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TSETSE AND TRYPANOSOMOSIS INFORMATION

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TSETSE AND TRYPANOSOMOSIS INFORMATION

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TSETSE AND TRYPANOSOMOSIS INFORMATION

The Tsetse and Trypanosomosis Information periodical has been established to disseminate current information on all aspects of tsetse and trypanosomosis research and control to institutions and individuals involved in the problems of African trypanosomosis. This service forms an integral part of the Programme Against African Trypanosomosis (PAAT) and is jointly sponsored by the Food and Agriculture Organization (FAO) of the United Nations, 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) and the British Government's Department for International Development (DFID).

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 trypanosomosis research and control, and requests for enrolment may be sent to: Ms Maria Grazia Solari, AGAH, FAO, Viale delle Terme di Caracalla, 00100 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*

a.i.	active ingredient
ACTH	adrenocorticotrophic hormone
ALAT	alanine aminotransaminase
ASAT	aspartic acid aminotransaminase
b.w.	body weight
BIIT	blood incubation infectivity test
CATT	card agglutination test for trypanosomiasis
CD_{50}	median curative dose
CNS	central nervous system
CSF	cerebrospinal fluid
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
HAT	human African trypanosomiasis
HCT	haematocrit centrifugation technique
GIS	geographic information system(s)
GPS	global positioning system(s)
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_{50}	median lethal concentration
LD_{50}	median lethal dose
M	molar
mAEC	miniature anion-exchange centrifugation technique
McAb	monoclonal antibody
MW	molecular weight
NARS	National Agricultural Research Services/Systems
p.i.	post-infection
PCR	polymerase chain reaction
PCV	packed cell volume
ppb	parts per billion (10^9)
ppm	parts per million
r.h.	relative humidity
RNA	ribonucleic acid
SIT	sterile insect technique
sp(p).	species (plural)
ssp(p).	subspecies (plural)
UV	ultra-violet
VAT	variable antigen type
VSG	variant surface glycoprotein
WBC	white blood cell

Organizations

ANDE	Agence Nationale de Développement de l'Elevage
AU	African Union
AU/STRC	African Union/Scientific, Technical and Research Commission
BICOT	Biological Control of Tsetse by the Sterile Insect Technique
CEBV	Communauté Economique du Bétail et de la Viande
CEMV	Centre Universitaire de Formation en Entomologie Médicale et Vétérinaire
IAR	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
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

FAO	Food and Agriculture Organization of the United Nations
FITCA	Farming in Tsetse Control Areas of Eastern Africa
GTZ	Deutsche Gesellschaft für Technische Zusammenarbeit
IAEA	International Atomic Energy Agency
IBAR	Interafrican Bureau for Animal Resources
ICIPE	International Centre of Insect Physiology and Ecology
ICPTV	Integrated Control of Pathogenic Trypanosomes and their Vectors
IFAD	International Fund for Agricultural Development
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
KARI	Kenya Agricultural Research Institute
KETRI	Kenya Trypanosomiasis Research Institute
LCV	Laboratoire Central Vétérinaire
LNERV	Laboratoire National de l'Elevage et de Recherches Vétérinaires
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 Trypanosomosis
PATTEC	Pan-African Tsetse and Trypanosomiasis Eradication Campaign
PRCT	Projet de Recherches Cliniques sur la Trypanosomiase
RDI	Rural Development International
RUCA	Rijksuniversitair Centrum Antwerpen
SADC	Southern African Development Community
SIDA	Swedish International Development Authority
SODEPRA	Société pour le Développement des Productions Animales
TDR	UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases
TDRC	Tropical Diseases Research Centre
TPRI	Tropical Pesticides Research Institute
TTRI	Tsetse and Trypanosomiasis Research Institute
UNDP	United Nations Development Programme
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
UTRO	Uganda Trypanosomiasis Research Organisation
WHO	World Health Organization

