-333.

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Toxicity and therapeutic trials using Cymelarsan (an arsenical compound) against *Trypanosoma evansi* infection were carried out using chronically infected goats. For the toxicity trial, 40 goats were divided into four groups of 10 animals each; the first three groups received subcutaneous injections of 5, 10, and 15 mg/kg bw of Cymelarsan, respectively, and the last one served as control. No systemic reaction was observed in any goat throughout the experiment. For the therapeutic trial, 15 adult female goats were inoculated intravenously with at least 1 x 10^5 *T. evansi* isolated in the Canary Islands. Six months after inoculation, the animals were treated with Cymelarsan at single dose of 0.3 mg/kg (5 animals), 0.5 mg/kg (5 animals), and 0.625 mg/kg (5 animals). At 4 and 6 weeks after treatment, two goats belonging to 0.3 mg/kg group showed recurrence of trypanosomes. Parasitaemia, however, was negative in all animals belonging to 0.5 and 0.625 mg/kg groups until the end of the experiment (6 months after treatment). Thus, it can be concluded that Cymelarsan is a safe trypanocidal drug for goats and that the curative dose is 0.5 mg/kg or above.

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

[See also **32**: 14820]

14857. **Chiliza, T. E., Van Wyngaardt, W. & Du Plessis, D. H., 2008**. Single-chain antibody fragments from a display library derived from chickens immunized with a mixture of parasite and viral antigens. *Hybridoma,* **27** (6): 413-421.

Immunology Section, ARC-Onderstepoort Veterinary Research Institute, Onderstepoort, Republic of South Africa.

Phage-displayed chicken single-chain antibody fragment libraries can provide useful diagnostic and research reagents. Using avian immunoglobulin genes simplifies the construction of such repertoires since far fewer primer sets are required to access the avian antibody repertoire than is the case with mice or humans. Libraries constructed using mRNA from an immune source are enriched in affinity-matured sequences and consequently need not be as large as "universal" non-immune repertoires to have a reasonable probability of yielding high-affinity binders. Repertoires focused on a number of defined targets can be constructed using lymphocyte mRNA from chickens immunized with a mixture of several

different antigens. This approach was evaluated with the aim of economically and rapidly deriving immunodiagnostic reagents for malaria, trypanosomiasis, and malignant catarrhal fever, all of which are important to health or food security in Africa. Two chickens were each immunized with a mixture comprised of recombinantly expressed histidine-rich protein, the aldolase and the lactate dehydrogenase of *Plasmodium falciparum*, the variant surface glycoprotein of *Trypanosoma* sp., and purified malignant catarrhal fever virus, a herpesvirus that causes an economically important disease of cattle and other ruminants. Immune responses to each of the individual antigens were determined by extracting egg-yolk IgY and testing for antigen-specific antibodies in ELISA. The chicken splenocytes were then recovered, RNA was extracted, and after reverse transcription, the immunoglobulin VH and VL regions were amplified by PCR and joined via a single glycyl residue for surface expression on a collection of filamentous bacteriophages. The resulting display library was then screened by panning to isolate binders. The immunized chickens did not, however, respond equally well to all the different antigens, nor was it possible to derive antibody fragments against all the targets. These limitations notwithstanding, several useful binders with the potential to be used in malaria diagnosis were obtained.

14858. **Thumbi, S. M., McOdimba, F. A., Mosi, R. O. & Jung'a, J. O., 2008**. Comparative evaluation of three PCR base diagnostic assays for the detection of pathogenic trypanosomes in cattle blood. *Parasites and Vectors,* **1** (1): 46.

Department of Animal Production, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053, Nairobi, Kenya. [owinojj@hotmail.com].

Currently, several PCR based diagnostic assays have been developed to improve the detection of pathogenic trypanosomes. These tests include use of species specific primers, single and nested PCRs based on primers amplifying the internal transcribed spacer (ITS) regions of ribosomal DNA. This study compares three PCR based diagnostic assays and assesses the agreement of these three assays by screening 103 cattle blood samples randomly collected from trypanosome endemic areas in western Kenya. The nested ITS based PCR, the single ITS based PCR and the species specific based PCR detected 28.1 percent, 26.2 percent and 10.7 percent of the samples respectively as positive for trypanosome infection. Nested ITS and single ITS PCRs picked 3.8 percent and 1.9 percent as mixed infections respectively. Cohen kappa statistic used to compare agreements beyond chance between the assays showed highest degree of agreement (0.6) between the two ITS based tests, and the lowest (0.2) between the nested PCR test and the species specific PCR. The single ITS and nested ITS based diagnostic assays detected higher numbers of positive cases, and reduced the number of PCR reactions per sample to one and two respectively, compared to the five PCR reactions carried out using the species specific primers. This significantly reduced the labour, time and the cost of carrying out PCR tests, indicating the superiority of the ITS multi-species detection techniques. Reliable epidemiological studies are a prerequisite to designing effective tsetse and trypanosomiasis control programmes. The present study established the suitability of using ITS based PCR assays for large-scale epidemiological studies.

14859. **Tran, T., Claes, F., Verloo, D., De Greve, H. & Buscher, P., 2009.** Towards a new reference test for surra in camels. *Clinical and Vaccine Immunology*. **e Publication ahead of print, April 29.**

Department of Parasitology, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium; Department of Molecular and Cellular Interactions, VIB, Brussels, Belgium; Laboratory of Molecular and Cellular Immunology, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium; Department of Biosystems, Katholieke Universiteit Leuven, Kasteelpark Arenberg 30 - bus 2456, B-3001 Heverlee, Belgium; Department of Scientific Cooperation and Assistance, European Food Safety Authority, Largo N. Palli 5/A, I-43100 Parma, Italy.

Current serological diagnosis of *Trypanosoma evansi* infection in camels is based on the native variable antigen type RoTat 1.2. The goal of this study is to develop a novel serological diagnostic test based on a non-variable protein and freed from the use of rats or mice for its production. An enzyme-linked immunosorbent assay using recombinant extracellular domain of invariant surface glycoprotein 75 (ELISA/ISG75) was developed and tested on a collection of 184 camel sera. The results were compared to those obtained from three established antibody detection tests based on variable surface glycoprotein RoTat 1.2, *in casu* ELISA/*T. evansi*, card agglutination test for trypanosomiasis (CATT/*T. evansi*) and immune trypanolysis assay (TL). ELISA/ISG75 and ELISA/*T. evansi* showed a sensitivity of 94.6 percent (95 percent confidence interval, CI, 87.8 - 98.2, at 19 percent positivity (PP) cut-off value) and 98.9 percent (95 percent CI 94.1 - 99.8, at 12 PP cut-off value) respectively. ELISA/ISG75 has 100 percent specificity (CI 95.9 - 100), while ELISA/*T. evansi* showed 98.9 percent specificity (CI 95.9 - 100). ELISA/ISG75 demonstrates an almost perfect agreement with TL, CATT/*T. evansi*, and ELISA/*T. evansi*, with Kappa scores of at least 0.94. The ELISA/ISG75 having a comparable performance as the gold standard (TL) and being independent of antigenic variation may become a new reference test for surra in camels. It opens avenues for diagnosis of *T. evansi* infections in other hosts as well as the development of a pan-*Trypanozoon* test for detection of *T. b. brucei, T. b. gambiense, T. b. rhodesiense, T. evansi* and *T. equiperdum*.

(b) PATHOLOGY AND IMMUNOLOGY

14860. **Boothroyd, C. E., Dreesen, O., Leonova, T., Ly, K. I., Figueiredo, L. M., Cross, G. A. & Papavasiliou, F. N., 2009**. A yeast-endonuclease-generated DNA break induces antigenic switching in *Trypanosoma brucei*. *Nature,* **459***:* 278-281.

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Trypanosoma brucei is the causative agent of African sleeping sickness in humans and one of the causes of nagana in cattle. This protozoan parasite evades the host immune

system by antigenic variation, a periodic switching of its variant surface glycoprotein (VSG) coat. VSG switching is spontaneous and occurs at a rate of about 10^{-2}-10^{-3} per population doubling in recent isolates from nature, but at a markedly reduced rate (10^{-5}-10^{-6}) in laboratory-adapted strains. VSG switching is thought to occur predominantly through gene conversion, a form of homologous recombination initiated by a DNA lesion that is used by other pathogens (for example, *Candida albicans, Borrelia* sp. and *Neisseria gonorrhoeae*) to generate surface protein diversity, and by B lymphocytes of the vertebrate immune system to generate antibody diversity. Very little is known about the molecular mechanism of VSG switching in *T. brucei*. Here we demonstrate that the introduction of a DNA double-stranded break (DSB) adjacent to the approximately 70-base-pair (bp) repeats upstream of the transcribed VSG gene increases switching *in vitro* approximately 250-fold, producing switched clones with a frequency and features similar to those generated early in an infection. We were also able to detect spontaneous DSBs within the 70-bp repeats upstream of the actively transcribed VSG gene, indicating that a DSB is a natural intermediate of VSG gene conversion and that VSG switching is the result of the resolution of this DSB by break-induced replication.

14861. **Chessler, A. D., Ferreira, L. R., Chang, T. H., Fitzgerald, K. A. & Burleigh, B. A., 2008**. A novel IFN regulatory factor 3-dependent pathway activated by trypanosomes triggers IFN-beta in macrophages and fibroblasts. *Journal of Immunology,* **181** (11): 7917-7924.

Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA 02115, USA.

Innate immune recognition of intracellular pathogens involves both extracellular and cytosolic surveillance mechanisms. The intracellular protozoan parasite *Trypanosoma cruzi* triggers a robust type 1 IFN response in both immune and non-immune cell types. In this study, we report that signalling through TBK1 and IFN regulatory factor 3 is required for *T. cruzi*-mediated expression of IFN-beta. The TLR adaptors MyD88 and TRIF, as well as TLR4 and TLR3, were found to be dispensable, demonstrating that *T. cruzi* induces IFN-beta expression in a TLR-independent manner. The potential role for cytosolic dsRNA sensing pathways acting through RIG-I and MDA5 was ruled out because *T. cruzi* was shown to trigger robust expression of IFN-beta in macrophages lacking the MAVS/IPS1/VISA/CARDif adaptor protein. The failure of *T. cruzi* to activate HEK293-IFN-beta-luciferase cells, which are highly sensitive to cytosolic triggers of IFN-beta expression including *Listeria, Sendai* virus, and transfected dsRNA and dsDNA, further indicates that the parasite does not engage currently recognized cytosolic surveillance pathways. Together, these findings identify the existence of a novel TLR-independent pathogen-sensing mechanism in immune and nonimmune cells that converges on TBK1 and IFN regulatory factor 3 for activation of IFN-beta gene expression.

14862. **Delgado, M., Anderson, P., Garcia-Salcedo, J. A., Caro, M. & Gonzalez-Rey, E., 2009**. Neuropeptides kill African trypanosomes by targeting intracellular compartments and inducing autophagic-like cell death. *Cell Death and Differentiation,* **16** (3): 406-416.

Instituto de Parasitologia y Biomedicina Lopez-Neyra, CSIC, Granada, Spain.

Trypanosoma brucei is the causative agent of African sleeping sickness. Available treatments are ineffective, toxic and susceptible to resistance by the parasite. Here we show that various endogenous neuropeptides act as potent antitrypanosome agents. Neuropeptides exerted their trypanolytic activity through an unusual mechanism that involves peptide uptake by the parasite, disruption of lysosome integrity and cytosolic accumulation of glycolytic enzymes. This promotes an energetic metabolism failure that initiates an autophagic-like cell death. Neuropeptide-based treatment improved clinical signs in a chronic model of trypanosomiasis by reducing the parasite burden in various target organs. Of physiological importance is the fact that hosts respond to trypanosome infection producing neuropeptides as part of their natural innate defence. From a therapeutic point of view, targeting of intracellular compartments by neuropeptides provides a new promising strategy for the treatment of trypanosomiasis.

14863. **Fatihu, M. Y., Adamu, S., Umar, I. A., Ibrahim, N. D., Eduvie, L. O. & Esievo, K. A., 2008**. Studies on effects of lactose on experimental *Trypanosoma vivax* infection in Zebu cattle. 2. Packed cell volume. *Onderstepoort Journal of Veterinarz Research,* **75** (3): 181-187.

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The ability of intravenously administered lactose in normal saline to prevent a decline in packed cell volume (PCV) during experimental trypanosomosis was studied in Zebu cattle. During the lactose infusion period, the PCV was stable up to day 5 post-infection (p.i.) in a lactose-infused group, compared to that in an uninfused group in which the PCV dropped significantly ($p < 0.05$) as shown by the values of cumulative percentage change. Furthermore the mean rate of change in PCV was significantly ($p < 0.05$) higher in the uninfused group relative to the lactose-infused group during the same period. While the PCV fell markedly in the lactose-infused group a day after lactose infusion was stopped (day 13 p.i.), subsequent PCV values were significantly ($p < 0.05$) higher compared to those in the uninfused group, up to the end of experiment on day 17 p.i. However the mean rates of change in PCV did not vary significantly ($p > 0.05$) between the groups during the period in which lactose infusion was stopped. The mean levels of parasitaemic waves and parasitaemia were higher, more prolonged and more frequent in the lactose-infused group. It was inferred that the lactose was able to prevent an early onset of anaemia in the *Trypanosoma vivax*-infected Zebu cattle.

14864. **Grebaut, P., Chuchana, P., Brizard, J. P., Demettre, E., Seveno, M., Bossard, G., Jouin, P., Vincendeau, P., Bengaly, Z., Boulange, A., Cuny, G. & Holzmuller, P., 2009.** Identification of total and differentially expressed excreted-secreted proteins from *Trypanosoma congolense* strains exhibiting different virulence and pathogenicity. *International Journal of Parasitology.* **e Publication ahead of print March 13.**

CIRAD UMR 17 Trypanosomes [UMR 177 IRD-CIRAD "Interactions Hôtes-Vecteurs-Parasites dans les Trypanosomoses"], TA A-17/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France.

Animal trypanosomosis is a major constraint to livestock productivity in the tropics and has a significant impact on the life of millions of people globally (mainly in Africa, South America and south-east Asia). In Africa, the disease in livestock is caused mainly by *Trypanosoma congolense, Trypanosoma vivax, Trypanosoma evansi* and *Trypanosoma brucei brucei*. The extracellular position of trypanosomes in the bloodstream of their host requires consideration of both the parasite and its naturally excreted-secreted factors (secretome) in the course of pathophysiological processes. We therefore developed and standardised a method to produce purified proteomes and secretomes of African trypanosomes. In this study, two strains of *T. congolense* exhibiting opposite properties of both virulence and pathogenicity were further investigated through their secretome expression and its involvement in host-parasite interactions. We used a combined proteomic approach (one-dimensional SDS-PAGE and two-dimensional differential in-gel electrophoresis coupled to mass spectrometry) to characterise the whole and differentially expressed protein contents of secretomes. The molecular identification of differentially expressed trypanosome molecules and their correlation with either the virulence process or pathogenicity are discussed with regard to their potential as new diagnostic or therapeutic tools against animal trypanosomosis.

14865. **Guilliams, M., Movahedi, K., Bosschaerts, T., VandenDriessche, T., Chuah, M. K., Herin, M., Acosta-Sanchez, A., Ma, L., Moser, M., Van Ginderachter, J. A., Brys, L., De Baetselier, P. & Beschin, A., 2009.** IL-10 dampens TNF/inducible nitric oxide synthase-producing dendritic cell-mediated pathogenicity during parasitic infection. *Journal of Immunology*, **182** (2): 1107-1118.

Department of Molecular and Cellular Interactions, VIB, Brussels, Belgium.

Antiparasite responses are associated with the recruitment of monocytes that differentiate to macrophages and dendritic cells at the site of infection. Although classically activated monocytic cells are assumed to be the major source of TNF and NO during *Trypanosoma brucei brucei* infection, their cellular origin remains unclear. In this study, we show that bone marrow-derived monocytes accumulate and differentiate to TNF/inducible NO synthase-producing dendritic cells (TIP-DCs) in the spleen, liver, and lymph nodes of *T. brucei brucei*-infected mice. Although TIP-DCs have been shown to play a beneficial role in the elimination of several intracellular pathogens, we report that TIP-DCs, as a major source of TNF and NO in inflamed organs, could contribute actively to tissue damage during the chronic stage of *T. brucei brucei* infection. In addition, the absence of IL-10 leads to enhanced differentiation of monocytes to TIP-DCs, resulting in exacerbated pathogenicity and early death of the host. Finally, we demonstrate that sustained production of IL-10 following IL-10 gene delivery treatment with an adeno-associated viral vector to chronically infected mice limits the differentiation of monocytes to TIP-DCs and protects the host from tissue damage.

14866. **Kibugu, J. K., Ngeranwa, J. J., Makumi, J. N., Gathumbi, J. K., Kagira, J. M., Mwangi, J. N., Muchiri, M. W. & Mdachi, R. E., 2009**. Aggravation of pathogenesis mediated by ochratoxin A in mice infected with *Trypanosoma brucei rhodesiense. Parasitology,* **136** (3): 273-281.

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Mice fed 1.5 mg ochratoxin A (OTA) per kg body weight and infected with *Trypanosoma brucei rhodesiense* were compared with trypanosome-infected placebo-fed and uninfected OTA-fed controls. Uninfected OTA-fed mice showed fever, lethargy, facial and eyelid oedemas, mild hepatitis and nephritis, and high survival. Infected placebo-fed controls had mean pre-patent period (PPP) of 3.26 days, lethargy, dyspnoea, fever, facial and scrotal oedema, survival of 33-65 days, reduced red cell counts (RCC: 10.96-6.87x10^6 cells/µL of blood), packed cell volume (PCV: 43.19-26.36 percent), haemoglobin levels (Hb: 13.37-7.92 g/dL) and mean corpuscular volume (MCV) of 37.96-41.31 fL, hepatosplenomegaly, generalized oedemas, heart congestion, hepatitis and nephritis. Compared to infected placebo-fed controls, infected OTA-fed mice had significantly ($p<0.05$) shorter mean PPP (2.58 days), reduced survival (6-47 days), more pronounced fever and dyspnoea. The latter had significantly ($p<0.05$) reduced RCC (10.74-4.56 x 10^6 cells/µL of blood), PCV (43.90-20.78 percent), Hb (13.06-5.74 g/dL), increased MCV (39.10-43.97 fL), severe generalized oedemas, haemorrhages, congestion, hepatic haemosiderosis, hepatitis, nephritis, endocarditis, pericarditis and exclusively, splenic macrophage and giant cell hyperplasia, expanded red pulp and splenic erythrophagocytosis. It was concluded that OTA aggravated the pathogenesis of *T. b. rhodesiense* infection in mice, and should therefore be taken into consideration during trypanosomosis control programmes.

14867. **Margolles-Clark, E., Jacques-Silva, M. C., Ganesan, L., Umland, O., Kenyon, N. S., Ricordi, C., Berggren, P. O. & Buchwald, P., 2009.** Suramin inhibits the CD40-CD154 co-stimulatory interaction: a possible mechanism for immunosuppressive effects. *Biochemical Pharmacology,* **77** (7): 1236-1245.

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Suramin is a symmetric polysulphonated naphthylamine-benzamide urea derivative approved for the treatment of trypanosomiasis and onchocerciasis and a known P2 (ATP/UTP purine receptor) antagonist. Here, we report its ability to inhibit the important CD40-CD154 co-stimulatory interaction required for T cell activation and the development of an effective immune response. *In vitro*, it inhibited the binding of both human and murine CD154 (CD40L) to their receptor (CD40) even in the presence of protein-containing media and prevented the CD154-induced proliferation of human B cells as well as the corresponding increase in surface expression of CD86, CD80, CD40, and MHC class II in a concentration-dependent manner. Furthermore, in isolated human islets, it also decreased the CD154-induced release of inflammatory cytokines such as IFN-g, interleukin-6 (IL-6), and IL-8. Suramin was selected for investigation because it has been reported to be an inhibitor of the interaction of TNF-a with its receptor and CD154 is a member of the TNF family. However, it turned out to be a considerably, about 30-fold, more effective inhibitor of the CD40-CD154

protein-protein interaction than of the corresponding TNF interaction. Its median inhibitory concentration (IC50 50 mM) is somewhat higher than for the P2-receptor, but well within the range of its therapeutic concentration levels. Suramin shows considerable polypharmacology, but its interference with the positive co-stimulatory interaction might provide a possible, not yet identified mechanism for its ability to suppress T cell activity and induce immunosuppression, which might also have limited its clinical usefulness in the treatment of AIDS and cancer.

14868. **Namangala, B., De Baetselier, P. & Beschin, A., 2009**. Both type-I and type-II responses contribute to murine trypanotolerance. *Journal of Veterinary Medical Science,* **71** (3): 313-318.

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The host immune system has been documented to influence the course and outcome of infection with the phospholipase-C-deficient (PLC(-/-)) *Trypanosoma brucei brucei*. We addressed the resistance mechanisms during trypanosomosis by comparing the immune response to variant-specific surface glycoprotein (VSG) in relatively susceptible C3H mice and trypanotolerant (C57BL/6 x BALB/c)-F1 (B6B-F1) mice infected with PLC(-/-) parasites. During the early stage of infection, lymphoid cells from both PLC(-/-)-susceptible C3H and -tolerant B6B-F1 mice mainly secreted VSG-specific IFN-gamma. Although C3H mice remained locked in a type-I cytokine environment (IFN-gamma, TNF-alpha) during late stage of infection, B6B-F1 mice switched to production of type-II cytokines (IL-4, IL-10) from late stage of infection onwards. It seems that VSG-specific cytokine responses associated with resistance to murine African trypanosomosis are infection-stage dependent, with type-I cytokine responses being critical during the early stage of infection while type-II cytokine responses appear to be more important during the late and chronic phases of the disease. Because of the striking similarities in the course of the PLC(-/-)infection in B6B-F1 mice with that of the trypanotolerant N'Dama cattle naturally-infected with *T. congolense*, the PLC(-/-)-infected B6B-F1 mice represents a suitable model to study the course of infection and immune responses during bovine trypanosomosis.

14869. **Ngure, R. M., Eckersall, P. D., Mungatana, N. K., Mburu, J. N., Jennings, F. W., Burke, J. & Murray, M., 2009**. Lipopolysaccharide binding protein in the acute phase response of experimental murine *Trypanosoma brucei brucei* infection. *Research in Veterinary Science,* **86** (3): 394-398.

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Cellular responses to lipopolysaccharide (LPS) are enhanced by LPS-binding protein (LBP). The present study investigated the acute phase response of LBP during *Trypanosoma brucei brucei* infection in mice. Mean plasma concentrations of LBP increased two-fold by the seventh day following infection, but decreased to intermediate levels by the 14th day. There were no significant differences in LBP concentrations of infected/antibiotic-treated and infected/untreated mice. At 35 days post-infection, the infected mice were treated with the

anti-trypanosomal diminazine aceturate (Berenil). LBP levels of the mice then decreased to pre-infection levels within one-week. This demonstrated that LBP is an acute phase protein during murine trypanosomosis. Furthermore, opportunistic secondary bacterial infection during trypanosomosis did not seem to play an important role in the changes in plasma LBP levels. We speculate that the marked concomitant increases in plasma LBP and endotoxin-like activity following murine trypanosome infection might play an important role in the pathogenesis of trypanosomosis.

14870. **Oliveira, M. P., Cortez, M., Maeda, F. Y., Fernandes, M. C., Haapalainen, E. F., Yoshida, N. & Mortara, R. A., 2009**. Unique behaviour of *Trypanosoma dionisii* interacting with mammalian cells: invasion, intracellular growth, and nuclear localization. *Acta Tropica,* **110** (1): 65-74.

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The phylogenetic proximity between *Trypanosoma cruzi* and *Trypanosoma (Schizotrypanum) dionisii* suggests that these parasites might explore similar strategies to complete their life cycles. *T. cruzi* is the aetiological agent of the life-threatening Chagas disease, whereas *T. dionisii* is a bat trypanosome and probably not capable of infecting humans. Here we sought to compare mammalian cell invasion and intracellular traffic of both trypanosomes and determine the differences and similarities in this process. The results presented demonstrate that *T. dionisii* is highly infective *in vitro*, particularly when the infection process occurs without serum and that the invasion is similarly affected by agents known to interfere with *T. cruzi* invasion process. Our results indicate that the formation of lysosomal-enriched compartments is part of a cell-invasion mechanism retained by related trypanosomatids, and that residence and further escape from a lysosomal compartment may be a common requisite for successful infection. During intracellular growth, parasites share a few epitopes with *T. cruzi* amastigotes and trypomastigotes. Unexpectedly, in heavily infected cells, amastigotes and trypomastigotes were found inside the host cell nucleus. These findings suggest that *T. dionisii*, although sharing some features in host cell invasion with *T. cruzi*, has unique behaviours that deserve to be further explored.

14871. **Rodgers, J., Stone, T. W., Barrett, M. P., Bradley, B. & Kennedy, P. G., 2009**. Kynurenine pathway inhibition reduces central nervous system inflammation in a model of human African trypanosomiasis. *Brain*. **Advance Access published on line March 31.**

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Human African trypanosomiasis, or sleeping sickness, is caused by the protozoan parasites *Trypanosoma brucei rhodesiense* or *Trypanosoma brucei gambiense*, and is a major cause of systemic and neurological disability throughout sub-Saharan Africa. Following early-stage disease, the trypanosomes cross the blood-brain barrier to invade the central nervous system leading to the encephalitic, or late stage, infection. Treatment of human African trypanosomiasis currently relies on a limited number of highly toxic drugs, but untreated, is invariably fatal. Melarsoprol, a trivalent arsenical, is the only drug that can be

used to cure both forms of the infection once the central nervous system has become involved, but unfortunately, this drug induces an extremely severe post-treatment reactive encephalopathy (PTRE) in up to 10 percent of treated patients, half of whom die from this complication. Since it is unlikely that any new and less toxic drug will be developed for treatment of human African trypanosomiasis in the near future, increasing attention is now being focussed on the potential use of existing compounds, either alone or in combination chemotherapy, for improved efficacy and safety. The kynurenine pathway is the major pathway in the metabolism of tryptophan. A number of the catabolites produced along this pathway show neurotoxic or neuroprotective activities, and their role in the generation of central nervous system inflammation is well documented. In the current study, Ro-61-8048, a high affinity kynurenine-3-monooxygenase inhibitor, was used to determine the effect of manipulating the kynurenine pathway in a highly reproducible mouse model of human African trypanosomiasis. It was found that Ro-61-8048 treatment had no significant effect ($P = 0.4445$) on the severity of the neuroinflammatory pathology in mice during the early central nervous system stage of the disease when only a low level of inflammation was present. However, a significant ($p = 0.0284$) reduction in the severity of the neuroinflammatory response was detected when the inhibitor was administered in animals exhibiting the more severe, late central nervous system stage, of the infection. *In vitro* assays showed that Ro-61-8048 had no direct effect on trypanosome proliferation suggesting that the anti-inflammatory action is due to a direct effect of the inhibitor on the host cells and not a secondary response to parasite destruction. These findings demonstrate that kynurenine pathway catabolites are involved in the generation of the more severe inflammatory reaction associated with the late central nervous system stages of the disease and suggest that Ro-61-8048 or a similar drug may prove to be beneficial in preventing or ameliorating the PTRE when administered as an adjunct to conventional trypanocidal chemotherapy.

14872. **Samanovic, M., Molina-Portela, M. P., Chessler, A. D., Burleigh, B. A. & Raper, J., 2009**. Trypanosome lytic factor, an antimicrobial high-density lipoprotein, ameliorates *Leishmania* infection. *PLoS Pathogens,* **5** (1): e1000276.

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Innate immunity is the first line of defence against invading microorganisms. Trypanosome lytic factor (TLF) is a minor sub-fraction of human high-density lipoprotein that provides innate immunity by completely protecting humans from infection by most species of African trypanosomes, which belong to the Kinetoplastida order. Herein, we demonstrate the broader protective effects of human TLF, which inhibits intracellular infection by *Leishmania*, a kinetoplastid that replicates in phagolysosomes of macrophages. We show that TLF accumulates within the parasitophorous vacuole of macrophages *in vitro* and reduces the number of *Leishmania* metacyclic promastigotes, but not amastigotes. We do not detect any activation of the macrophages by TLF in the presence or absence of *Leishmania*, and therefore propose that TLF directly damages the parasite in the acidic parasitophorous vacuole. To investigate the physiological relevance of this observation, we have reconstituted lytic activity *in vivo* by generating mice that express the two main protein components of TLFs: human apolipoprotein L-I and haptoglobin-related protein. Both proteins are expressed in mice at levels equivalent to those found in humans and circulate within high-density lipoproteins. We find that TLF mice can ameliorate an infection with

Leishmania by significantly reducing the pathogen burden. In contrast, TLF mice were not protected against infection by the kinetoplastid *Trypanosoma cruzi*, which infects many cell types and transiently passes through a phagolysosome. We conclude that TLF not only determines species specificity for African trypanosomes, but can also ameliorate an infection with *Leishmania*, while having no effect on *T. cruzi*. We propose that TLFs are a component of the innate immune system that can limit infections by their ability to selectively damage pathogens in phagolysosomes within the reticuloendothelial system.

(c) CHEMOTHERAPEUTICS

14873. **Alloatti, A., Testero, S. A. & Uttaro, A. D., 2009**. Chemical evaluation of fatty acid desaturases as drug targets in *Trypanosoma cruzi*. *International Journal of Parasitology*. **In press, corrected proof**.

Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET, Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmaceuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Santa Fe, Argentina.

Four positional isomers of thiastearate (TS) and isoxyl (thiocarlide) were assayed as fatty acid desaturase inhibitors in *Trypanosoma cruzi* epimastigotes. 9-TS did not exert a significant effect on growth of *T. cruzi*, nor on the fatty acid profile of the parasite cells. One hundred µM of 10-TS totally inhibited growth, with an effective concentration for 50 percent growth inhibition (EC(50)) of 3.0+/-0.2 mµM. Growth inhibition was reverted by supplementing the culture media with oleate. The fatty acid profile of treated cells revealed that conversion of stearate to oleate and palmitate to palmitoleate were drastically reduced and, as a consequence, the total level of unsaturated fatty acids decreased from 60 percent to 32 percent. Isoxyl, a known inhibitor of stearoyl-CoA Delta9 desaturase in mycobacteria, had similar effects on *T. cruzi* growth (EC(50) 2.0+/-0.3 mµM) and fatty acid content, indicating that Delta9 desaturase was the target of both drugs. 12- and 13-TS were inhibitors of growth with EC(50) values of 50+/-2 and 10+/-3 mµM, respectively, but oleate or linoleate were unable to revert the effect. Both drugs increased the percentage of oleate and palmitate in the cell membrane and drastically reduced the content of linoleate from 38 percent to 16 percent and 12 percent, respectively, which is in agreement with a specific inhibition of oleate Delta12 desaturase. The absence of corresponding enzyme activity in mammalian cells and the significant structural differences between trypanosome and mammalian Delta9 desaturases, together with our results, highlight these enzymes as promising targets for selective chemotherapeutic intervention.

14874. **Bakunova, S. M., Bakunov, S. A., Patrick, D. A., Kumar, E. V., Ohemeng, K. A., Bridges, A. S., Wenzler, T., Barszcz, T., Jones, S. K., Werbovetz, K. A., Brun, R. & Tidwell, R. R., 2009**. Structure-activity study of pentamidine analogues as antiprotozoal agents. *Journal of Medicinal Chemistry,* **52** (7): 2016-2035.

Department of Pathology and Laboratory Medicine, School of Medicine, The University of North Carolina, Chapel Hill, North Carolina 27599, USA.

Diamidine 1 (pentamidine) and 65 analogues (2-66) have been tested for *in vitro* antiprotozoal activities against *Trypanosoma brucei rhodesiense, Plasmodium falciparum*, and *Leishmania donovani*, and for cytotoxicity against mammalian cells. Dications 32, 64, and 66 exhibited antitrypanosomal potencies equal or greater than melarsoprol (IC(50) = 4 nM). Nine congeners (2-4, 12, 27, 30, and 64-66) were more active against *P. falciparum* than artemisinin (IC(50) = 6 nM). Eight compounds (12, 32, 33, 44, 59, 62, 64, and 66) exhibited equal or better antileishmanial activities than 1 (IC(50) = 1.8 µM). Several congeners were more active than 1 *in vivo*, curing at least 2/4 infected animals in the acute mouse model of trypanosomiasis. The diimidazoline 66 was the most promising compound in the series, showing excellent *in vitro* activities and high selectivities against *T. b. rhodesiense, P. falciparum*, and *L. donovani* combined with high antitrypanosomal efficacy *in vivo*.

14875. **Baliani, A., Peal, V., Gros, L., Brun, R., Kaiser, M., Barrett, M. P. & Gilbert, I. H., 2009**. Novel functionalized melamine-based nitroheterocycles: synthesis and activity against trypanosomatid parasites. *Organic and Biomolecular Chemistry,* **7** (6): 1154-1166.

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Human African trypanosomiasis (HAT), caused by the protozoan parasite *Trypanosoma brucei* sp., is a major health problem in sub-Saharan Africa. New drugs are urgently required for the disease. Selective uptake of toxic compounds into trypanosomes has been achieved by exploiting plasma membrane transporters. For example, the P2 aminopurine transporter, along with other transporters, selectively concentrates melamine and benzamidine moieties into trypanosomes. We have previously reported the use of the melamine motif to selectively target nitrofuran to the trypanosome. In this paper we report the further investigation of the structure activity relationships and the effect of the introduction of different functionalized substituents onto the melamine unit. Most of the compounds tested *in vitro* for their trypanocidal activity showed activities in the sub µM range against *T. b. rhodesiense*.

14876. **Barker, R. H., Jr., Liu, H., Hirth, B., Celatka, C. A., Fitzpatrick, R., Xiang, Y., Willert, E. K., Phillips, M. A., Kaiser, M., Bacchi, C. J., Rodriguez, A., Yarlett, N., Klinger, J. D. & Sybertz, E., 2009**. Novel S-adenosylmethionine decarboxylase inhibitors for the treatment of human African trypanosomiasis. *Antimicrobial Agents and Chemotherapy*, **53** (5): 2052-2058.

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Trypanosomiasis remains a significant disease across the sub-Saharan African continent, with 50 000 to 70 000 individuals infected. The utility of current therapies is limited by issues of toxicity and the need to administer compounds intravenously. We have begun a program to pursue lead optimization around MDL 73811, an irreversible inhibitor of

S-adenosylmethionine decarboxylase (AdoMetDC). This compound is potent but in previous studies cleared rapidly from the blood of rats. One of the analogues synthesized (Genz-644131) was shown to be highly active against *Trypanosoma brucei rhodesiense in vitro* (50 percent inhibitory concentration, 400 pg/ml). Enzyme kinetic studies showed Genz-644131 to be approximately fivefold more potent than MDL 73811 against the *T. brucei brucei* AdoMetDC-prozyme complex. This compound was stable *in vitro* in rat and human liver microsomal and hepatocyte assays, was stable in rat whole-blood assays, did not significantly inhibit human cytochrome P450 enzymes, had no measurable efflux in CaCo-2 cells, and was only 41 percent bound by serum proteins. Pharmacokinetic studies of mice following intraperitoneal dosing showed that the half-life of Genz-644131 was threefold greater than that of MDL 73811 (7.4 h versus 2.5 h). Furthermore, brain penetration of Genz-644131 was 4.3-fold higher than that of MDL 73811. Finally, *in vivo* efficacy studies of *T. b. brucei* strain STIB 795-infected mice showed that Genz-644131 significantly extended survival (from 6.75 days for controls to >30 days for treated animals) and cured animals infected with *T. b. brucei* strain LAB 110 EATRO. Taken together, the data strengthen validation of AdoMetDC as an important parasite target, and these studies have shown that analogues of MDL 73811 can be synthesized with improved potency and brain penetration.

14877. **Benitez, J., Guggeri, L., Tomaz, I., Arrambide, G., Navarro, M., Pessoa, J. C., Garat, B. & Gambino, D., 2009**. Design of vanadium mixed-ligand complexes as potential antiprotozoa agents. *Journal of Inorganic Biochemistry,* **103** (4): 609-616.

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In the search for new therapeutic tools against Chagas' disease (American Trypanosomiasis) four novel mixed-ligand vanadyl complexes, [V(IV)O(L(2)-2H)(L(1))], including a bidentate polypyridyl DNA intercalator (L(1)) and a tridentate salycylaldehyde semicarbazone derivative (L(2)) as ligands were synthesized, characterized by a combination of techniques, and *in vitro* evaluated. EPR suggest a distorted octahedral geometry with the tridentate semicarbazone occupying three equatorial positions and the polypyridyl ligand coordinated in an equatorial/axial mode. Both complexes including dipyrido[3,2-a: 2',3'-c]phenazine (dppz) as polypyridyl coligand showed IC(50) values in the mμM range against Dm28c strain (epimastigotes) of *Trypanosoma cruzi*, causative agent of the disease, being as active as the anti-trypanosomal reference drug Nifurtimox. To get an insight into the trypanocidal mechanism of action of these compounds, DNA was evaluated as a potential parasite target and EPR, and (51)V NMR experiments were also carried out upon aging aerated solutions of the complexes. Data obtained by electrophoretic analysis suggest that the mechanism of action of these complexes could include DNA interactions.

14878. **Cameron, S., Martini, V. P., Iulek, J. & Hunter, W. N., 2009**. *Geobacillus stearothermophilus* 6-phosphogluconate dehydrogenase complexed with 6-phosphogluconate. *Acta Crystallographia Section F Structural Biology and Crystalization Communications,* **65** (Pt 5): 450-454.

University of Dundee, Scotland, UK.

Two crystal structures of recombinant *Geobacillus stearothermophilus* 6-phosphogluconate dehydrogenase (Gs6PDH) in complex with the substrate 6-phosphogluconate have been determined at medium resolution. Gs6PDH shares significant sequence identity and structural similarity with the enzymes from *Lactococcus lactis*, sheep liver and the protozoan parasite *Trypanosoma brucei*, for which a range of structures have previously been reported. Comparisons indicate that amino-acid sequence conservation is more pronounced in the two domains that contribute to the architecture of the active site, namely the N-terminal and C-terminal domains, compared with the central domain, which is primarily involved in the subunit-subunit associations required to form a stable dimer. The active-site residues are highly conserved, as are the interactions with the 6-phosphogluconate. There is interest in 6PDH as a potential drug target for the protozoan parasite *T. brucei*, the pathogen responsible for African sleeping sickness. The recombinant *T. brucei* enzyme has proven to be recalcitrant to enzyme-ligand studies and a surrogate protein might offer new opportunities to investigate and characterize 6PDH inhibitors. The high degree of structural similarity, efficient level of expression and straightforward crystallization conditions mean that Gs6PDH may prove to be an appropriate model system for structure-based inhibitor design targeting the enzyme from *Trypanosoma* species.

14879. **Chauhan, S. C., Padmanabhan, P. K. & Madhubala, R., 2008**. Glyoxalase pathway of trypanosomatid parasites: a promising chemotherapeutic target. *Current Drug Targets,* **9** (11): 957-965.

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Trypanosomatids are pathogenic protozoa of the order Kinetoplastida. A unique feature of these parasitic protozoa is the presence of a unique dithiol trypanothione (N(1), N(8) -bis(glutathionyl)spermidine) and the flavoenzyme trypanothione reductase. This is in contrast to human and other eukaryotes, which contain ubiquitous glutathione/glutathione reductase system. An important function of thiols is to protect cells from toxic metabolic by-products such as methylglyoxal, a reactive 2-oxoaldehyde. Methylglyoxal is a mutagenic and a cytotoxic compound. The glyoxalase system is involved in the detoxification of methylglyoxal. The exceptionality of the glyoxalase enzyme in the parasitic protozoa is the dependence on the dithiol -trypanothione for detoxifying the toxic methylglyoxal. The detoxification process by the glyoxalase enzyme in eukaryotes and most other organisms is dependent on the tripeptide glutathione. The glyoxalase enzyme of trypanosomatids are also exceptional in a way that they use the divalent cation nickel as a cofactor like the glyoxalase enzyme of *E. coli*, whereas in eukaryotes the cofactor is zinc. This reflects that both the substrate as well as the cofactor of the kinetoplastids glyoxalase enzyme are distinct from that of the glyoxalase enzyme of eukaryotes. These differences reveal that the active site of the glyoxalase enzyme of the parasite and its mammalian counterpart are significantly different thereby proposing that the glyoxalase enzyme of the protozoan parasite can be a potential chemotherapeutic target.