CONTENTS

SECTION A – NEWS

Peter Van den Bossche

It is with much regret that we announce the death of Dr Peter Van den Bossche (1962) in a ghastly motor accident, on November 11, which was caused by a drunken driver. Peter was one of the few individuals with considerable field and laboratory experience in tsetse and trypanosomosis control in Africa. He served for many years in the ASVEZA project (Assistance to the Veterinary Services in Zambia) in Chipata Zambia, after which he moved to the Regional Project on Tsetse and Trypanosomiasis Control (RTTCP) in Southern Africa. He played a key role in this project and contributed significantly to its success. In 2000 Peter joined the Animal Health Department of the Institute of Tropical Medicine in Antwerp and in 2005 he became Head of the Disease Control Unit of the Department. He was also Adjunct Professor at the Veterinary Faculty of the University of Pretoria. Peter was a hard working, highly motivated and very productive researcher, always enthusiastic about his work and remained an optimist. His personal warmth brought him friendship, especially in the family of Tsetse and Trypanosomosis which will sadly miss him. His sudden death is a great loss, in the first place to his wife and three children, the Institute of Tropical Medicine and the Programme Against African Trypanosomosis (PAAT), on whose External Review Panel he served last year, and the whole scientific and tsetse and trypanosomosis community.

CHANGES IN NOMENCLATURE

Readers should note that since the establishment of TTI and various other initiatives related to tsetse and trypanosomiasis (e.g. the Programme Against African Trypanosomiasis, PAAT), FAO now uses the term trypanosomosis to describe the animal disease caused by trypanosomes. The decision to do so is consistent with the decision made in 1990 by the World Federation of Parasitologists to adopt for all parasitic diseases, the principles prepared by a Terminological ad hoc Committee that was set up in 1985 by the World Association for the Advancement of Veterinary Parasitology (WAAVP) to develop principles for a Standardised Nomenclature of Animal Parasitic Diseases (SNOAPAD). Since then the reference to "Animal" was dropped, thereby changing the acronym to SNOPAD, the essential rule proposed being that construction of disease names would be done by adding the suffix "-osis" to the stem of the name of the parasite taxon. Readers interested in the background to FAO's decision should consult both the WAAVP website (http://www.waavp.org/node/40), and the paper by Tibor Kassai published in Veterinary Parasitology in 2006 (http://www.waavp.org/files/Nomenclature%20for%20parasitic%20diseases.pdf).

From now on therefore, TTI is renamed ***Tsetse and Trypanosomosis Information*** and all references to PAAT and to the disease within PAAT and related FAO publications will use the term trypanosomosis. However, unless otherwise communicated officially to FAO, WHO and the IAEA, the names of national and international institutions and programmes containing the term trypanosomiasis are retained. Also retained is the use of the term trypanosomiasis when appearing in the abstracts of scientific papers published in journals and referred to in TTI.

EXTERNAL EVALUATION OF THE PROGRAMME AGAINST AFRICAN TRYPANOSOMOSIS (PAAT)[1]

1. INTRODUCTION

During November-December 2009, FAO commissioned an external evaluation of the inter-Agency (i.e. FAO/AU-IBAR/IAEA/WHO) Programme Against African Trypanosomosis (PAAT) that was established by the 29th FAO Conference in 1997 to assist countries affected by tsetse and trypanosomosis both to understand the constraints and intervene appropriately to improve animal and human health and productivity and thereby promote sustainable agriculture and rural development.

- To collectively identify, develop and disseminate standards, principles, guidelines, information and other strategic tools for assisting all affected African countries to better analyse their policy, strategy and technical options, thereby improving their capacities for prioritising and implementing interventions, *i.e. its normative role*.
- To provide countries and donors - individually, and collectively when dealing with transboundary issues - with "one stop" advisory/quality assurance services for planning and implementing national, bi-national and regional programmes, *i.e. its operational role*.