14880. **Chollet, C., Baliani, A., Wong, P. E., Barrett, M. P. & Gilbert, I. H., 2009**. Targeted delivery of compounds to *Trypanosoma brucei* using the melamine motif. *Bioorganic and Medicinal Chemistry,* **17** (6): 2512-2523.

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There is an urgent need for the development of new drugs for the treatment of human African trypanosomiasis. The causative organism, *Trypanosoma brucei*, has been shown to have some unusual plasma membrane transporters, in particular the P2 aminopurine transporter and related permeases, which have been used for the selective targeting of trypanocidal compounds to the organism. In this paper, we report the addition of melamine-based P2-targeting motifs to three different classes of compound in order to try and improve activity through increased selective uptake. The classes reported here are fluoroquinolones, difluoromethylornithine and artesunate derivatives.

14881. **Field, M. C., 2009**. Drug screening by crossing membranes: a novel approach to identification of trypanocides. *Biochemical Journal,* **419** (2): e1-3.

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Trypanosomes are a group of protozoan parasites that inflict huge health and economic burdens across the globe. The African trypanosome, *Trypanosoma brucei*, the causative agent of sleeping sickness, has a highly sophisticated mechanism of antigenic variation that facilitates chronic survival in the mammalian host, and also all but eliminates any realistic hope for vaccination-based control. However, trypanosomes are also highly divergent organisms, with many biochemical processes setting them apart from their hosts, and there remains great optimism that these features may be exploited for development of new drugs. Unfortunately, the compounds that are in use at present are decades old and resistance has emerged. The article in this issue of the Biochemical Journal by Patham *et. al.*, a joint team from the universities of Pittsburgh and Georgia, represents one approach to exploiting this divergence. The authors of the study have exploited novel aspects of the biochemistry within the system for translocation of nascent polypeptides across the endoplasmic reticulum membrane to identify three compounds that are able to inhibit the process. They then demonstrate that these same compounds are both trypanocidal, but well tolerated by human tissue culture cells. These observations may present interesting new leads in the fight against trypanosomiasis, and potentially identify a new target that can be explored for therapeutic potential.

14882. **Haines, L. R., Thomas, J. M., Jackson, A. M., Eyford, B. A., Razavi, M., Watson, C. N., Gowen, B., Hancock, R. E. & Pearson, T. W., 2009.** Killing of trypanosomatid parasites by a modified bovine host defence peptide, BMAP-18. *PLoS Neglected Tropical Diseases*, **3** (2): e373.

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Tropical diseases caused by parasites continue to cause socioeconomic devastation that reverberates worldwide. There is a growing need for new control measures for many of these diseases due to increasing drug resistance exhibited by the parasites and problems with drug toxicity. One new approach is to apply host defence peptides (HDP; formerly called antimicrobial peptides) to disease control, either to treat infected hosts, or to prevent disease transmission by interfering with parasites in their insect vectors. A potent anti-parasite effector is bovine myeloid antimicrobial peptide-27 (BMAP-27), a member of the cathelicidin family. Although BMAP-27 is a potent inhibitor of microbial growth, at higher concentrations it also exhibits cytotoxicity to mammalian cells. We tested the anti-parasite activity of BMAP-18, a truncated peptide that lacks the hydrophobic C-terminal sequence of the BMAP-27 parent molecule, an alteration that confers reduced toxicity to mammalian cells. BMAP-18 showed strong growth inhibitory activity against several species and life cycle stages of African trypanosomes, fish trypanosomes and *Leishmania* parasites *in vitro*. When compared to native BMAP-27, the truncated BMAP-18 peptide showed reduced cytotoxicity on a wide variety of mammalian and insect cells and on *Sodalis glossindius*, a bacterial symbiont of the tsetse vector. The fluorescent stain rhodamine 123 was used in immunofluorescence microscopy and flow cytometry experiments to show that BMAP-18 at low concentrations rapidly disrupted mitochondrial potential without obvious alteration of parasite plasma membranes, thus inducing death by apoptosis. Scanning electron microscopy revealed that higher concentrations of BMAP-18 induced membrane lesions in the parasites as early as 15 min. after exposure, thus killing them by necrosis. In addition to direct killing of parasites, BMAP-18 was shown to inhibit LPS-induced secretion of tumour necrosis factor alpha (TNF-alpha), a cytokine that is associated with inflammation and cachexia (wasting) in sleeping sickness patients. As a prelude to *in vivo* applications, high affinity antibodies to BMAP-18 were produced in rabbits and used in immuno-mass spectrometry assays to detect the intact peptide in human blood and plasma. In conclusion, BMAP-18, a truncated form of the potent antimicrobial BMAP-27, showed low toxicity to mammalian cells, insect cells and the tsetse bacterial symbiont *Sodalis glossinidius* while retaining an ability to kill a variety of species and life cycle stages of pathogenic kinetoplastid parasites *in vitro*. BMAP-18 also inhibited secretion of TNF-alpha, an inflammatory cytokine that plays a role in the cachexia associated with African sleeping sickness. These findings support the idea that BMAP-18 should be explored as a candidate for therapy of economically important trypanosome-infected hosts, such as cattle, fish and humans, and for paratransgenic expression in *Sodalis glossinidius*, a bacterial symbiont in the tsetse vector, as a strategy for interference with trypanosome transmission.

14883. **Ishiyama, A., Otoguro, K., Namatame, M., Nishihara, A., Furusawa, T., Masuma, R., Shiomi, K., Takahashi, Y., Ichimura, M., Yamada, H. & Omura, S., 2008.** *In vitro* and *in vivo* antitrypanosomal activity of two microbial metabolites, KS-505a and alazopeptin. *Journal of Antibiotics (Tokyo),* **61** (10): 627-632.

Research Center for Tropical Diseases, Center for Basic Research, Kitasato University, Tokyo, Japan.

Our on-going screening programme to discover new antitrypanosomal antibiotics has been evaluating compounds isolated from soil microorganisms as well as investigating the antibiotic libraries of the Kitasato Institute for Life Sciences and BioFrontier Laboratories of

Kyowa Hakko Kogyo Co., Ltd. We have now discovered two compounds, KS-505a and alazopeptin, which exhibit moderate anti-trypanosomal characteristics. We report here the *in vitro* and *in vivo* antitrypanosomal activities and cytotoxicities of KS-505a and alazopeptin, compared with some commonly-used antitrypanosomal drugs. This is the first report of *in vitro* and *in vivo* antitrypanosomal activities of either KS-505a or alazopeptin.

14884. **Karioti, A., Skaltsa, H., Kaiser, M. & Tasdemir, D., 2009**. Trypanocidal, leishmanicidal and cytotoxic effects of anthecotulide-type linear sesquiterpene lactones from *Anthemis auriculata*. *Phytomedicine*. **In press, corrected proof.**

Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, 157 71 Athens, Greece.

Trypanosomiasis and leishmaniasis pose major public health threats for many countries, particularly those in sub-Saharan Africa and South America. In the present study, we evaluated the *in vitro* antiprotozoal activity of three irregular, linear sesquiterpene lactones recently isolated from Greek *Anthemis auriculata*, namely anthecotulide (1), 4-hydroxyanthecotulide (2) and 4-acetoxyanthecotulide (3). Trypomastigote forms of *Trypanosoma brucei rhodesiense* and *T. cruzi* as well as axenic amastigotes of *Leishmania donovani* were used for testing. The cytotoxic potential of the compounds was also assessed against mammalian (rat) skeletal myoblasts (L6 cells). All compounds showed potent trypanocidal and leishmanicidal activity. 4-Hydroxyanthecotulide (2) appeared to be the most active compound against all parasites, particularly towards *T. b. rhodesiense* (IC(50) 0.56mug/ml), whereas 4-acetoxyanthecotulide (3) was the least active. All three metabolites possessed toxicity on mammalian cells, which might limit their use as antiprotozoal agents.

14885. **Kaur, S., Shivange, A. V. & Roy, N., 2009**. Structural analysis of trypanosomal sirtuin: an insight for selective drug design. *MolecularDiversity*. **e Publication ahead of print, April 29.**

Department of Biotechnology, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S Nagar, Mohali, Punjab, 160062, India.

The Trypanosomatidae family continues to create burdens on countries that are least equipped to bring new medicines to the clinic. For sickness caused by this family of parasites (African trypanosomiasis, Chagas disease, and leishmaniasis) no vaccines are available, and currently available drugs suffer from insufficient efficacy, excessive toxicity, and steady loss of effectiveness due to resistance. Availability of the genome sequence of pathogens of this family offers a unique avenue for the identification of novel common drug targets for all three pathogens. The sirtuin family of nicotinamide adenine dinucleotide (NAD)-dependent deacetylases is remarkably conserved throughout evolution from archaebacteria to eukaryotes and plays an important role in trypanosomatidae biology and virulence. In order to gain insight for selective drug design, three-dimensional (3D) models of *L. major, L. infantum, T. brucei,* and *T. cruzi* sirtuin were constructed by homology modeling and compared with human sirtuin. The molecular electrostatic potentials and cavity depth analysis of these models suggest that the inhibitor binding catalytic domain has various minor structural differences in the active site of trypanosomal and human sirtuin, regardless of sequence

similarity. These studies have implications for designing effective strategies to identify inhibitors that can be developed as novel broad-spectrum antitrypanosomal drugs.

14886. **Kodama, H., Denso, Okazaki, F. & Ishida, S., 2008**. Protective effect of humus extract against *Trypanosoma brucei* infection in mice. *Journal of Veterinary Medical Science,* **70** (11): 1185-1190.

Laboratory of Veterinary Immunology, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan. [kodama@vet.osakafu-u.ac.jp].

Humic substances are formed during the decomposition of organic matter in humus, and are found in many natural environments in which organic materials and microorganisms are present. Oral administration of humus extract to mice successfully induced effective protection against experimental challenge by the two subspecies, *Trypanosoma brucei brucei* and *T. brucei gambiense*. Mortality was most reduced among mice who received a 3 percent humus extract for 21 days in drinking water *ad libitum*. Spleen cells from humus-administered mice exhibited significant non-specific cytotoxic activity against L1210 mouse leukemia target cells. Also, spleen cells produced significantly higher amounts of Interferon-gamma when stimulated *in vitro* with Concanavalin A than cells from normal controls. These results clearly show that administration to mice of humus extract induced effective resistance against *Trypanosoma* infection. Enhancement of the innate immune system may be involved in host defence against trypanosomiasis.

14887. **Link, A., Heidler, P., Kaiser, M. & Brun, R., 2009**. Synthesis of a series of N(6)-substituted adenosines with activity against trypanosomatid parasites. *European Journal of Medicinal Chemistry*. **In press, corrected proof.**

Institute of Pharmacy, Ernst-Moritz-Arndt-University, Friedrich-Ludwig-Jahn-Strasse 17, 17487 Greifswald, Germany.

The involvement of purine salvage in the accumulation of current trypanocidal drugs is important for the treatment of African sleeping sickness. The substrate specificity of essential nucleoside transporters is therefore of physiological and pharmacological interest. With the intention to contribute to the knowledge in the field, a series of 16 adenosine derivatives with substituents in N(6)-position were prepared in order to evaluate their potential to inhibit *Trypanosoma brucei* sp. *in vitro*. An unmodified ribose moiety was selected to conserve key molecular recognition motifs of the arsenal of integral membrane proteins expressed in large numbers on the protozoan plasma membrane. Two of the new compounds prepared using a polymer-assisted acylation protocol showed antitrypanosomal activities in the single digit µmolar concentration range.

14888. **Mdachi, R. E., Thuita, J. K., Kagira, J. M., Ngotho, J. M., Murilla, G. A., Ndung'u, J. M., Tidwell, R. R., Hall, J. E. & Brun, R., 2009**. Efficacy of the novel diamidine compound 2,5-Bis(4-amidinophenyl)- furan-bis-O-methlylamidoxime (pafuramidine, DB289) against *Trypanosoma brucei rhodesiense* infection in vervet monkeys after oral administration. *Antimicrobial Agents and Chemotherapy,* **53** (3): 953-957.

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Owing to the lack of oral drugs for human African trypanosomiasis, patients have to be hospitalized for 10 to 30 days to facilitate treatment with parenterally administered medicines. The efficacy of a novel orally administered prodrug, 2,5-bis(4-amidinophenyl)-furan-bis-O-methlylamidoxime (pafuramidine, DB289), was tested in the vervet monkey (*Chlorocebus* [*Cercopithecus*] *aethiops*) model of sleeping sickness. Five groups of three animals each were infected intravenously with 10^4 *Trypanosoma brucei rhodesiense* KETRI 2537 cells. On the seventh day post infection (p.i.) in an early-stage infection, animals in groups 1, 2, and 3 were treated orally with pafuramidine at dose rates of 1, 3, or 10 mg/kg of body weight, respectively, for five consecutive days. The animals in groups 4 and 5 were treated with 10 mg/kg for 10 consecutive days starting on the 14^{th} day p.i. (group 4) or on the 28^{th} day p.i. (group 5), when these animals were in the late stage of the disease. In the groups treated in the early stage, 10 mg/kg of pafuramidine completely cured all three monkeys, whereas lower doses of 3 mg/kg and 1 mg/kg cured only one of three and zero of three monkeys, respectively. Treatment of late-stage infections resulted in cure rates of one of three (group 4) and zero of three (group 5) monkeys. These studies demonstrated that pafuramidine was orally active in monkeys with early-stage *T. brucei rhodesiense* infections at dose rates above 3 mg/kg for 5 days. It was also evident that the drug attained only minimal efficacy against late-stage infections, indicating the limited ability of the molecule to cross the blood-brain barrier. This study has shown that oral diamidines have potential for the treatment of early-stage sleeping sickness.

14889. **Mogi, T., Ui, H., Shiomi, K., Omura, S., Miyoshi, H. & Kita, K., 2009**. Antibiotics LL-Z1272 identified as novel inhibitors discriminating bacterial and mitochondrial quinol oxidases. *Biochimica Biophysica Acta,* **1787** (2): 129-133.

Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. [tmogi@m.u-tokyo.ac.jp].

To counter antibiotic-resistant bacteria, we screened the Kitasato Institute for Life Sciences Chemical Library with bacterial quinol oxidase, which does not exist in the mitochondrial respiratory chain. We identified five prenylphenols, LL-Z1272beta, gamma, delta, varepsilon and zeta, as new inhibitors for the *Escherichia coli* cytochrome bd. We found that these compounds also inhibited the *E. coli* bo-type ubiquinol oxidase and trypanosome alternative oxidase, although these three oxidases are structurally unrelated. LL-Z1272beta and varepsilon (dechlorinated derivatives) were more active against cytochrome bd while LL-Z1272gamma, delta, and zeta (chlorinated derivatives) were potent inhibitors of cytochrome bo and trypanosome alternative oxidase. Thus prenylphenols are useful for the

selective inhibition of quinol oxidases and for understanding the molecular mechanisms of respiratory quinol oxidases as a probe for the quinol oxidation site. Since quinol oxidases are absent from mammalian mitochondria, LL-Z1272beta and delta, which are less toxic to human cells, could be used as lead compounds for development of novel chemotherapeutic agents against pathogenic bacteria and African trypanosomiasis.

14890. **Moreira, O. C., Rios, P. F., Esteves, F. F., Meyer-Fernandes, J. R. & Barrabin, H., 2009**. CrATP as a new inhibitor of ecto-ATPases of trypanosomatids. *Parasitology,* **136** (1): 35-44.

Instituto de Bioquímica Médica, Programa de Biologia Estrutural, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro Rio de Janeiro, Brazil.

Trypanosomatid protozoa include heteroxenic species some of them pathogenic for men, animals and plants. Parasite membrane contains ecto-enzymes whose active sites face the external medium rather than the cytoplasm. *Herpetomonas* sp. displayed a Mg2+-dependent ecto-ATPase activity, a Mg-independent ecto-ADPase and an ecto-phosphatase activity. Both, the ecto-ADPase and phosphatase activities were insensitive to CrATP (chromium(III) adenosine 5'-triphosphate complex). Ecto-ATPase activity was reversibly inhibited. At 2 µM ATP the apparent Ki was 4.7+/-1.0 µM but a fraction of about 40-50 percent was insensitive to CrATP. Remarkably, at low substrate concentration (0.2 mM) more than 90 percent of the ecto-ATPase was inhibited with Ki=0.33+/-1.0 µM. These parameter dependences are interpreted as the presence of 2 ecto-ATPases activities, one of them with high ATP apparent affinity and sensitivity to CrATP. DIDS (4,4 diisothiocyanatostilbene 2,2' disulphonic acid), suramin and ADP were also effective as inhibitors. Only ADP presented no additive inhibition with CrATP. The pattern of partial inhibition by CrATP was also observed for the ecto-ATPase activities of *Leishmania amazonensis*, *Trypanosoma cruzi* and *Trypanosoma rangeli*. CrATP emerges as a new inhibitor of ecto-ATPases and as a tool for a better understanding of properties and role of ecto-ATPases in the biology of parasites.

14891. **Oluwafemi, A. J., Okanla, E. O., Camps, P., Munoz-Torrerob, D., Mackey, Z. B., Chiang, P. K., Seville, S. & Wright, C. W., 2009**. Evaluation of cryptolepine and huperzine derivatives as lead compounds towards new agents for the treatment of human African trypanosomiasis. *Natural Product Communications,* **4** (2): 193-198.

Department of Zoology, University of Ilorin, Ilorin, Nigeria.

The alkaloid cryptolepine (1) and eight synthetic analogues (2-8) were assessed for *in vitro* activities against *Trypanosoma brucei*. Four of the analogues were found to be highly potent with IC50 values of less than 3 nM and three of these were assessed against *T. brucei brucei* infection in rats. The most effective compound was 2, 7-dibromocryptolepine 7; a single oral dose of 20 mg/kg suppressed parasitaemia and increased the mean survival time to 13.6 days compared with 8.4 days for untreated controls. In addition, four huperzine derivatives (9-12) were shown to have *in vitro* anti-trypanosomal activities with IC50 values ranging from 303-377 nM.

14892. **Patham, B., Duffy, J., Lane, A., Davis, R. C., Wipf, P., Fewell, S. W., Brodsky, J. L. & Mensa-Wilmot, K., 2009**. Post-translational import of protein into the endoplasmic reticulum of a trypanosome: an *in vitro* system for discovery of anti-trypanosomal chemical entities. *Biochemical Journal,* **419** (2): 507-517.

Department of Cellular Biology, University of Georgia, 724 Biological Sciences Building, Athens, GA 30602, USA.

HAT (human African trypanosomiasis), caused by the protozoan parasite *Trypanosoma brucei*, is an emerging disease for which new drugs are needed. Expression of plasma membrane proteins e.g. VSG (variant surface glycoprotein) is crucial for the establishment and maintenance of an infection by *T. brucei*. Transport of a majority of proteins to the plasma membrane involves their translocation into the ER (endoplasmic reticulum). Thus inhibition of protein import into the endoplasmic reticulum of *T. brucei* would be a logical target for discovery of lead compounds against trypanosomes. We have developed a *Tb*RM (*T. brucei* microsome) system that imports VSG_117 post-translationally. Using this system, MAL3-101, equisetin and CJ-21 058 were discovered to be small molecule inhibitors of VSG_117 translocation into the ER. These agents also killed bloodstream *T. brucei in vitro*; the concentrations at which 50 percent of parasites were killed (IC50) were 1.5 µM (MAL3-101), 3.3 µM (equisetin) and 7 µM (CJ-21 058). Thus VSG_117 import into *Tb*RMs is a rapid and novel assay to identify "new chemical entities" (e.g. MAL3-101, equisetin and CJ-21 058) for anti-trypanosome drug development.

14893. **Prasanna, S. & Doerksen, R. J., 2009**. Topological polar surface area: a useful descriptor in 2D-QSAR. *Current Medicinal Chemistry,* **16** (1): 21-41.

Department of Medicinal Chemistry, University of Mississippi, MS 38677-1848, USA.

Topological polar surface area (TPSA), which makes use of functional group contributions based on a large database of structures, is a convenient measure of the polar surface area that avoids the need to calculate ligand 3D structure or to decide which is the relevant biological conformation or conformations. We demonstrate the utility of TPSA in 2D-QSAR for 14 sets of diverse pharmacological activity data. Even though a large pool of reports showing the importance of the classic 2D descriptors such as calculated logP (ClogP) and calculated molar refractivity (CMR) exists in the 2D-QSAR literature, this is the first report to demonstrate the value of TPSA as a relevant descriptor applicable to a large, structurally and pharmacologically diverse set of classes of compounds. We also address the limitations of applicability of this descriptor for 2D-QSAR analysis. We observed a negative correlation of TPSA with activity data for anticancer alkaloids, MT1 and MT2 agonists, MAO-B and tumour necrosis factor-alpha inhibitors and a positive correlation with inhibitory activity data for telomerase, PDE-5, GSK-3, DNA-PK, aromatase, malaria, trypanosomatids and CB2 agonists.

14894. **Rubio, B. K., Tenney, K., Ang, K. H., Abdulla, M., Arkin, M., McKerrow, J. H. & Crews, P., 2009**. The marine sponge *Diacarnus bismarckensis* as a source of peroxiterpene inhibitors of *Trypanosoma brucei*, the causative agent of sleeping sickness. *Journal of Natural Products,* **72**(2):218-222.

Department of Chemistry and Biochemistry and Institute for Marine Sciences, University of California Santa Cruz, Santa Cruz, California, USA, Sandler Center for Basic Research in Parasitic Disease, University of California San Francisco, San Francisco, California, USA, and Small Molecule Discovery Center, University of California San Francisco, San Francisco, California, USA.

Human African trypanosomiasis, also known as African sleeping sickness, is a neglected tropical disease with inadequate therapeutic options. We have launched a collaborative new lead discovery venture using our repository of extracts and natural product compounds as input into our growth inhibition primary screen against *Trypanosoma brucei.* Careful evaluation of the spectral data of the natural products and derivatives allowed for the elucidation of the absolute configuration (using the modified Mosher's method) of two new peroxiterpenes: (+)-muqubilone B and (-)-ent-muqubilone. Five known compounds were also isolated: (+)-sigmosceptrellin A, (+)-sigmosceptrellin A methyl ester, (-)-sigmosceptrellin B, (+)-epi-muqubillin A, and (-)-epi-nuapapuin B methyl ester. The isolated peroxiterpenes demonstrated activities in the range IC(50) = 0.2-2 µg/mL.

14895. **Sanderson, L., Dogruel, M., Rodgers, J., De Koning, H. & Thomas, S. A., 2009**. Pentamidine movement across the murine blood-brain and blood-CSF barriers; effect of trypanosome infection, combination therapy, P-glycoprotein and MRP. *Journal of Pharmacology and Experimental Therapeutics*. **Fast Forward Publication, March 4.**

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During the first stage of human African trypanosomiasis (HAT), *T. b. gambiense* are found mainly in the blood and pentamidine treatment is used. Pentamidine is predominately ineffective once the parasites have invaded the CNS. This lack of efficacy is thought to be due to the inability of pentamidine to cross the blood-brain barrier, although this has never been directly explored. This study addresses this using brain perfusion in healthy mice, P-glycoprotein-deficient mice and in a murine model of HAT (*T. b. brucei*). The influence of additional anti-trypanosomal drugs on pentamidine delivery to the CNS was also investigated. Results revealed that ^{3}H pentamidine can cross the blood-brain barrier, although a proportion was retained by the capillary endothelium and failed to reach the healthy or trypanosome-infected brain (up to day 21 p.i.). The CNS distribution of pentamidine was increased in the final (possibly terminal) stage of trypanosome infection partly due to loss of barrier integrity (day 28-35 p.i.) as measured by ^{14}C sucrose and ^{3}H suramin. Furthermore, pentamidine distribution to the CNS involved influx and efflux (via P-glycoprotein and multi-drug resistance associated protein (MRP)) transporters and was affected by the other anti-trypanosomal agents, suramin, melarsoprol and nifurtimox, but not eflornithine. These interactions could contribute to side effects or lead to the development of parasite resistance to the drugs. Thus great care must be taken when designing drug combinations containing

pentamidine or other diamidine analogues. However, co-administration of P-glycoprotein and/or MRP inhibitors with pentamidine, or other diamidines, might provide a means of improving efficacy against CNS stage HAT.

14896. **Smith, T. K., Young, B. L., Denton, H., Hughes, D. L. & Wagner, G. K., 2009**. First small molecular inhibitors of *T. brucei* dolicholphosphate mannose synthase (DPMS), a validated drug target in African sleeping sickness. *Bioorganic and Medicinal Chemistry Letters,* **19** (6): 1749-1752.

Centre for Biomolecular Sciences, The North Haugh, The University, St. Andrews, Scotland, UK.

Drug-like molecules with activity against *Trypanosoma brucei* are urgently required as potential therapeutics for the treatment of African sleeping sickness. Starting from known inhibitors of other glycosyltransferases, we have developed the first small molecular inhibitors of dolicholphosphate mannose synthase (DPMS), a mannosyltransferase critically involved in glycoconjugate biosynthesis in *T. brucei*. We show that these DPMS inhibitors prevent the biosynthesis of glycosylphosphatidylinositol (GPI) anchors, and possess trypanocidal activity against live trypanosomes.

14897. **Steverding, D. & Wang, X., 2009**. Evaluation of anti-sleeping-sickness drugs and topoisomerase inhibitors in combination on *Trypanosoma brucei*. *Journal of Antimicrobial Chemotherapy,* **63***:*1293-1295.

BioMedical Research Centre, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich NR4 7TJ, UK.

No abstract available.

14898. **Toriizuka, Y., Kinoshita, E., Kogure, N., Kitajima, M., Ishiyama, A., Otoguro, K., Yamada, H., Omura, S. & Takayama, H., 2008**. New lycorine-type alkaloid from *Lycoris traubii* and evaluation of antitrypanosomal and antimalarial activities of lycorine derivatives. *Bioorganic and Medicinal Chemistry,* **16** (24): 10182-10189.

Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan.

A new lycorine derivative LT1 (4) was isolated from the aerial part and bulbs of *Lycoris traubii* Hayward (*Amaryllidaceae*). Its structure including absolute configuration was established by spectroscopic analysis and semi-synthesis to be 1-O-(3'S)-hydroxybutanoyllycorine. Some lycorine ester derivatives including LT1 were examined for their inhibitory activity against *Trypanosoma brucei brucei*, the parasite associated with sleeping sickness, and against *Plasmodium falciparum*, the causative agent of malaria. Among them, 2-O-acetyllycorine (6) showed the most potent activity against parasitic *T. b. brucei,* and LT1 (4), 1-O-(3'R)-hydroxybutanoyllycorine (8), 1,2-di-O-butanoyllycorine (11), and 1-O-propanoyllycorine (12) showed significant activity against *P. falciparum* in an *in vitro* experiment.

14899. **Vanhamme, L., 2008**. Trypanosome RNA polymerases and transcription factors: sensible trypanocidal drug targets? *Current Drug Targets,* **9** (11): 979-996.

Laboratoire de Parasitologie Moléculaire, Institut de Biologie et Médecine Moléculaire (IBMM), Université Libre de Bruxelles (ULB), 12 rue des Professeurs Jeener et Brachet, 6041 Gosselies, Belgium. [luc.vanhamme@ulb.ac.be].

Trypanosomes and *Leishmania* are the agents of several important parasitic diseases threatening hundreds of million human beings worldwide. As they diverged early in evolution, they display original molecular characteristics. These peculiarities are each defining putative specific targets for anti-parasitic drugs. Transcription displays its lot of unique characteristics in trypanosomes and will be taken as an example to uncover these targets. Unique features of transcription in trypanosomes include constitutive and poly-cistronic transcription by RNA polymerase II as well as transcription of protein-coding genes by RNA polymerase I. It is becoming clear that these unique mechanisms are performed by dedicated molecular players. The first of them have been recently characterized. They are reviewed and their suitability as drug targets is commented.

14900. **Weis, R. & Seebacher, W., 2009**. New bicyclic amines: synthesis and SARs of their action against the causative organisms of malaria and sleeping sickness. *Current Medicinal Chemistry,* **16** (11): 1426-1441.

Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens-University, Universitatsplatz 1, A-8010 Graz, Austria. [we.seebacher@uni-graz.at].

Diaryl-substituted bicyclic amines are a scarcely investigated class of compounds. Only few of them are described and their biological activities are reported poorly. During our work in the field of heterocyclic chemistry, we found that 4-dialkylaminobicyclo[2.2.2]octan-2-ones and -ols show antiprotozoal properties against *Plasmodium falciparum* K(1) and *Trypanosoma brucei rhodesiense*, the causative organisms of tropical malaria and of human African trypanosomiasis. Therefore, we synthesized over 200 derivatives in order to investigate their anti-trypanosomal and anti-plasmodial activities as well as their cytotoxicity using *in vitro* microplate assays. Even if the target and the mechanism of action of these compounds are still unknown, we can at least provide several structure-activity relationships for this interesting class of compounds. Moreover, we achieved a distinct improvement of their antiplasmodial and antitrypanosomal properties.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

14901. **Delespaux, V., Dinka, H., Masumu, J., Van den Bossche, P. & Geerts, S., 2008**. Five-fold increase in *Trypanosoma congolense* isolates resistant to diminazene aceturate over a seven-year period in Eastern Zambia. *Drug Resistance Updates,* **11** (6): 205-209.

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Two groups of *Trypanosoma congolense* isolates collected from cattle in 1996 (n=39) and 2003 (n=38) in the Eastern Province of Zambia were analyzed by BclI-PCR-RFLP to assess the evolution of diminazene aceturate (DA) resistance over a period of seven years. The results show a significant increase of DA resistance in this relatively short period of time. In 1996, among the 39 isolates, 61.5 percent were found sensitive, 12.8 percent resistant and 25.7 percent had a mixed BclI-PCR-RFLP profile. In 2003, among the 38 isolates, 10.5 percent were found sensitive, 63.2 percent were resistant and 26.3 percent showed a mixed BclI-PCR-RFLP profile. *In vivo* tests in mice showed that isolates with a sensitive or mixed RFLP profile were sensitive to DA whereas isolates with a resistant RFLP profile were resistant. Since there are no indications that the drug pressure has increased between 1996 and 2003, it is suggested that genetic exchange of resistance genes might explain the increased frequency of resistance to DA.

14902. **Hamilton, P. B., Adams, E. R., Njiokou, F., Gibson, W. C., Cuny, G. & Herder, S., 2009**. Phylogenetic analysis reveals the presence of the *Trypanosoma cruzi* clade in African terrestrial mammals. *Infection, Genetics and Evolution,* **9** (1): 81-86.

School of Biosciences, University of Exeter, Exeter, UK. [p.b.hamilton@exeter.ac.uk].

Despite the impact of some trypanosome species on human and livestock health, the full diversity of trypanosomes in Africa is poorly understood. A recent study examined the prevalence of trypanosomes among a wide variety of wild vertebrates in Cameroon using species-specific PCR tests, but six trypanosome isolates remained unidentified. Here they have been re-examined using fluorescent fragment length barcoding (FFLB) and phylogenetic analysis of glycosomal glyceraldehyde phosphate dehydrogenase gGAPDH and 18S ribosomal RNA (rRNA) genes. Isolates from a monkey (*Cercopithecus nictitans*) and a palm civet (*Nandinia binotata*) belonged to the *Trypanosoma cruzi* clade, known previously only from New World and Australian terrestrial mammals, and bats from Africa, Europe and South America. Of the four other isolates, three from antelope were identified as *Trypanosoma theileri*, and one from a crocodile as *T. grayi*. This is the first report of trypanosomes of the *T. cruzi* clade in African terrestrial mammals and expands the clade's

known global distribution in terrestrial mammals. Previously it has been hypothesized that African and New World trypanosomes diverged after continental separation, dating the divergence to around 100 million years ago. The new evidence instead suggests that intercontinental transfer occurred well after this, possibly via bats or rodents, allowing these trypanosomes to establish and evolve in African terrestrial mammals, and questioning the validity of calibrating trypanosome molecular trees using continental separation.

14903. **Madeira, M. F., Sousa, M. A., Barros, J. H., Figueiredo, F. B., Fagundes, A., Schubach, A., CC, D. E. P., Faissal, B. N., Fonseca, T. S., Thoma, H. K. & Marzochi, M. C., 2009.** *Trypanosoma caninum* n. sp. (Protozoa: Kinetoplastida) isolated from intact skin of a domestic dog *(Canis familiaris*) captured in Rio de Janeiro, Brazil. *Parasitology,* **136** (4): 411-423.

Laboratório de Vigiläncia em Leishmanioses, Instituto de Pesquisa Clinica Evandro Chagas, Fundaçáo Oswaldo Cruz, Rio de Janeiro, RJ, Brazil. [fatima.madeira@ipec.fiocruz.br].

An unknown *Trypanosoma* species was isolated from an axenic culture of intact skin from a domestic dog captured in Rio de Janeiro, Brazil, which was co-infected with *Leishmania (Viannia) braziliensis*. Giemsa-stained smears of cultures grown in different media revealed the presence of epimastigotes, trypomastigotes, spheromastigotes, transitional stages, and dividing forms (epimastigotes or spheromastigotes). The highest frequency of trypomastigotes was observed in RPMI (15.2 percent) and DMEM (9.2 percent) media containing 5 percent FCS, with a mean length of these forms of 43.0 and 36.0 mµm, respectively. Molecular analysis by sequential application of PCR assays indicated that this trypanosome differs from *Trypanosoma cruzi* and *T. rangeli* when specific primers were applied. On the other hand, a PCR strategy targeted to the D7 domain of 24salpha rDNA, using primers D75/D76, amplified products of about 250 bp in that isolate (stock A-27), different from the amplification products obtained with *T. cruzi* and *T. rangeli*. This organism differs from *T. cruzi* mainly by the size of its trypomastigote forms and kinetoplasts and the absence of infectivity for macrophages and triatomine bugs. It is also morphologically distinct from salivarian trypanosomes reported in Brazil. Isoenzyme analysis at 8 loci demonstrated a very peculiar banding pattern clearly distinct from those of *T. rangeli* and *T. cruzi*. We conclude that this isolate is a new *Trypanosoma* species. The name *T. caninum* is suggested.

14904. **Masumu, J., Geysen, D. & Bossche, P. V., 2009**. Endemic type of animal trypanosomiasis is not associated with lower genotype variability of *Trypanosoma congolense* isolates circulating in livestock. *Research in Veterinary Science.* **In press, corrected proof.**

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In order to verify whether the low impact on livestock production in endemic areas is related to a low number of trypanosome strains circulating in livestock, 37 *Trypanosoma congolense* isolates collected from cattle in 11 sites in an endemic trypanosomiasis area in Eastern Zambia were characterised for genotype variability using a modified amplified

fragment length polymorphism technique (AFLP). Isolates were further cloned to evaluate the occurrence of mixed infections in individuals. The results obtained revealed a high genotype diversity (94.6 percent) among these isolates. Apart from one site, all isolates gave different AFLP profiles in each of the sites. When clones were compared, three (8 percent) of the 37 isolates had mixed infections. These results indicate the circulation of a high number of strains in this trypanosomiasis endemic area despite the low impact the disease has on livestock production.

14905. **Poinar Jr, G., 2008**. *Leptoconops nosopheris* sp. n. (Diptera: *Ceratopogonidae*) and *Paleotrypanosoma burmanicus* gen. n., sp. n. (Kinetoplastida: *Trypanosomatidae*), a biting midge-trypanosome vector association from the Early Cretaceous. *Memórias do Instituto Oswaldo Cruz,* **103** (5): 468-471.

Department of Zoology, Oregon State University, Corvallis, OR 97331, USA. [poinarg@science.oregonstate.edu].

Leptoconops nosopheris sp. n. is described from a blood-filled female biting midge in Early Cretaceous Burmese amber. The new species is characterized by a very elongate terminal flagellomere, elongated cerci, and an indistinct spur on the metatibia. This biting midge contained digenetic trypanosomes (Kinetoplastida: *Trypanosomatidae*) in its alimentary tract and salivary glands. These trypanosomes are described as *Paleotrypanosoma burmanicus* gen. n., sp. n., which represents the first fossil record of a *Trypanosoma* generic lineage.

14906. **Sato, H., Takano, A., Kawabata, H., Une, Y., Watanabe, H. & Mukhtar, M. M., 2009**. *Trypanosoma cf. varani* in an imported ball python (*Python reginus*) from Ghana. *Journal of Parasitology***. e Publication ahead of print, January 12.**

Yamaguchi University and National Institute of Infectious Diseases, Japan and Azabu University and University of Khartoum, Sudan.

Peripheral blood from a ball python (*Python reginus*) imported from Ghana was cultured in Barbour-Stoenner-Kelly (BSK) medium for *Borrelia* sp. isolation, resulting in the prominent appearance of free, and clusters of, trypanosomes in a variety of morphological forms. The molecular phylogenetic characterization of these cultured trypanosomes using the small subunit rDNA indicated that this python was infected with a species closely related to *Trypanosoma varani* Wenyon, 1908, originally described in the Nile monitor lizard (*Varanus niloticus*) from Sudan. Furthermore, nucleotide sequences of glycosomal glyceraldehyde-3-phosphate dehydrogenase gene of both isolates showed few differences. Giemsa-stained blood smears prepared from the infected python 8 months after the initial observation of trypanosomes in haemoculture contained trypomastigotes with a broad body and a short, free flagellum, which most closely resembled to the original description of *T. varani*, or *T. voltariae* Macfie, 1919 recorded in a black-necked spitting cobra (*Naja nigricollis*) from Ghana. It is highly possible that lizards and snakes could share naturally an identical trypanosome species. Alternatively, lizards and snakes in the same region might have closely-related, but distinct, *Trypanosoma* species as a result of sympatric speciation. From multiple viewpoints, including molecular phylogenetic analyses, reappraisal of trypanosome species

from a wide range of reptiles in Africa is needed to clarify the relationship of recorded species or to unmask unrecorded species.

14907. **Yurchenko, V. Y., Lukes, J., Jirku, M. & Maslov, D. A., 2009**. Selective recovery of the cultivation-prone components from mixed trypanosomatid infections: a case of several novel species isolated from *Neotropical heteroptera*. *International Journal of Systemic Evolution and Microbiology,* **59** (4): 893-909.

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Mixed trypanosomatid infections (a simultaneous presence of two or more parasites in the same host) have long been suspected to represent an obstacle for recovering cultures that would faithfully represent original species descriptions. However, without the means to directly compare the parasites in the host and in culture, this would remain just a possibility. Here we have used PCR-based genotyping of spliced leader RNA gene repeats to analyse several novel species of insect trypanosomatids isolated from heteropteran hosts and to compare them with the parasites that had been detected in the gut smears of the same hosts. We have found that, whereas the original infections were dominated by some blastocrithidia-like parasites, most of the respective axenic cultures contained novel species of *Crithidia* and *Leptomonas*. Therefore, we concluded that, in each case, this replacement was caused by differences in cultivation properties between the original predominant blastocrithidia and the less fastidious parasite that was later recovered in culture. The properties of the new organisms, including their morphology and ultrastructure, as well as their phylogenetic affinities within the family, were investigated and used to describe five novel species.