This evaluation was conducted by a Team consisting of Drs. James Dargie (Animal Production and Health consultant and Leader), Peter Van den Bossche (specialist in Tsetse and Trypanosomosis Interventions based at the Institute of Tropical Medicine, Antwerp, Belgium) and Oumar Diall, specialist in Tsetse Biology and Trypanosomosis Epidemiology based at the Laboratoire Central Vétérinaire, Bamako, Mali). Their report, summarized below for the purposes of informing TTI readers of the main conclusions and recommendations was submitted for consideration to the management of the organizations primarily concerned, as appropriate in January 2010. Readers should note that the views expressed are those of the Evaluation Team alone and may not necessarily reflect those of FAO and other organizations mentioned.

The Terms of Reference of the evaluation were essentially:

- to assess the performance of PAAT since its creation in 1997;
- to provide a considered opinion about its continuing relevance for addressing the current and likely future needs of its stakeholders and beneficiaries against the backdrop of scientific/technical, institutional and political changes within its founding Agencies and the countries and institutions with which they partner; and
- to provide recommendations - primarily to FAO as the principal "driver" of the inter-Agency alliance but also as considered appropriate to others within and outside that alliance - for adjustments to the structures and institutional arrangements that underpin PAAT, and to the planning and implementation of the support provided to it by FAO itself as well as by the other Agencies contributing to the Programme's Secretariat.

[1] See Terminology Changes in News Section

In carrying out the evaluation, the Team visited FAO Headquarters, Burkina Faso, Ghana, Ethiopia and Kenya and had extensive discussions with policy and technical decision-makers dealing with tsetse and trypanosomosis control, and livestock and wider agricultural development issues within these and other organizations/institutions and countries. Discussions by telephone were held with WHO and IAEA members of the PAAT Secretariat and with the PAAT Chairman. The Team was also provided with a rich variety of written materials concerning relevant developments within and outside PAAT, and examined information available on the PAAT and related web sites. Additionally, the Team Leader had the opportunity of presenting and obtaining feedback on the Team's major findings from members of the Panel of PAAT Advisory Group (PAG) Coordinators during their 15th. meeting held in Mombasa, Kenya in December 2009. These discussions coupled with the written inputs served to shape the Team's analyses and considerations and ultimately the conclusions and recommendations it reached concerning both the past performance and future opportunities for PAAT and FAO in assisting African countries and the international community to deal effectively with the direct and indirect consequences of animal and human trypanosomosis. The Team therefore wishes to thank all concerned for sharing their knowledge, experience and perspectives, without which the considerations underpinning and the conclusions and recommendations made in this report would not have been possible.

Particular thanks go to Mr. Raffaele Mattioli of FAO's Animal Production & Health Division (AGA) for his many technical inputs, insightful observations and unflinching commitment to supporting the Team's work, and to Ms Maria Grazia Solari of AGA for carrying out the many associated administrative arrangements in such an efficient and friendly manner. The excellent arrangements made and generous hospitality provided by Dr. R. Saini and other staff of ICIPE at the PAG meeting in Mombasa are also acknowledged.

In carrying out its work, the Evaluation Team noted that as a mechanism for fostering concerted international planning and action, FAO Members recognised two inter-related roles for PAAT:

- To collectively identify, develop and disseminate standards, principles, guidelines, information and other strategic tools for assisting all affected African countries to better analyse their policy, strategy and technical options, thereby improving their capacities for prioritising and implementing interventions, *i.e. its normative role*.
- To provide countries and donors - individually, and collectively when dealing with transboundary issues - with "one stop" advisory/quality assurance services for planning and implementing national, bi-national and regional programmes, *i.e. its operational role*.