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14908. **Adhiambo, C., Blisnick, T., Toutirais, G., Delannoy, E. & Bastin, P., 2009**. A novel function for the atypical small G protein Rab-like 5 in the assembly of the trypanosome flagellum. *Journal of Cell Science,* **122** (6): 834-841.

Trypanosome Cell Biology Unit, Pasteur Institute and CNRS, Paris, France.

The atypical small G protein Rab-like 5 has been shown to traffic in sensory cilia of *Caenorhabditis elegans*, where it participates in signalling processes but not in cilia construction. In this report, we demonstrate that RABL5 co-localises with intraflagellar transport (IFT) proteins at the basal body and in the flagellum matrix of the protist *Trypanosoma brucei*. RABL5 fused to GFP exhibits anterograde movement in the flagellum of live trypanosomes, suggesting it could be associated with IFT. Accordingly, RABL5 accumulates in the short flagella of the retrograde IFT140(RNAi) mutant and is restricted to the basal body region in the IFT88(RNAi) anterograde mutant, a behaviour that is identical to other IFT proteins. Strikingly, RNAi silencing reveals an essential role for RABL5 in trypanosome flagellum construction. RNAi knock-down produces a phenotype similar to inactivation of retrograde IFT with formation of short flagella that are filled with a high amount of IFT proteins. These data reveal for the first time a functional difference for a

conserved flagellar matrix protein between two different ciliated species and raise questions related to cilia diversity.

14909. **Allen, J. W., Ferguson, S. J. & Ginger, M. L., 2008**. Distinctive biochemistry in the trypanosome mitochondrial intermembrane space suggests a model for stepwise evolution of the MIA pathway for import of cysteine-rich proteins. *FEBS Letters,* **582** (19): 2817-2825.

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Mia 40-dependent disulphide bond exchange is used by animals, yeast, and probably plants for import of small, cysteine-rich proteins into the mitochondrial intermembrane space (IMS). During import, electrons are transferred from the imported substrate to Mia40 then, via the sulphydryl oxidase Erv1, into the respiratory chain. Curiously, however, there are protozoa which contain substrates for Mia 40-dependent import, but lack Mia 40. There are also organisms where Erv1 is present in the absence of respiratory chain components. In accommodating these and other relevant observations pertaining to mitochondrial cell biology, we hypothesise that the ancestral IMS import pathway for disulphide-bonded proteins required only Erv1 (but not Mia 40) and identify parasites in which O_2 is the likely physiological oxidant for Erv1.

14910. **Archer, S., Queiroz, R., Stewart, M. & Clayton, C., 2008**. Trypanosomes as a model to investigate mRNA decay pathways. *Methods in Enzymology,* **448**: 359-377.

Zentrum fur Molekulare Biologie der Universitat Heidelberg, Heidelberg, Germany.

In trypanosomes, individual mRNAs arise by the processing of primary polycistronic transcripts. Consequently, mRNA degradation rates are critical determinants of mRNA abundance. In this chapter, we summarize the various options for genetic manipulation in trypanosomes with the goal of analyzing mRNA stability, including RNA interference. We describe a method for measuring the half-lives of trypanosome mRNAs, including those that are very unstable, and also the isolation of tagged protein-RNA complexes by IgG affinity chromatography. Last, we detail our current methods for RNA analysis with microarrays.

14911. **Barquilla, A., Crespo, J. L. & Navarro, M., 2008**. Rapamycin inhibits trypanosome cell growth by preventing TOR complex 2 formation. *Proceedings of the National Academy of Sciences U S A,* **105** (38): 14579-14584.

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Target of rapamycin (TOR) kinases control cell growth through two functionally distinct multiprotein complexes. TOR complex 1 (TORC1) controls temporal cell growth and is sensitive to rapamycin, whereas TOR complex 2 (TORC2) is rapamycin resistant and

regulates spatial cell growth. Here, we identified two TOR orthologues, *Tb*TOR1 and *Tb*TOR2, in the protozoan parasite *Trypanosoma brucei*, as well as orthologues of the well-known TORC1 and TORC2 partners, KOG1/raptor and AVO3/rictor. *Tb*TOR proteins differ in their functions, subcellular localization, and rapamycin sensitivity. *Tb*TOR1 controls cell growth by regulating cell cycle, nucleolus structure, and protein synthesis, whereas *Tb*TOR2 coordinates cell polarization and cytokinesis. Rapamycin treatment of bloodstream trypanosomes resulted in a pronounced reduction of cell proliferation, with an EC(50) of 152 nM. Unique for a eukaryote, we observed that rapamycin acted exclusively by preventing TORC2 formation, with no effect on TORC1. Our findings on TOR signalling in this protozoan, which is located in a distal position in the eukaryotic cell lineage, highlight the clinical possibilities of rapamycin derivates and provide valuable insights into understanding rapamycin-mediated inhibition of TORC2.

14912. **Barquilla, A. & Navarro, M., 2009**. Trypanosome TOR as a major regulator of cell growth and autophagy. *Autophagy,* **5** (2): 256-258.

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Trypanosomatid protozoa parasites are responsible for tropical diseases, and undergo complex life cycles involving developmental forms adapted to insect vectors and vertebrate hosts. During their life cycle these parasites proceed through different forms in response to dramatic environmental changes and/or developmentally regulated programs. Successful progression of the parasite through its life cycle is highly dependent on the capacity of adaptation to distinct stresses involving processes such as autophagy. In eukaryotes, target of rapamycin (TOR) protein kinases act as a sensor, which integrates the nutritional and energetic status, adjusting cell metabolism and growth. Compromising cell viability in yeast and mammals leads to a reduction of TOR function, triggering processes aimed to overcome unfavourable conditions. This is partly achieved by TOR-mediated regulation of protein synthesis and recycling of cellular components by autophagy. In the last few years, autophagy has been described during developmental differentiation processes in *Trypanosomatidae.* However, no link between TOR signalling, autophagy, and differentiation has been described so far. This addendum is a commentary to the work published by our group, in which we discuss the possible role of TOR kinases, as a controller of cell growth and autophagy, in the regulation of differentiation processes during Trypanosomatids life cycles.

14913. **Barquilla, A. & Navarro, M., 2009**. Trypanosome TOR complex 2 functions in cytokinesis. *Cell Cycle,* **8** (5): 697-699.

Instituto de Parasitologia y Biomedicina Lopez-Neyra, Consejo Superior de Investigaciones Cientificas, CSIC, (Spanish National Research Council), Avda. del Conocimiento s/n, Granada, Spain.

TOR (target of rapamycin) is a kinase of the phosphatidylinositol kinase-related kinase (PIKK) family that controls cell growth in eukaryotes in response to nutrients, energy conditions and growth factors. We have recently identified two trypanosome TOR

orthologues, named *Tb*TOR1 and *Tb*TOR2, and two other proteins with significant homology to yeast or mammalian TORs, named *Tb*TOR-like 1 and *Tb*TOR-like 2. TbTOR1 depletion results in arrest of bloodstream trypanosomes in G(1), concomitant to protein synthesis inhibition; however, *Tb*TOR2 depletion leads to dramatic morphological defects in cell polarization, endocytosis and cytokinesis. Rapamycin inhibits *T. brucei* cell growth by prevention of TORC2 complex formation, without any effect on TORC1 contrary to what generally occurs in other eukaryotes. Based on the unique features of *T. brucei* and its distal position in the eukaryotic cell lineage, we describe our views on the function of the TOR protein as a major regulator of cell growth and cytokinesis and discuss a possible role in the developmental differentiation processes.

14914. **Bercovich, N., Levin, M. J. & Vazquez, M. P., 2009**. The FIP-1 like polyadenylation factor in trypanosomes and the structural basis for its interaction with CPSF30. *Biochemica and Biophysica Research Communications,* **380** (4): 850-855.

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In trypanosomes transcription is polycistronic and individual mRNAs are generated by a trans-splicing/polyadenylation coupled reaction. We identified a divergent trypanosome FIP1-like, a factor required for mRNA 3' end formation from yeasts to human. Here we show that it is a nuclear protein with a dotted distribution essential for trypanosome viability. A strong interaction was found between *Tc*FIP1-like and *Tc*CPSF30, a component of the polyadenylation complex. We determined the specific amino acids in each protein involved in the interaction. Significant differences were found between the trypanosome interaction surface and its human counterpart. Although CPSF30/FIP1 interaction is known in other organisms, this is the first report mapping the interaction surface at the amino acid level.

14915. **Casanova, M., Crobu, L., Blaineau, C., Bourgeois, N., Bastien, P. & Pages, M., 2009**. Microtubule-severing proteins are involved in flagellar length control and mitosis in Trypanosomatids. *Molecular Microbiology,* **71** (6): 1353-1370.

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Microtubules are key players in the biology of Trypanosomatid parasites, not only as classical components of the mitotic spindle, microtubule-organizing centres and flagellum but also as the essential constituent of the cytoskeleton. Their length dynamics are regulated by, among others, microtubule-severing proteins. Four and six genes encoding microtubule-severing proteins can be found bioinformatically in the *Leishmania major* and *Trypanosoma brucei* genome respectively. We investigated all these proteins in these organisms, which include the katanin, katanin-like, spastin and fidgetin, and looked at their subcellular localization as well as their putative function by examining “loss-of-function” phenotypes. The katanin-like KAT60b was found implicated in flagellar length reduction, but not in its size increase, while the katanin p80 subunit appeared clearly involved in cytokinesis. Fidgetin and spastin homologues were both localized in the nucleus: the first as a discrete and variable number of dots during most of the cell cycle, redistributing to the spindle and midbody during

mitosis; the second concentrated as < or = 5 perinucleolar punctuations, similar to the electron-dense plaques identified in *T. brucei*, which were assimilated to kinetochores. This first study of microtubule-severing proteins in 'divergent' eukaryotes gives further insight into the multiple functions of these proteins identified in the hitherto studied models.

14916. **Charriere, F., O'Donoghue, P., Helgadottir, S., Marechal-Drouard, L., Cristodero, M., Horn, E. K., Soll, D. & Schneider, A., 2009.** Dual targeting of a tRNA ASP requires two different aspartyl-tRNA synthetases in *Trypanosoma brucei*. *Journal of Biological Chemistry*. **e Publication ahead of print, April 22.**

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The mitochondrion of the parasitic protozoan *Trypanosoma brucei* does not encode any tRNAs. This deficiency is compensated for by partial import of nearly all of its cytosolic tRNAs. Most trypanosomal aminoacyl-tRNA synthetases are encoded by single copy genes suggesting the use of the same enzyme in the cytosol and in the mitochondrion. However, the *T. brucei* genome encodes two distinct genes for eukaryotic aspartyl-tRNA synthetase (AspRS) even though the cell has a single tRNA(Asp) isoacceptor only. Phylogenetic analysis showed that the two *T. brucei* AspRSs evolved from a duplication early in kinetoplastid evolution and also revealed that 8 other major duplications of AspRS occurred in the eukaryotic domain. RNAi analysis established that both *Tb*-AspRS1 and *Tb*-AspRS2 are essential for growth and required for cytosolic and mitochondrial Asp-tRNA(Asp) formation, respectively. *In vitro* charging assays demonstrated that the mitochondrial *Tb*-AspRS2 aminoacylates both cytosolic and mitochondrial tRNA(Asp), whereas the cytosolic *Tb*-AspRS1 selectively recognizes cytosolic but not mitochondrial tRNA(Asp). This indicates that cytosolic and mitochondrial tRNA(Asp), even though derived from the same nuclear gene, are physically different most likely due to a mitochondria-specific nucleotide modification. Mitochondrial *Tb*-AspRS2 defines a novel group of eukaryotic AspRSs with an extended substrate specificity that is restricted to trypanosomatids and therefore may be exploited as a novel drug target.

14917. **Cliffe, L. J., Kieft, R., Southern, T., Birkeland, S. R., Marshall, M., Sweeney, K. & Sabatini, R., 2009**. JBP1 and JBP2 are two distinct thymidine hydroxylases involved in J biosynthesis in genomic DNA of African trypanosomes. *Nucleic Acids Research,* **37** (5): 1452-1462.

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Genomic DNA of African trypanosomes contains a hypermodified thymidine residue termed base J (beta-d-glucosyl-HOMedU). This modified base is localized primarily to repetitive DNA, namely the telomeres, and is implicated in the regulation of antigenic variation. The base is synthesized in a two-step pathway. Initially, a thymidine residue in DNA is hydroxylated by a thymidine hydroxylase (TH). This intermediate (HOMedU) is then glucosylated to form base J. Two proteins involved in J synthesis, JBP1 (J binding protein 1) and JBP2, contain a putative TH domain related to the family of Fe(2+)/2-oxoglutarate-dependent hydroxylases. We have previously shown that mutations in the TH domain of JBP1 kill its ability to stimulate J synthesis. Here we show that mutation of key residues in

the TH domain of JBP2 ablate its ability to induce *de novo* J synthesis. While the individual JBP1 null and JBP2 null trypanosomes have reduced J levels, the deletion of both JBP1 and JBP2 generates a cell line that completely lacks base J but still contains glucosyl-transferase activity. Reintroduction of JBP2 in the J-null trypanosome stimulates HOMedU formation and site-specific synthesis of base J. We conclude that JBP2 and JBP1 are the TH enzymes involved in J biosynthesis.

14918. **Cordeiro, A. T., Thiemann, O. H. & Michels, P. A., 2009**. Inhibition of *Trypanosoma brucei* glucose-6-phosphate dehydrogenase by human steroids and their effects on the viability of cultured parasites. *Bioorganic and Medicinal Chemistry,* **17** (6): 2483-2489.

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Dehydroepiandrosterone (DHEA) is known as an intermediate in the synthesis of mammalian steroids and a potent uncompetitive inhibitor of mammalian glucose-6-phosphate dehydrogenase (G6PDH), but not of the enzyme from plants and lower eukaryotes. G6PDH catalyzes the first step of the pentose-phosphate pathway supplying cells with ribose 5-phosphate, a precursor of nucleic acid synthesis, and NADPH for biosynthetic processes and protection against oxidative stress. In this paper we demonstrate that also G6PDH of the protozoan parasite *Trypanosoma brucei* is uncompetitively inhibited by DHEA and epiandrosterone (EA), with K(i) values in the lower µM range. A viability assay confirmed the toxic effect of both steroids on cultured *T. brucei* bloodstream form cells. Additionally, RNAi mediated reduction of the G6PDH level in *T. brucei* bloodstream forms validated this enzyme as a drug target against human African trypanosomiasis. Together these findings show that inhibition of G6PDH by DHEA derivatives may lead to the development of a new class of anti-trypanosomatid compounds.

14919. **Denninger, V., Koopmann, R., Muhammad, K., Barth, T., Bassarak, B., Schonfeld, C., Kilunga, B. K. & Duszenko, M., 2008.** Kinetoplastida: model organisms for simple autophagic pathways? *Methods in Enzymology,* **451**: 373-408.

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Phylogenetic analyses based on defined proteins or different RNA species have revealed that the order Kinetoplastida belongs to the early-branching eukaryotes and may thus contain organisms in which complex cellular events are easier to analyze. This view was further supported by results from a bioinformatic survey that suggested that nearly half of the autophagy-related proteins existent in yeast are missing in trypanosomatids. On the other hand, these organisms have evolved a highly sophisticated machinery to escape from the different host immune-response strategies and have learned to cope with extremely variable environmental conditions by morphological and functional reorganization of the cell. For both the stress response and the differentiation processes, autophagy seems to be an indispensable prerequisite. So far autophagy has not been systematically investigated in

trypanosomatids. Here we present technical information on how to handle the different parasites belonging to this order and give an overview of the current status of autophagy research in these organisms.

14920. **de Souza, W., Attias, M. & Rodrigues, J. C., 2009**. Particularities of mitochondrial structure in parasitic protozoa (Apicomplexa and Kinetoplastida). *International Journal of Biochemistry and Cell Biology*. **In press, corrected proof.**

Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, CCS-Bloco G, Ilha do Fundao, 21941-902, Rio de Janeiro-RJ, Brazil; Diretoria de Programas, Instituto Nacional de Metrologia e Qualidade Industrial-INMETRO, Rio de Janeiro, Brazil..

Without mitochondria, eukaryotic cells would depend entirely on anaerobic glycolysis for ATP generation. This also holds true for Protists, both free-living and parasitic. Parasitic Protists include agents of human and animal diseases that have a huge impact on world populations. In the phylum Apicomplexa, several species of *Plasmodium* cause malaria, whereas *Toxoplasma gondii* is a cosmopolite parasite found on all continents. Flagellates of the order Kinetoplastida include the genera *Leishmania* and *Trypanosoma* causative agents of human leishmaniasis and (depending on the species) African trypanosomiasis and Chagas disease. Although clearly distinct in many aspects, the members of these two groups bear a single and usually well developed mitochondrion. The single mitochondrion of Apicomplexa has a dense matrix and many cristae with a circular profile. The organelle is even more peculiar in the order kinetoplastida, exhibiting a condensed network of DNA at a specific position, always close to the flagellar basal body. This arrangement is known as Kinetoplast and the name of the Order is derived from it. Kinetoplastids also bear glycosomes, peroxisomes that concentrate enzymes of the glycolytic cycle. Mitochondrial volume and activity are maximum when glycosomal activity is low and *vice versa*. In both Apicomplexa and Trypanosomatids, mitochondria show particular features that are absent in other eukaryotic organisms. These peculiar features make them an attractive target for therapeutic drugs for the diseases they cause.

14921. **Duclert-Savatier, N., Poggi, L., Miclet, E., Lopes, P., Ouazzani, J., Chevalier, N., Nilges, M., Delarue, M. & Stoven, V., 2009**. Insights into the enzymatic mechanism of 6-phosphogluconolactonase from *Trypanosoma brucei* using structural data and molecular dynamics simulation. *Journal of Molecular Biology,* **366** (3): 868-881.

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Trypanosoma brucei is the causative agent of African sleeping sickness. Current work for the development of new drugs against this pathology includes evaluation of enzymes of the pentose phosphate pathway (PPP), which first requires a clear understanding of their function and mechanism of action. In this context, we focused on *T. brucei* 6-phosphogluconolactonase (*Tb*6PGL), which converts delta-6-phosphogluconolactone into 6-phosphogluconic acid in the second step of the PPP. We have determined the crystal structure

of *Tb*6PGL is complex with two ligands, 6-phosphogluconic acid and citrate, at 2.2 A and 2.0 A resolution, respectively. We have performed molecular dynamics (MD) simulations on *Tb*6PGL in its empty form and complexed with delta-6-phosphogluconolactone, its natural ligand. Analysis of the structural data and MD simulations allowed us to propose a detailed enzymatic mechanism for 6PGL enzymes.

14922. **Dutra, P. M., Dias, F. A., Santos, M. A., Rodrigues, C. O., Romeiro, A., Attias, M., De Souza, W., Lopes, A. H. & Meyer-Fernandes, J. R., 2001.** Secreted phosphatase activities in trypanosomatid parasites of plants modulated by platelet-activating factor. *Phytopathology,* **91** (4): 408-414.

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The secreted phosphatase activities of two trypanosomatid parasites were characterized and compared with supernatants of living cells. The plant parasite *Phytomonas francai* and the phytophagous hemipteran parasite *Herpetomonas* sp. hydrolyzed p-nitrophenylphosphate at a rate of 15.54 and 6.51 nmol Pi/mg of protein per min, respectively. Sodium orthovanadate (N(a)VO(3)) and sodium fluoride (NaF) decreased the phosphatase activities. The phosphatase activity of *P. francai* was drastically diminished (73 percent inhibition) in the presence of sodium tartrate, whereas the phosphatase activity of *Herpetomonas* sp. was inhibited by 23 percent. Cytochemical analysis showed the localization of these enzymes on the external surface and in the flagellar pocket of the two trypanosomatids. Sodium tartrate inhibited this reaction, confirming the biochemical data. Platelet-activating factor modulated the phosphatase activities, inhibiting *P. francai* activity and stimulating *Herpetomonas* sp. phosphatase activity.

14923. **Farr, H. & Gull, K., 2009**. Functional studies of an evolutionarily conserved, cytochrome b5 domain protein reveal a specific role in axonemal organisation and the general phenomenon of post-division axonemal growth in trypanosomes. *Cell Motility and the Cytoskeleton,* **66** (1): 24-35.

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Eukaryotic cilia and flagella are highly conserved structures composed of a canonical 9+2 microtubule axoneme. Several recent proteomic studies of cilia and flagella have been published, including a proteome of the flagellum of the protozoan parasite *Trypanosoma brucei.* Comparing proteomes reveals many novel proteins that appear to be widely conserved in evolution. Amongst these, we found a previously uncharacterised protein which localised to the axoneme in *T. brucei*, and therefore named it trypanosome axonemal protein (TAX)-2. Ablation of the protein using RNA interference in the procyclic form of the parasite has no effect on growth but causes a reduction in motility. Using transmission electron microscopy, various structural defects were seen in some axonemes, most frequently with microtubule doublets missing from the 9+2 arrangement. RNAi knockdown of TAX-2 expression in the bloodstream form of the parasite caused defects in growth and cytokinesis, a further example of the effects caused by loss of flagellar function in bloodstream form *T. brucei.* In procyclic cells we used a new set of vectors to ablate protein expression in cells

expressing a GFP:TAX-2 fusion protein, which enabled us to easily quantify protein reduction and visualise axonemes made before and after RNAi induction. This establishes a useful generic technique but also revealed a specific observation that the new flagellum on the daughter trypanosome continues growth after cytokinesis. Our results provide evidence for TAX-2 function within the axoneme, where we suggest that it is involved in processes linking the outer doublet microtubules and the central pair.

14924. **Fisk, J. C., Sayegh, J., Zurita-Lopez, C., Menon, S., Presnyak, V., Clarke, S. G. & Read, L. K., 2009**. A type III protein arginine methyltransferase from the protozoan parasite *Trypanosoma brucei*. *Journal of Biological Chemistry,* **284** (17): 11590-11600.

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Arginine methylation is a widespread post-translational modification of proteins catalyzed by a family of protein arginine methyltransferases (PRMTs). The ancient protozoan parasite, *Trypanosoma brucei*, possesses five putative PRMTs, a relatively large number for a single-celled eukaryote. Trypanosomatids lack gene regulation at the level of transcription, instead relying on post-transcriptional control mechanisms that act at the levels of RNA turnover, translation, and editing, all processes that likely involve multiple RNA-binding proteins, which are common targets of arginine methylation. Here, we report the characterization of a trypanosome PRMT, TbPRMT7, which is homologous to human PRMT7. Interestingly, trypanosomatids are the only single-celled eukaryotes known to harbour a PRMT7 homologue. *Tb*PRMT7 differs dramatically from all known metazoan PRMT7 homologues in lacking the second AdoMet binding-like domain that is required for activity of the human enzyme. Nevertheless, bacterially expressed *Tb*PRMT7 exhibits robust methyltransferase activity toward multiple targets *in vitro*. High resolution ion exchange chromatography analysis of methylated substrates reveals that *Tb*PRMT7 is a type III PRMT, catalyzing the formation of only monomethylarginine, thereby representing the only exclusively type III PRMT identified to date. *Tb*PRMT7 is expressed in both mammalian and insect stage *T. brucei* and is apparently dispensable for growth in both life cycle stages. The enzyme is cytoplasmically localized and is a component of several higher order complexes *in vivo*. Together, our studies indicate that *Tb*PRMT7 is a Type III PRMT, and its robust activity and presence in numerous complexes suggest it plays multiple roles during the complex *T. brucei* life cycle.

14925. **Forsythe, G. R., McCulloch, R. & Hammarton, T. C., 2009.** Hydroxyurea-induced synchronisation of bloodstream stage *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*, **164** (2): 131-136.

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Synchronisation of the *Trypanosoma brucei* cell cycle proved elusive for many years. A recent report demonstrated that synchronisation of procyclic form cells was possible following treatment with hydroxyurea. Here, that work is extended to the disease-relevant,

mammalian-infective bloodstream stage trypanosome. Treatment of bloodstream stage Lister 427 *T. brucei* cells growing *in vitro* with 10 µg ml^{-1} hydroxyurea for 6h led to an enrichment of cells in S phase. Following removal of the drug, cells proceeded uniformly through one round of the cell cycle, providing a much needed tool to enrich for specific cell cycle stages, in a manner similar to hydroxyurea treatment of procyclic form *T. brucei*.

14926. **Gawryluk, R. M. & Gray, M. W., 2009**. A split and rearranged nuclear gene encoding the iron-sulphur subunit of mitochondrial succinate dehydrogenase in *Euglenozoa*. *BMC Research Notes,* **2**: 16.

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Analyses based on phylogenetic and ultrastructural data have suggested that euglenids (such as *Euglena gracilis*), trypanosomatids and diplonemids are members of a monophyletic lineage termed Euglenozoa. However, many uncertainties are associated with phylogenetic reconstructions for ancient and rapidly evolving groups; thus, rare genomic characters become increasingly important in reinforcing inferred phylogenetic relationships. We discovered that the iron-sulphur subunit (SdhB) of mitochondrial succinate dehydrogenase is encoded by a split and rearranged nuclear gene in *Euglena gracilis* and trypanosomatids, an example of a rare genomic character. The two subgenic modules are transcribed independently and the resulting mRNAs appear to be independently translated, with the two protein products imported into mitochondria, based on the presence of predicted mitochondrial targeting peptides. Although the inferred protein sequences are in general very divergent from those of other organisms, all of the required iron-sulphur cluster-coordinating residues are present. Moreover, the discontinuity in the euglenozoan SdhB sequence occurs between the two domains of a typical, covalently continuous SdhB, consistent with the inference that the euglenozoan "half" proteins are functional. The discovery of this unique molecular marker provides evidence for the monophyly of *Euglenozoa* that is independent of evolutionary models. Our results pose questions about the origin and timing of this novel gene arrangement and the structure and function of euglenozoan SdhB.

14927. **Greig, N., Wyllie, S., Patterson, S. & Fairlamb, A. H., 2009**. A comparative study of methylglyoxal metabolism in trypanosomatids. *Febs Journal,* **276** (2): 376-386.

Division of Biological Chemistry and Drug Discovery, Wellcome Trust Biocentre, College of Life Sciences, University of Dundee, UK.

The glyoxalase system, comprising the metalloenzymes glyoxalase I (GLO1) and glyoxalase II (GLO2), is an almost universal metabolic pathway involved in the detoxification of the glycolytic by-product methylglyoxal to d-lactate. In contrast to the situation with the trypanosomatid parasites *Leishmania major* and *Trypanosoma cruzi*, this trypanothione-dependent pathway is less well understood in the African trypanosome, *Trypanosoma brucei*. Although this organism possesses a functional GLO2, no apparent GLO1 gene could be identified in the *T. brucei* genome. The absence of GLO1 in *T. brucei* was confirmed by the lack of GLO1 activity in whole cell extracts, failure to detect a GLO1-like protein on immunoblots of cell lysates, and lack of d-lactate formation from methylglyoxal as compared to *L. major* and *T. cruzi*. *T. brucei* procyclics were found to be

2.4-fold and 5.7-fold more sensitive to methylglyoxal toxicity than *T. cruzi* and *L. major*, respectively. *T. brucei* also proved to be the least adept of the "Tritryp" parasites in metabolizing methylglyoxal, producing l-lactate rather than d-lactate. Restoration of a functional glyoxalase system by expression of *T. cruzi* GLO1 in *T. brucei* resulted in increased resistance to methylglyoxal and increased conversion of methylglyoxal to d-lactate, demonstrating that GLO2 is functional *in vivo*. Procyclic forms of *T. brucei* possess NADPH-dependent methylglyoxal reductase and NAD(+)-dependent l-lactaldehyde dehydrogenase activities sufficient to account for all of the methylglyoxal metabolized by these cells. We propose that the predominant mechanism for methylglyoxal detoxification in the African trypanosome is via the methylglyoxal reductase pathway to l-lactate.

14928. **Harrington, J. M., Howell, S. & Hajduk, S. L., 2009**. Membrane permeabilization by trypanosome lytic factor, a cytolytic human high-density lipoprotein. *Journal of Biological Chemistry***, 284**(20): 13505-13512.

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Trypanosome lytic factor (TLF) is a subclass of human high-density lipoprotein (HDL) that mediates an innate immune killing of certain mammalian trypanosomes, most notably *Trypanosoma brucei brucei*, the causative agent of a wasting disease in cattle. Mechanistically, killing is initiated in the lysosome of the target trypanosome where the acidic pH facilitates a membrane disrupting activity by TLF. Here we utilize a model liposome system to characterize the membrane binding and permeabilizing activity of TLF and its protein constituents, haptoglobin related protein (Hpr), apolipoprotein L-1 (apoL-1) and apolipoprotein A-1 (apoA-1). We show that TLF efficiently binds and permeabilizes unilamellar liposomes at lysosomal pH whereas non-lytic human HDL exhibits inefficient permeabilizing activity. Purified, delipidated Hpr and apoL-1 both efficiently permeabilize lipid bilayers at low pH. Trypanosome lytic factor, apoL-1 and apoA-1 exhibit specificity for anionic membranes while Hpr permeabilizes both anionic and zwitterionic membranes. Analysis of the relative particle sizes of susceptible liposomes reveals distinctly different membrane-active behaviour for native TLF and the delipidated protein components. We propose that lysosomal membrane damage in TLF susceptible trypanosomes is initiated by the stable association of the TLF particle with the lysosomal membrane and that this is a property unique to this subclass of human HDL.

14929. **Hart, S. R., Lau, K. W., Hao, Z., Broadhead, R., Portman, N., Huhmer, A., Gull, K., McKean, P. G., Hubbard, S. J. & Gaskell, S. J., 2009.** Analysis of the trypanosome flagellar proteome using a combined electron transfer/collisionally activated dissociation strategy. *Journal of the American Society of Mass Spectrometry,* **20** (2): 167-175.

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The use of electron-transfer dissociation as an alternative peptide ion activation method for generation of protein sequence information is examined here in comparison with

the conventional method of choice, collisionally activated dissociation, using a linear ion trapping instrument. Direct comparability between collisionally and electron-transfer-activated product ion data was ensured by employing an activation-switching method during acquisition, sequentially activating precisely the same precursor ion species with each fragmentation method in turn. Sequest (Thermo Fisher Scientific, San Jose, CA) searching of product ion data generated an overlapping yet distinct pool of polypeptide identifications from the products of collisional and electron-transfer-mediated activation. To provide a highly confident set of protein recognitions, identification data were filtered using parameters that achieved a peptide false discovery rate of 1 percent, with two or more independent peptide assignments required for each protein. The use of electron transfer dissociation (ETD) has allowed us to identify additional peptides where the quality of product ion data generated by collisionally activated dissociation (CAD) was insufficient to infer peptide sequence. Thus, a combined ETD/CAD approach leads to the recognition of more peptides and proteins than are achieved using peptide analysis by CAD- or ETD-based tandem mass spectrometry alone.

14930. **Helm, J. R., Wilson, M. E. & Donelson, J. E., 2009**. Differential expression of a protease gene family in African trypanosomes. *Molecular and Biochemical Parasitology,* **163** (1): 8-18.

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During their life cycle African trypanosomes must quickly adapt to the different environments of the tsetse fly midgut and the mammalian bloodstream by modulating expression of many of their genes. One group of these differentially expressed genes encodes different forms of a major surface protease. Using a luciferase reporter gene transiently or permanently transfected into trypanosomes, we show here that the 3'-UTRs of these protease genes are responsible for their differential expression. Deletion analysis of the 389-bp 3'-UTR of one of the protease genes, MSP-B, demonstrated that it contains a U-rich regulatory region of about 23bp (UCGUCUGUUAUUUCUUAGUCCAG), which suppresses expression of the reporter protein in bloodstream trypanosomes by as much as 25-fold, but has little effect on the reporter expression in procyclic (tsetse fly) trypanosomes. Replacing the entire 3'-UTR with just this 23-bp element mimicked most of the suppression effect of the complete 3'-UTR. Northern blots showed that the 23-bp element influences the steady state RNA level, but not enough to account for the 25-fold suppression effect. Polysome analyses showed that in procyclic trypanosomes more of the total protease mRNA is associated with intermediate-sized and large polysomes than in bloodstream trypanosomes. Thus, the 23-bp element of this protease gene affects both the level of RNA and its translation.

14931. **Hertz-Fowler, C., Figueiredo, L. M., Quail, M. A., Becker, M., Jackson, A., Bason, N., Brooks, K., Churcher, C., Fahkro, S., Goodhead, I., Heath, P., Kartvelishvili, M., Mungall, K., Harris, D., Hauser, H., Sanders, M., Saunders, D., Seeger, K., Sharp, S., Taylor, J. E., Walker, D., White, B., Young, R., Cross, G. A., Rudenko, G., Barry, J. D., Louis, E. J. & Berriman, M., 2008**. Telomeric expression sites are highly conserved in *Trypanosoma brucei*. *PLoS ONE,* **3** (10): e3527.

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Sub-telomeric regions are often under-represented in genome sequences of eukaryotes. One of the best known examples of the use of telomere proximity for adaptive purposes are the bloodstream expression sites (BESs) of the African trypanosome *Trypanosoma brucei*. To enhance our understanding of BES structure and function in host adaptation and immune evasion, the BES repertoire from the Lister 427 strain of *T. brucei* was independently tagged and sequenced. BESs are polymorphic in size and structure but reveal a surprisingly conserved architecture in the context of extensive recombination. Very small BESs do exist and many functioning BESs do not contain the full complement of expression site associated genes (ESAGs). The consequences of duplicated or missing ESAGs, including ESAG9, a newly named ESAG12, and additional variant surface glycoprotein genes (VSGs) were evaluated by functional assays after BESs were tagged with a drug-resistance gene. Phylogenetic analysis of constituent ESAG families suggests that BESs are sequence mosaics and that extensive recombination has shaped the evolution of the BES repertoire. This work opens important perspectives in understanding the molecular mechanisms of antigenic variation, a widely used strategy for immune evasion in pathogens, and telomere biology.

14932. **Holzmuller, P., Grebaut, P., Peltier, J. B., Brizard, J. P., Perrone, T., Gonzatti, M., Bengaly, Z., Rossignol, M., Aso, P. M., Vincendeau, P., Cuny, G., Boulange, A. & Frutos, R., 2008**. Secretome of animal trypanosomes. *Annals of the New York Academy of Sciences,* **1149**: 337-342.

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Animal trypanosomosis is one of the most severe constraints to agricultural development in sub-Saharan Africa and is also an important disease of livestock in Latin America and Asia. The causative agents are various species of protozoan parasites belonging to the genus *Trypanosoma*, among which *T. congolense* and *T. evansi* are the major pathogenic species. The extracellular position of trypanosomes obliges us to consider both the parasite and its excreted/secreted factors in the course of the physiopathologic process. The advent of proteomics led us to propose a comparative approach of the proteome (i.e. the whole parasite content) and the secretome (i.e., naturally excreted/secreted molecules) of *T. congolense* and *T. evansi* with particular attention to common and specific molecules between strains of differing virulence and pathogenicity. The molecular identification of differentially expressed trypanosome molecules correlated with either the virulence process or the pathogenicity will provide new potential molecular targets for improved field diagnosis and chemotherapy of animal trypanosomosis.

14933. **Hury, A., Goldshmidt, H., Tkacz, I. D. & Michaeli, S., 2009**. Trypanosome spliced-leader-associated RNA (SLA1) localization and implications for spliced-leader RNA biogenesis. *Eukaryotic Cell,* **8** (1): 56-68.

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Spliced-leader-associated RNA (SLA1) guides the pseudouridylation at position -12 (relative to the 5' splice site) of the spliced-leader (SL) RNA in all trypanosomatid species. Nevertheless, the exact role of this RNA is currently unknown. Here, we demonstrate that the absence of pseudouridine on *Leptomonas collosoma* SL RNA has only a minor effect on the ability of this RNA to function in trans splicing *in vivo*. To investigate the possible role of SLA1 during SL RNA biogenesis, the structure of the SL RNA was examined in permeable *Trypanosoma brucei* cells depleted for CBF5, the H/ACA pseudouridine synthase, lacking SLA1. Our results suggest that in the absence of SLA1, the SL RNA secondary structure is changed, as was detected by differential sensitivity to oligonucleotide-directed RNase H cleavage, suggesting that the association of SLA1 maintains the SL RNA in a structural form which is distinct from the structure of the SL RNA in the steady state. In *T. brucei* cells depleted for the SL RNA core protein SmD1, SL RNA first accumulates in large amounts in the nucleus and then is expelled to the cytoplasm. Here, we demonstrate by *in vivo* aminomethyltrimethyl UV cross-linking studies that under SmD1 depletion, SLA1 remains bound to SL RNA and escorts the SL RNA to the cytoplasm. *In situ* hybridization with SLA1 and SL RNA demonstrates colocalization between SLA1 and the SL RNA transcription factor tSNAP42, as well as with Sm proteins, suggesting that SLA1 associates with SL RNA early in its biogenesis. These results demonstrate that SLA1 is a unique chaperonic RNA that functions during the early biogenesis of SL RNA to maintain a structure that is most probably suitable for cap 4 modification.

14934. **Izquierdo, L., Atrih, A., Rodrigues, J. A., Jones, D. C. & Ferguson, M. A., 2009**. *Trypanosoma brucei* UDP-glucose:glycoprotein glucosyltransferase has unusual substrate specificity and protects the parasite from stress. *Eukaryotic Cell,* **8** (2): 230-240.

Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.

In this paper, we describe the range of N-linked glycan structures produced by wild-type and glucosidase II null mutant bloodstream form *Trypanosoma brucei* parasites and the creation and characterization of a bloodstream form *Trypanosoma brucei* UDP-glucose:glycoprotein glucosyltransferase null mutant. These analyses highlight peculiarities of the *Trypanosoma brucei* UDP-glucose:glycoprotein glucosyltransferase, including an unusually wide substrate specificity, ranging from Man(5)GlcNAc(2) to Man(9)GlcNAc(2) glycans, and an unusually high efficiency *in vivo*, quantitatively glucosylating the Asn263 N-glycan of variant surface glycoprotein (VSG) 221 and 75 percent of all non-VSG N glycosylation sites. We also show that although *Trypanosoma brucei* UDP-glucose:glycoprotein glucosyltransferase is not essential for parasite growth at 37 ^{o}C, it is essential for parasite growth and survival at 40 ^{o}C. The null mutant was also shown to be hypersensitive to the effects of the N glycosylation inhibitor tunicamycin. Further analysis of bloodstream form *Trypanosoma brucei* under normal conditions and stress conditions suggests that it does not have a classical unfolded protein response triggered by sensing unfolded proteins in the endoplasmic reticulum. Rather, judging by its uniform Grp78/BiP levels, it appears to have an unregulated and constitutively active endoplasmic reticulum protein folding system. We suggest that the latter may be particularly appropriate for this organism, which has an extremely high flux of glycoproteins through its secretory pathway.

14935. **Jackson, A. P., Quail, M. A. & Berriman, M., 2008**. Insights into the genome sequence of a free-living Kinetoplastid: *Bodo saltans* (Kinetoplastida: *Euglenozoa*). *BMC Genomics,* **9**: 594.

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Bodo saltans is a free-living kinetoplastid and among the closest relatives of the trypanosomatid parasites, which cause such human diseases as African sleeping sickness, leishmaniasis and Chagas disease. A *B. saltans* genome sequence will provide a free-living comparison with parasitic genomes necessary for comparative analyses of existing and future trypanosomatid genomic resources. Various coding regions were sequenced to provide a preliminary insight into the bodonid genome sequence relative to trypanosomatid sequences. 0.4 Mbp of *B. saltans* genome were sequenced from 12 distinct regions and contained 178 coding sequences. As in trypanosomatids, introns were absent and percentGC was elevated in coding regions, greatly assisting in gene finding. In the regions studied, roughly 60 percent of all genes had homologues in trypanosomatids, while 28 percent were *Bodo*-specific. Intergenic sequences were typically short, resulting in higher gene density than in trypanosomatids. Although synteny was typically conserved for those genes with trypanosomatid homologues, strict co-linearity was rarely observed because gene order was regularly disrupted by *Bodo*-specific genes. The results show that the *B. saltans* genome contains both sequences homologous to trypanosomatids and sequences never seen before. Structural similarities suggest that its assembly should be solvable, and, although *de novo* assembly will be necessary, existing trypanosomatid projects will provide some guide to annotation. A complete genome sequence will provide an effective ancestral model for understanding the shared and derived features of known trypanosomatid genomes, but it will also identify those kinetoplastid genome features lost during the evolution of parasitism.

14936. **Jetton, N., Rothberg, K. G., Hubbard, J. G., Wise, J., Li, Y., Ball, H. L. & Ruben, L., 2009**. The cell cycle as a therapeutic target against *Trypanosoma brucei*: hesperadin inhibits Aurora kinase-1 and blocks mitotic progression in bloodstream forms. *Molecular Microbiology,* **72** (2): 442-458.

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Aurora kinase family members co-ordinate a range of events associated with mitosis and cytokinesis. Anti-cancer therapies are currently being developed against them. Here, we evaluate whether Aurora kinase-1 (*Tb*AUK1) from pathogenic *Trypanosoma brucei* might be targeted in anti-parasitic therapies as well. Conditional knockdown of *Tb*AUK1 within infected mice demonstrated its essential contribution to infection. An *in vitro* kinase assay was developed which used recombinant trypanosome histone H3 as a substrate. Tandem mass spectroscopy identified a novel phosphorylation site in the carboxyl-tail of recombinant trypanosome histone H3. Hesperadin, an inhibitor of human Aurora B, prevented the phosphorylation of substrate with IC(50) of 40 nM. Growth of cultured bloodstream forms was also sensitive to Hesperadin (IC(50) of 50 nM). Hesperadin blocked nuclear division and cytokinesis but not other aspects of the cell cycle. Consequently, growth arrested cells accumulated multiple kinetoplasts, flagella and nucleoli, similar to the effects of RNAi-

dependent knockdown of *Tb*AUK1 in cultured bloodstream forms cells. Molecular models predicted high-affinity binding of Hesperadin to both conserved and novel sites in *Tb*AUK1. Collectively, these data demonstrate that cell cycle progression is essential for infections with *T. brucei* and that parasite Aurora kinases can be targeted with small-molecule inhibitors.

14937. **Kawahara, T., Siegel, T. N., Ingram, A. K., Alsford, S., Cross, G. A. & Horn, D., 2008**. Two essential MYST-family proteins display distinct roles in histone H4K10 acetylation and telomeric silencing in trypanosomes. *Molecular Microbiology,* **69** (4): 1054-1068.

London School of Hygiene and Tropical Medicine, London, UK.

Chromatin modification is important for virtually all aspects of DNA metabolism but little is known about the consequences of such modification in trypanosomatids, early branching protozoa of significant medical and veterinary importance. MYST-family histone acetyltransferases in other species function in transcription regulation, DNA replication, recombination and repair. *Trypanosoma brucei* HAT3 was recently shown to acetylate histone H4K4 and we now report characterization of all three *T. brucei* MYST acetyltransferases (HAT1-3). First, GFP-tagged HAT1-3 all localize to the trypanosome nucleus. While HAT3 is dispensable, both HAT1 and HAT2 are essential for growth. Strains with HAT1 knock-down display mitosis without nuclear DNA replication and also specific de-repression of a telomeric reporter gene, a rare example of transcription control in an organism with widespread and constitutive polycistronic transcription. Finally, we show that HAT2 is responsible for H4K10 acetylation. By analogy to the situation in *Saccharomyces cerevisiae*, we discuss low-level redundancy of acetyltransferase function in *T. brucei* and suggest that two MYST-family acetyltransferases are essential due to the absence of a Gcn5 homologue. The results are also consistent with the idea that HAT1 contributes to establishing boundaries between transcriptionally active and repressed telomeric domains in *T. brucei*.

14938. **Lee, J. H., Jung, H. S. & Gunzl, A., 2009**. Transcriptionally active TFIIH of the early-diverged eukaryote *Trypanosoma brucei* harbours two novel core subunits but not a cyclin-activating kinase complex. *Nucleic Acids Research*. **Advanced Access, published on line, April 22.**

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Trypanosoma brucei is a member of the early-diverged, protistan family *Trypanosomatidae* and a lethal parasite causing African sleeping sickness in humans. Recent studies revealed that *T. brucei* harbours extremely divergent orthologues of the general transcription factors TBP, TFIIA, TFIIB and TFIIH and showed that these factors are essential for initiating RNA polymerase II-mediated synthesis of spliced leader (SL) RNA, a trans splicing substrate and key molecule in trypanosome mRNA maturation. In yeast and metazoans, TFIIH is composed of a core of seven conserved subunits and the ternary cyclin-

activating kinase (CAK) complex. Conversely, only four TFIIH subunits have been identified in *T. brucei*. Here, we characterize the first protistan TFIIH which was purified in its transcriptionally active form from *T. brucei* extracts. The complex consisted of all seven core subunits but lacked the CAK sub-complex; instead it contained two trypanosomatid-specific subunits, which were indispensable for parasite viability and SL RNA gene transcription. These findings were corroborated by comparing the molecular structures of trypanosome and human TFIIH. While the ring-shaped core domain was surprisingly congruent between the two structures, trypanosome TFIIH lacked the knob-like CAK moiety and exhibited extra densities on either side of the ring, presumably due to the specific subunits.

14939. **Leite, M. S., Thomaz, R., Oliveira, J. H., Oliveira, P. L. & Meyer-Fernandes, J. R., 2009**. *Trypanosoma brucei brucei*: effects of ferrous iron and haeme on ecto-nucleoside triphosphate diphosphohydrolase activity. *Experimental Parasitology,* **121** (2): 137-143.

Laboratório de Bioquímica Celular, Instituto de Bioquímica Medica, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, UFRJ, Cidade Universitaria, Ilha do Fundão, Rio de Janeiro, Brazil.

Trypanosoma brucei brucei is the causative agent of animal African trypanosomiasis, also called nagana. Procyclic vector form resides in the midgut of the tsetse fly, which feeds exclusively on blood. Haemoglobin digestion occurs in the midgut resulting in an intense release of free haeme. In the present study we show that the magnesium-dependent ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) activity of procyclic *T. brucei brucei* is inhibited by ferrous iron and haeme. The inhibition of E-NTPDase activity by ferrous iron, but not by haeme, was prevented by pre-incubation of cells with catalase. However, antioxidants that permeate cells, such as PEG-catalase and N-acetyl-cysteine prevented the inhibition of E-NTPDase by haeme. Ferrous iron was able to induce an increase in lipid peroxidation, while haeme did not. Therefore, both ferrous iron and haeme can inhibit E-NTPDase activity of *T. brucei brucei* by means of formation of reactive oxygen species, but apparently acting through distinct mechanisms.

14940. **Maia da Silva, F., Marcili, A., Lima, L., Cavazzana, M., Jr., Ortiz, P. A., Campaner, M., Takeda, G. F., Paiva, F., Nunes, V. L., Camargo, E. P. & Teixeira, M. M., 2009.** *Trypanosoma rangeli* isolates of bats from Central Brazil: genotyping and phylogenetic analysis enable description of a new lineage using spliced-leader gene sequences. *Acta Tropica,* **109** (3): 199-207.

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Trypanosoma rangeli infects several mammalian orders but has never confidently been described in *Chiroptera*, which are commonly parasitized by many trypanosome species. Here, we described trypanosomes from bats captured in Central Brazil identified as *T. rangeli, T. dionisii, T. cruzimarinkellei* and *T. cruzi*. Two isolates, Tra643 from *Platyrrhinus lineatus* and Tra1719 from *Artibeus planirostris* were identified as *T. rangeli* by morphological, biological and molecular methods, and confirmed by phylogenetic analyses. Analysis using SSU rDNA sequences clustered these bat trypanosomes together with *T.*

rangeli from other hosts, and separated them from other trypanosomes from bats. Genotyping based on length and sequence polymorphism of PCR-amplified intergenic spliced-leader gene sequences assigned Tra1719 to the lineage A whereas Tra643 was shown to be a new genotype and was assigned to the new lineage E. To our knowledge, these two isolates are the earliest *T. rangeli* from bats and the first isolates from Central Brazil molecularly characterized. *Rhodnius stali* captured for this study was found infected by *T. rangeli* and *T. cruzi.*

14941. **Marin, C., Dollet, M., Pages, M. & Bastien, P., 2009**. Large differences in the genome organization of different plant Trypanosomatid parasites (*Phytomonas* sp.) reveal wide evolutionary divergences between taxa. *Infection, Genetics and Evolution,* **9** (2): 235-240.

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All currently known plant trypanosomes have been grouped in the genus *Phytomonas* sp., although they can differ greatly in terms of both their biological properties and effects upon the host. Those parasitizing the phloem sap are specifically associated with lethal syndromes in Latin America, such as, phloem necrosis of coffee, “Heart rot” of coconut and “Marchitez sorpresiva” of oil palm, that inflict considerable economic losses in endemic countries. The genomic organization of one group of *Phytomonas* (D) considered as representative of the genus has been published previously. The present work presents the genomic structure of two representative isolates from the pathogenic phloem-restricted group (H) of *Phytomonas,* analyzed by pulsed field gel electrophoresis followed by hybridization with chromosome-specific DNA markers. It came as a surprise to observe an extremely different genomic organization in this group as compared with that of group D. Most notably, the chromosome number is 7 in this group (with a genome size of 10 Mb) versus 21 in the group D (totalling 25 Mb). These data unravel an unsuspected genomic diversity within plant trypanosomatids that may justify a further debate about their division into different genera.

14942. **Monnerat, S., Clucas, C., Brown, E., Mottram, J. C. & Hammarton, T. C., 2009**. Searching for novel cell cycle regulators in *Trypanosoma brucei* with an RNA interference screen. *BMC Research Notes,* **2**: 46.

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The protozoan parasite *Trypanosoma brucei* is spread by the tsetse fly and causes human African trypanosomiasis. Its cell cycle is complex and not fully understood at the molecular level. The *T. brucei* genome contains over 6 000 protein coding genes with >50 percent having no predicted function. A small scale RNA interference (RNAi) screen was carried out in *Trypanosoma brucei* to evaluate the prospects for identifying novel cycle regulators. Procyclic form *T. brucei* was transfected with a genomic RNAi library and 204 clones isolated. However, only 76 RNAi clones were found to target a protein coding gene of potential interest. These clones were screened for defects in proliferation and cell cycle progression following RNAi induction. Sixteen clones exhibited proliferation defects upon

RNAi induction, with eight clones displaying potential cell cycle defects. To confirm the phenotypes, new RNAi cell lines were generated and characterised for five genes targeted in these clones. While we confirmed that the targeted genes are essential for proliferation, we were unable to unambiguously classify them as cell cycle regulators. Our study identified genes essential for proliferation, but did not, as hoped, identify novel cell cycle regulators. Screening of the RNAi library for essential genes was extremely labour-intensive, which was compounded by the suboptimal quality of the library. For such a screening method to be viable for a large scale or genome wide screen, a new, significantly improved RNAi library will be required, and automated phenotyping approaches will need to be incorporated.

14943. **Mugasa, C. M., Laurent, T., Schoone, G. J., Kager, P. A., Lubega, G. W. & Schallig, H. D., 2009**. Nucleic acid sequence-based amplification with oligochromatography for detection of *Trypanosoma brucei* in clinical samples. *Journal of Clinical Microbiology,* **47** (3): 630-635.

Department of Veterinary Parasitology and Microbiology, Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda.

Molecular tools such as real-time nucleic acid sequence-based amplification (NASBA) and PCR have been developed to detect *Trypanosoma brucei* parasites in blood for the diagnosis of human African trypanosomiasis (HAT). Despite good sensitivity, these techniques are not implemented in HAT control programs due to the high cost of the equipment, which is unaffordable for laboratories in developing countries where HAT is endemic. In this study, a simplified technique, oligochromatography (OC), was developed for the detection of amplification products of *T. brucei* 18S rRNA by NASBA. The *T. brucei* NASBA-OC test has analytical sensitivities of 1- 10 parasites/ml on nucleic acids extracted from parasite culture and 10 parasites/ml on spiked blood. The test showed no reaction with nontarget pathogens or with blood from healthy controls. Compared with the composite standard applied in the present study, i.e., parasitological confirmation of a HAT case by direct microscopy or by microscopy after concentration of parasites using either a microhaematocrit centrifugation technique or a mini-anion-exchange centrifugation technique, NASBA-OC on blood samples had a sensitivity of 73.0 percent (95 percent confidence interval, 60 to 83 percent), while standard expert microscopy had a sensitivity of 57.1 percent (95 percent confidence interval, 44 to 69 percent). On cerebrospinal fluid samples, NASBA-OC had a sensitivity of 88.2 percent (95 percent confidence interval, 75 to 95 percent) and standard microscopy had a sensitivity of 86.2 percent (95 percent confidence interval, 64 to 88 percent). The *T. brucei* NASBA-OC test developed in this study can be employed in field laboratories, because it does not require a thermocycler; a simple heat block or a water bath maintained at two different temperatures is sufficient for amplification.

14944. **Myslyuk, I., Doniger, T., Horesh, Y., Hury, A., Hoffer, R., Ziporen, Y., Michaeli, S. & Unger, R., 2008**. Psiscan: a computational approach to identify H/ACA-like and AGA-like non-coding RNA in trypanosomatid genomes. *BMC Bioinformatics,* **9**: 471.

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Detection of non coding RNA (ncRNA) molecules is a major bioinformatics challenge. This challenge is particularly difficult when attempting to detect H/ACA molecules which are involved in converting uridine to pseudouridine on rRNA in trypanosomes, because these organisms have unique H/ACA molecules (termed H/ACA-like) that lack several of the features that characterize H/ACA molecules in most other organisms. We present here a computational tool called Psiscan, which was designed to detect H/ACA-like molecules in trypanosomes. We started by analyzing known H/ACA-like molecules and characterized their crucial elements both computationally and experimentally. Next, we set up constraints based on this analysis and additional phylogenic and functional data to rapidly scan three trypanosome genomes (*T. brucei, T. cruzi* and *L. major*) for sequences that observe these constraints and are conserved among the species. In the next step, we used minimal energy calculation to select the molecules that are predicted to fold into a lowest energy structure that is consistent with the constraints. In the final computational step, we used a Support Vector Machine that was trained on known H/ACA-like molecules as positive examples and on negative examples of molecules that were identified by the computational analyses but were shown experimentally not to be H/ACA-like molecules. The leading candidate molecules predicted by the SVM model were then subjected to experimental validation. The experimental validation showed 11 molecules to be expressed (4 out of 25 in the intermediate stage and 7 out of 19 in the final validation after the machine learning stage). Five of these 11 molecules were further shown to be *bona fide* H/ACA-like molecules. As snoRNA in trypanosomes are organized in clusters, the new H/ACA-like molecules could be used as starting points to manually search for additional molecules in their neighbourhood. All together this study increased our repertoire by fourteen H/ACA-like and six C/D snoRNAs molecules from *T. brucei* and *L. major*. In addition the experimental analysis revealed that six ncRNA molecules that are expressed are not downregulated in CBF5 silenced cells, suggesting that they have structural features of H/ACA-like molecules but do not have their standard function. We termed this novel class of molecules AGA-like, and we are exploring their function. This study demonstrates the power of tight collaboration between computational and experimental approaches in a combined effort to reveal the repertoire of ncRNA molecules.

14945. **Nett, I. R., Martin, D. M., Miranda-Saavedra, D., Lamont, D., Barber, J. D., Mehlert, A. & Ferguson, M. A., 2009**. The phosphoproteome of bloodstream form *Trypanonosoma brucei,* causative agent of African sleeping sickness. *Molecular and Cellular Proteomics*. **e Publication ahead of print, April 4.**

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The protozoan parasite *Trypanosoma brucei* is the causative agent of human African sleeping sickness and related animal diseases and it has over 170 predicted protein kinases. Protein phosphorylation is a key regulatory mechanism for cellular function that, thus far, has been studied in *T. brucei* principally through putative kinase mRNA knockdown and observation of the resulting phenotype. However, despite the relatively large kinome of this organism, and the demonstrated essentiality of several *T. brucei* kinases, only 8 specific phosphorylation sites have been determined in this organism. Using a gel-free, phosphopeptide-enrichment, based proteomics approach we have performed the first large-scale phosphorylation site analyses for *T. brucei*. Serine, threonine and tyrosine

phosphorylation sites were determined for a cytosolic protein fraction of the bloodstream form of the parasite, resulting in the identification of 491 phosphoproteins based on the identification of 852 unique phosphopeptides and 1 204 phosphorylation sites. The phosphoproteins detected in this study are predicted from their genome annotations to participate in a wide variety of biological processes, including signal transduction, processing of DNA and RNA, protein synthesis and degradation and to a minor extent in metabolic pathways. The analysis of phoshopeptides and phosphorylation sites was facilitated by in-house developed software and this automated approach was validated by manual annotation of spectra of the kinase subset of proteins. Analysis of the cytosolic bloodstream form *T. brucei* kinome revealed the presence of 44 phosphorylated protein kinases in our dataset, which could be classified into the major eukaryotic protein kinase groups by applying a multi-level hidden Markov model (HMM) library of the kinase catalytic domain. Identification of the kinase phosphorylation sites showed conserved phosphorylation sequence motifs in several kinase activation segments, which supports the view that phosphorylation-based signalling is a general and fundamental regulatory process that extends to this highly divergent lower eukaryote.

14946. **Niemann, M., Kaibel, H., Schluter, E., Weitzel, K., Brecht, M. & Goringer, H. U., 2009**. Kinetoplastid RNA editing involves a 3' nucleotidyl phosphatase activity. *Nucleic Acids Research,* **37** (6): 1897-1906.

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Mitochondrial pre-messenger RNAs (pre-mRNAs) in African trypanosomes require RNA editing in order to mature into functional transcripts. The process involves the addition and/or removal of U nucleotides and is mediated by a high-molecular-mass complex, the editosome. Editosomes catalyze the reaction through an enzyme-driven pathway that includes endo/exoribonuclease, terminal uridylate transferase and RNA ligase activities. Here we show that editing involves an additional reaction step, a 3' nucleotidyl phosphatase activity. The activity is associated with the editing complex and we demonstrate that the editosomal proteins *Tb*MP99 and *Tb*MP100 contribute to the activity. Both polypeptides contain endo-exonuclease-phosphatase domains and we show that gene ablation of either one of the two polypeptides is compensated by the other protein. However, simultaneous knockdown of both genes results in trypanosome cells with reduced 3' nucleotidyl phosphatase and reduced editing activity. The data provide a rationale for the exoUase activity of the editosomal protein *Tb*MP42, which generates nonligatable 3' phosphate termini. Opposing phosphates at the two pre-mRNA cleavage fragments likely function as a roadblock to prevent premature ligation.

14947. **Pereira, F. M., Bernardo, P. S., Dias Junior, P. F., Silva, B. A., Romanos, M. T., d'Avila-Levy, C. M., Branquinha, M. H. & Santos, A. L., 2009.** Differential influence of gp63-like molecules in three distinct *Leptomonas* species on the adhesion to insect cells. *Parasitology Research***, 104** (2): 347-353.

Laboratório de Estudos Integrados em Bioquímica Microbiana, Departamento de Microbiologia Geral, Instituto de Microbiologia Prof. Paulo de Góes, Centro de Ciências da Saúde, Bloco E-subsolo, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Parasites belonging to the *Leptomonas* genus have been used as model organisms for studying biochemical, cellular, and genetic processes unique to members of the Trypanosomatidae family. In the present study, the cell-associated and extracellular peptidases of three *Leptomonas* species, *Leptomonas collosoma, Leptomonas samueli*, and *Leptomonas wallacei*, were assayed and characterized by gelatin-sodium dodecyl sulfate polyacrylamide gel electrophoresis. All parasites released metallopeptidases, whereas no cell-associated proteolytic activity could be detected in the cellular extracts from *L. collosoma*. Western blotting probed with a polyclonal antibody raised against gp63 from *Leishmania amazonensis* revealed two major reactive polypeptides of apparent molecular masses of 63 and 52 kDa, with different intensities in cellular extracts and released proteins from the studied trypanosomatids. Flow cytometry and fluorescence microscopy analyses showed that the gp63-like molecules have a surface location. This is the first report on the presence of gp63-like molecules in *L. collosoma, L. samueli*, and *L. wallacei*. The pretreatment of *L. samueli* and *L. wallacei* with anti-gp63 antibody significantly diminished their association index to *Aedes albopictus* cell line (C6/36), suggesting a potential involvement of the gp63-like molecules in the interaction process of these insect trypanosomatids with the vector.

14948. **Prohaska, K. & Williams, N., 2009**. Assembly of the *Trypanosoma brucei* 60S ribosomal subunit nuclear export complex requires trypanosome-specific proteins P34 and P37. *Eukaryotic Cell,* **8** (1): 77-87.

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We previously identified two *Trypanosoma brucei* RNA binding proteins, P34 and P37, and determined that they are essential for proper ribosomal assembly in this organism. Loss of these proteins via RNA interference is lethal and causes a decrease in both 5S rRNA levels and formation of 80S ribosomes, concomitant with a decrease in total cellular protein synthesis. These data suggest that these proteins are involved at some point in the ribosomal biogenesis pathway. In the current study, we have performed subcellular fractionation in conjunction with immune capture experiments specific for 60S ribosomal proteins and accessory factors in order to determine when and where P34 and P37 are involved in the ribosomal biogenesis pathway. These studies demonstrate that P34 and P37 associate with the 60S ribosomal subunit at the stage of the nucleolar 90S particle and remain associated subsequent to nuclear export. In addition, P34 and P37 associate with conserved 60S ribosomal subunit nuclear export factors exportin 1 and Nmd3, suggesting that they are components of the 60S ribosomal subunit nuclear export complex in *T. brucei*. Most significantly, the pre-60S complex does not associate with exportin 1 or Nmd3 in the absence of P34 and P37. These results demonstrate that, although *T. brucei* 60S ribosomal subunits utilize a nuclear export complex similar to that described for other organisms, trypanosome-specific factors are essential to the process.

14949. **Rotureau, B., Morales, M. A., Bastin, P. & Spath, G. F., 2009**. The flagellum-MAP kinase connection in Trypanosomatids: a key sensory role in parasite signaling and development? *Cellular Microbiology*. **e Publication ahead of print, February 4.**

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Trypanosomatid parasites are the causative agents of severe human diseases such as sleeping sickness, Chagas disease and leishmaniases. These microorganisms are transmitted via different insect vectors and hence are confronted to changing environments during their infectious cycle in which they activate specific and complex patterns of differentiation. Several studies in *Trypanosoma brucei* and in different sub-species of *Leishmania* have shed light on the role of mitogen activated protein (MAP) kinases in these processes. Surprisingly, several MAP kinases turned out to be involved in the control of flagellum length in the promastigote stage of *Leishmania.* Recently, a sensory function has been recognized for cilia and flagella in unicellular and multi-cellular eukaryotes. This review aims to stimulate discussions on the possibility that the Trypanosomatid flagellum could act as a sensory organ through the MAP kinase pathway, with the objective to encourage investigation of this new hypothesis through a series of proposed experimental approaches.

14950. **Sakurai, T., Tanaka, M., Kawazu, S. & Inoue, N., 2009**. Establishment of an *in vitro* transgene expression system in epimastigotes of *Trypanosoma congolense*. *Parasitology International,* **58** (1): 110-113.

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

Trypanosoma congolense epimastigote forms (EMFs) adhere to the tsetse fly proboscis, proliferate, and differentiate into animal-infective metacyclic forms (MCFs). This differentiation step, called metacyclogenesis, is indispensable for the cyclical transmission of the parasite. Although an *in vitro* metacyclogenesis culture system was established several decades ago, few genetic tools have been utilized to investigate the molecular mechanisms underlying *T. congolense* metacyclogenesis. This study established a transgene expression system using an *in vitro* derived EMF of *T. congolense* IL3000, and the transgenic EMF successfully underwent metacyclogenesis *in vitro*. The newly constructed expression vector pSAK was designed for integration into the alpha-beta tubulin locus, which is tandemly arranged in the *T. congolense* genome. The expression cassette of pSAK/enhanced green fluorescent protein (eGFP) was transfected into the EMF by electroporation. An EMF expressing eGFP was successfully generated and differentiated into an MCF that constitutively expressed eGFP. The *in vitro* metacyclogenesis system in combination with the transgenic EMF technique will be important tools to investigate the molecular mechanisms of metacyclogenesis.

14951. **Sela, D., Yaffe, N. & Shlomai, J., 2008**. Enzymatic mechanism controls redox-mediated protein-DNA interactions at the replication origin of kinetoplast DNA minicircles. *Journal of Biological Chemistry,* **283** (46): 32034-32044.

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Kinetoplast DNA (kDNA) is the mitochondrial DNA of trypanosomatids. Its major components are several thousand topologically interlocked DNA minicircles. Their replication origins are recognized by universal minicircle sequence-binding protein (UMSBP), a CCHC-type zinc finger protein, which has been implicated with minicircle

replication initiation and kDNA segregation. Interactions of UMSBP with origin sequences *in vitro* have been found to be affected by the protein's redox state. Reduction of UMSBP activates its binding to the origin, whereas UMSBP oxidation impairs this activity. The role of redox in the regulation of UMSBP *in vivo* was studied here in synchronized cell cultures, monitoring both UMSBP origin binding activity and its redox state, throughout the trypanosomatid cell cycle. These studies indicated that UMSBP activity is regulated *in vivo* through the cell cycle dependent control of the protein's redox state. The hypothesis that UMSBP's redox state is controlled by an enzymatic mechanism, which mediates its direct reduction and oxidation, was challenged in a multienzyme reaction, reconstituted with pure enzymes of the trypanosomal major redox-regulating pathway. Coupling *in vitro* of this reaction with a UMSBP origin-binding reaction revealed the regulation of UMSBP activity through the opposing effects of tryparedoxin and tryparedoxin peroxidase. In the course of this reaction, tryparedoxin peroxidase directly oxidizes UMSBP, revealing a novel regulatory mechanism for the activation of an origin-binding protein, based on enzyme-mediated reversible modulation of the protein's redox state. This mode of regulation may represent a regulatory mechanism, functioning as an enzyme-mediated, redox-based biological switch.

14952. **Smith, E. E. & Malik, H. S., 2009**. The apolipoprotein L family of programmed cell death and immunity genes rapidly evolved in primates at discrete sites of host-pathogen interactions. *Genome Research*, **19**(5) 850-858.

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Apolipoprotein L1 (APOL1) is a human protein that confers immunity to *Trypanosoma brucei* infections but can be countered by a trypanosome-encoded antagonist SRA. APOL1 belongs to a family of programmed cell death genes whose proteins can initiate host apoptosis or autophagic death. We report here that all six members of the APOL gene family (APOL1-6) present in humans have rapidly evolved in simian primates. APOL6, furthermore, shows evidence of an adaptive sweep during recent human evolution. In each APOL gene tested, we found rapidly evolving codons in or adjacent to the SRA-interacting protein domain (SID), which is the domain of APOL1 that interacts with SRA. In APOL6, we also found a rapidly changing 13-amino-acid cluster in the membrane-addressing domain (MAD), which putatively functions as a pH sensor and regulator of cell death. We predict that APOL genes are antagonized by pathogens by at least two distinct mechanisms: SID antagonists which include SRA that interact with the SID of various APOL proteins, and MAD antagonists that interact with the MAD hinge base of APOL6. These antagonists either block or prematurely cause APOL-mediated programmed cell death of host cells to benefit the infecting pathogen. These putative interactions must occur inside host cells, in contrast to secreted APOL1 that trafficks to the trypanosome lysosome. Hence, the dynamic APOL gene family appears to be an important link between programmed cell death of host cells and immunity to pathogens.

14953. **Stern, M. Z., Gupta, S. K., Salmon-Divon, M., Haham, T., Barda, O., Levi, S., Wachtel, C., Nilsen, T. W. & Michaeli, S., 2009**. Multiple roles for polypyrimidine tract binding (PTB) proteins in trypanosome RNA metabolism. *Rna*, **15** (4): 648-665.

Bar-Ilan University, Ramat-Gan, Israel.

Trypanosomatid genomes encode for numerous proteins containing an RNA recognition motif (RRM), but the function of most of these proteins in mRNA metabolism is currently unknown. Here, we report the function of two such proteins that we have named PTB1 and PTB2, which resemble the mammalian polypyrimidine tract binding proteins (PTB). RNAi silencing of these factors indicates that both are essential for life. PTB1 and PTB2 reside mostly in the nucleus, but are found in the cytoplasm, as well. Microarray analysis performed on PTB1 and PTB2 RNAi silenced cells indicates that each of these factors differentially affects the transcriptome, thus regulating a different subset of mRNAs. PTB1 and PTB2 substrates were categorized bioinformatically, based on the presence of PTB binding sites in their 5' and 3' flanking sequences. Both proteins were shown to regulate mRNA stability. Interestingly, PTB proteins are essential for trans-splicing of genes containing C-rich polypyrimidine tracts. PTB1, but not PTB2, also affects cis-splicing. The specificity of binding of PTB1 was established *in vivo* and *in vitro* using a model substrate. This study demonstrates for the first time that trans-splicing of only certain substrates requires specific factors such as PTB proteins for their splicing. The trypanosome PTB proteins, like their mammalian homologs, represent multivalent RNA binding proteins that regulate mRNAs from their synthesis to degradation.

14954. **Stevens, J. R., 2008**. Kinetoplastid phylogenetics, with special reference to the evolution of parasitic trypanosomes. *Parasite,* **15** (3): 226-232.

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To fully understand the evolutionary history of parasitic kinetoplastids and to understand the context within which the evolution of each parasite group has developed, an understanding not just of the parasites, but of all kinetoplastids is required. Accordingly, this paper provides an overview of kinetoplastid evolution and systematics, including coverage of the proposal by Moreira *et al.* (2004) to divide kinetoplasts into Prokinetoplastina (Ichthyobodo and Perkinsiella) and Metakinetoplastina (other bodonids and trypanosomatids). The implications of such a revision, with regard to correctly identifying outgroup taxa for studies of evolution within taxa of medical importance, are addressed, together with a more detailed review of the evolution and origins of the trypanosomes in the light of new phylogenies, new approaches and revisions in kinetoplastid systematics.

14955. **Vazquez, M. P., Mualem, D., Bercovich, N., Stern, M. Z., Nyambega, B., Barda, O., Nasiga, D., Gupta, S. K., Michaeli, S. & Levin, M. J., 2009**. Functional characterization and protein-protein interactions of trypanosome splicing factors U2AF35, U2AF65 and SF1. *Molecular and Biochemical Parasitology,* **164** (2): 137-146.

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Early in the assembly of eukaryotes the branch-point binding protein (BBP, also called SF1) recognizes the branch point sequence, whereas the heterodimer U2AF, consisting of a 65 and a 35 kDa subunit, contacts the polypyrimidine tract and the AG splice site, respectively. Herein, we identified, cloned and expressed the *Trypanosoma cruzi* and

Trypanosoma brucei U2AF35, U2AF65 and SF1. Trypanosomatid U2AF65 strongly diverged from yeast and human homologues. On the contrary, trypanosomatid SF1 was conserved but lacked the C-terminal sequence present in the mammalian protein. Yeast two hybrid approaches were used to assess their interactions. The interaction between U2AF35 and U2AF65 was very weak or not detectable. However, as in other eukaryotes, the interaction between U2AF65 and SF1 was strong. At the cellular level, these results were confirmed by fractionation and affinity-selection experiments in which SF1 and U2AF65 were affinity-selected with TAP tagged SF1, but not with TAP tagged U2AF35. Silencing one of the three factors affected growth and trans-splicing in the first step of this reaction. Trypanosomes are the first described example of eukaryotic cells in which the interaction of two expressed U2AF factors seemed to be very weak, or not detectable.

14956. **Verplaetse, E., Rigden, D. J. & Michels, P. A., 2009**. Identification, characterization and essentiality of the unusual peroxin 13 from *Trypanosoma brucei*. *Biochimica and Biophysica Acta,* **1793** (3): 516-527.

Research Unit for Tropical Diseases, de Duve Institute, Brussels, Belgium.

Peroxin 13 (PEX13) is one of the components of a peroxisomal membrane complex involved in import of proteins into the matrix of the organelles and has previously been characterized in a variety of organisms. Trypanosomatids (*Trypanosoma, Leishmania*), which are protozoan parasites having peroxisome-like organelles designated as glycosomes, and possess an unusual PEX13 which shares very low sequence identity with others and lacks some typical PEX13 characteristics. It was identified in the databases through its multiple YGx motifs present in a glycine-rich N-terminal region of low sequence complexity. Like other PEX13s, it contains predicted transmembrane segments and a SH3 domain in its C-terminal half. The localization of *T. brucei* PEX13 in the glycosomal membrane was confirmed by expression of a fusion construct with green fluorescent protein, and western blot analysis of purified organelles and membranes. The C-terminal half of the protein was shown to interact with the third of three pentapeptide repeats of the previously characterized PEX5, the receptor of glycosomal proteins with a type 1 peroxisome-targeting signal, and with PEX14, another component of the same peroxisomal protein import complex in the membrane. PEX13 is essential for the parasite; depletion by RNA interference results in mislocalization of glycosomal proteins and death of the parasites.

14957. **Walrad, P., Paterou, A., Acosta-Serrano, A. & Matthews, K. R., 2009**. Differential trypanosome surface coat regulation by a CCCH protein that co-associates with procyclin mRNA cis-elements. *PLoS Pathogens,* **5** (2): e1000317.

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The genome of *Trypanosoma brucei* is unusual in being regulated almost entirely at the post-transcriptional level. In terms of regulation, the best-studied genes are procyclins, which encode a family of major surface GPI-anchored glycoproteins (EP1, EP2, EP3, GPEET) that show differential expression in the parasite's tsetse fly vector. Although procyclin mRNA cis-regulatory sequences have provided the paradigm for post-

transcriptional control in kinetoplastid parasites, trans-acting regulators of procyclin mRNAs are unidentified, despite intensive effort over 15 years. Here we identify the developmental regulator, *Tb*ZFP3, a CCCH-class predicted RNA binding protein, as an isoform-specific regulator of Procyclin surface coat expression in trypanosomes. We demonstrate (i) that endogenous *Tb*ZFP3 shows sequence-specific co-precipitation of EP1 and GPEET, but not EP2 and EP3, procyclin mRNA isoforms, (ii) that ectopic overexpression of *Tb*ZFP3 does not perturb the mRNA abundance of procyclin transcripts, but rather that (iii) their protein expression is regulated in an isoform-specific manner, as evidenced by mass spectrometric analysis of the Procyclin expression signature in the transgenic cell lines. The *Tb*ZFP3 mRNA-protein complex (*Tb*ZFP3mRNP) is identified as a trans-regulator of differential surface protein expression in trypanosomes. Moreover, its sequence-specific interactions with procyclin mRNAs are compatible with long-established predictions for Procyclin regulation. Combined with the known association of *Tb*ZFP3 with the translational apparatus, this study provides a long-sought missing link between surface protein cis-regulatory signals and the gene expression machinery in trypanosomes.

14958. **Weng, J., Aphasizheva, I., Etheridge, R. D., Huang, L., Wang, X., Falick, A. M. & Aphasizhev, R., 2008**. Guide RNA-binding complex from mitochondria of trypanosomatids. *Molecular Cell,* **32** (2): 198-209.

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In the mitochondria of trypanosomatids, the majority of mRNAs undergo massive uracil-insertion/deletion editing. Throughout the processes of pre-mRNA polyadenylation, guide RNA (gRNA) uridylylation and annealing to mRNA, and editing reactions, several multiprotein complexes must engage in transient interactions to produce a template for protein synthesis. Here, we report the identification of a protein complex essential for gRNA stability. The gRNA-binding complex (GRBC) interacts with gRNA processing, editing, and polyadenylation machineries and with the mitochondrial edited mRNA stability (MERS1) factor. RNAi knockdown of the core subunits, GRBC1 and GRBC2, led to the elimination of gRNAs, thus inhibiting mRNA editing. Inhibition of MERS1 expression selectively abrogated edited mRNAs. Homologous proteins unique to the order of Kinetoplastida, GRBC1 and GRBC2, form a stable 200 kDa particle that directly binds gRNAs. Systematic analysis of RNA-mediated and RNA-independent interactions involving the GRBC and MERS1 suggests a unified model for RNA processing in the kinetoplast mitochondria.

14959. **Xiao, Y., McCloskey, D. E. & Phillips, M. A., 2009**. Ornithine decarboxylase and spermidine synthase RNAi-mediated gene silencing in *Trypanosoma brucei* provides insight into the regulation of polyamine biosynthesis. *Eukaryotic Cell,* **8**(5): 747-755.

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Polyamine biosynthesis is a drug target for the treatment of African sleeping sickness, however mechanisms regulating the pathway in *Trypanosoma brucei* are not well understood. Recently we showed that RNAi-mediated gene silencing or inhibition of S-adenosylmethionine decarboxylase (AdoMetDC) led to upregulation of the AdoMetDC activator, prozyme, and ornithine decarboxylase (ODC) proteins. To determine if this regulatory response is specific to AdoMetDC we studied the effects of RNAi-induced silencing of the spermidine synthase (SpdSyn) and ODC genes in bloodstream form T. brucei. Knockdown of either gene product led to depletion of polyamine and trypanothione pools, and to cell death. Decarboxylated AdoMet levels were elevated, while AdoMet was not affected. There was no significant effect on protein levels of other polyamine pathway enzymes. Treatment of parasites with the ODC inhibitor alpha-difluoromethylornithine (DFMO) gave similar results to those observed for ODC knockdown. Thus the cellular response to loss of AdoMetDC activity is distinctive, suggesting that AdoMetDC activity controls expression levels of the other spermidine biosynthetic enzymes. ODC RNAi-mediated cell death occurred more rapidly than for SpdSyn. Further the ODC RNAi cells were rescued by putrescine, but not spermidine, suggesting that depletion of both putrescine and spermidine is more detrimental than depletion of spermidine alone. This finding may contribute to the effectiveness of ODC as a target for the treatment of African sleeping sickness, thus providing important insight into the mechanism of action of a key anti-trypanosomal agent.

14960. **Zhaorigetu, S., Wan, G., Kaini, R., Jiang, Z. & Hu, C. A., 2008**. ApoL1, a BH3-only lipid-binding protein, induces autophagic cell death. *Autophagy,* **4** (8): 1079-1082.

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We recently reported the identification and characterization of a novel BH3-only pro-death protein, apolipoprotein L1 (ApoL1), that, when overexpressed, induces autophagic cell death (ACD) in a variety of cells, including those originated from normal and cancerous tissues. ApoL1 failed to induce ACD in autophagy-deficient Atg5(-/-) and Atg7(-/-) MEF cells, suggesting that ApoL1-induced cell death is indeed autophagy-dependent. In addition, a BH3 domain deletion allele of ApoL1 was unable to induce ACD, demonstrating that ApoL1 is a *bona fide* BH3-only pro-death protein. To further investigate regulation of ApoL1 expression, we showed that ApoL1 is inducible by interferon-gamma and tumour necrosis factor-alpha in human umbilical vein endothelial cells, suggesting that ApoL1 may play a role in cytokine-induced inflammatory response. Moreover, we observed that ApoL1 is a lipid-binding protein with high affinity for phosphatidic acid and cardiolipin and less affinity for various phosphoinositides. Functional genomics analysis identified 5 non-synonymous single nucleotide polymorphisms (NSNPs) in the coding exons of the human ApoL1 structural gene-all the 5 NSNPs may cause deleterious alteration of ApoL1 activity. Finally, we discuss the link between ApoL1 and various human diseases.

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