To assess its achievements, the Team examined PAAT's original Project Planning Matrix or log frame, noting its expected overall development and intermediate goals, the objective to which PAAT would contribute, the outputs it would generate and the activities that would be conducted to achieve these outputs. Also defined were verifiable indicators for achievement and the assumptions upon which these were based. The Team also examined issues like the structure and funding of PAAT and the changes that had taken place in the external environment since its inception. Particularly noteworthy here were the significant reductions in both government and donor budgets for agriculture in general, and in particular for agricultural R&D, and the endorsement of the ***Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC)*** by the AU Heads of State and Government with its

ultimate objective of eradicating tsetse and trypanosomosis from Africa through a strategy based on final elimination of tsetse populations using the sterile insect technique (SIT).

2. ASSESSMENT OF PAAT'S ACTIVITIES AND OUTPUTS

Output 1: Trypanosomosis and tsetse research and control coordinated

PAAT was expected to generate five outputs to deliver its objective of "Promoting and Facilitating Effective Control of Trypanosomosis". To produce these outputs, PAAT conducted a number of activities on a regular basis (e.g. convened annual meetings of its PC and PAG, attended the bi-annual ISCTRC meetings), it planned and implemented others on the basis of decisions made by the PC and PAG e.g. prepared guidelines, produced the Tsetse and Trypanosomosis Information (TTI) and developed the PAAT Information System (PAAT-IS), and it implemented yet others that were not planned, but were nevertheless considered necessary to achieve its outputs (e.g. harmonization meetings, visits by Secretariat staff to specific organizations and institutes, presentations at conferences etc.). Consultations and other interactions between members of the Secretariat and between the Secretariat and other stakeholders on technical, policy and financial matters were constant and sometimes intense.

Conclusions:

1.1 Individually and collectively, all of these activities - including in recent times the occasional attendance of the PATTEC Coordinator at PAAT meetings served to promote the sharing and exchange of information and views concerning trypanosomosis and tsetse research and control activities among and between the Secretariat and national and international T&T control and research communities. Also, by providing a neutral and relaxed forum that encouraged open discussion on several contentious issues, PAAT unquestionably helped to stop divisions becoming entrenched. *In all these respects, such meetings and the publication of their outputs and outcomes in TTI and on the PAAT web pages contributed greatly to objective information sharing among and co-ordination between stakeholders and identification of priorities for action, and hence towards Output 1.*

1.2. FAO therefore deserves high praise for adhering rigorously to its role as an "honest broker" throughout the history of PAAT. Its efforts to "keep the ship sailing" along sound technical and policy principles, often in the face of efforts by others to exert undue influence through their greater financial resources is recognised by African stakeholders and the donor community alike.

1.3. However, *it was never realistic to expect that PAAT could co-ordinate research and control of T&T at national or sub-regional/regional levels*. As in every other branch of science and technology, this is the rightful role of individual governments, ministries, national and international research institutes and funding bodies. Viewed in this light, PAAT's roles should therefore have been seen as (a) collecting, analysing and synthesising knowledge arising from T&T research and control including its related land use, environmental and socio-economic dimensions, and disseminating this to affected countries and the donor community; (b) identifying policies and options for intervention that could be

considered by individual governments based on available technologies and clear statements of the scientific, technical, financial, legal, managerial, logistical and infrastructural requirements for implementing these technologies (including their integration) through publications and by convening meetings with relevant stakeholders, and (c) assisting countries directly to build the capacities required to make sound policy and strategic decisions concerning interventions.

1.4. Feedback from stakeholders in the countries visited and at the PAG meeting was generally highly positive with respect to the first and to a lesser extent the second of these roles (primarily Outputs 2 and 3 below), particularly considering the resource constraints. *Most of these outputs were considered to be real "jewels" – high in quality, high in relevance and filling real "needs"*, although one or two were felt to be "over the top" in terms of detail and scientific/technical content and would now benefit from simplification for use at field as opposed to middle management levels i.e. from placing greater focus on what people "need" to know and less on what is "nice" to know, including for use in scientific journal publications.

1.5. Nevertheless, *the evaluation Team questions the extent to which PAAT's outputs and recommendations reach certain important groups of stakeholders/end-users- most importantly, those involved in higher level policy-making* e.g. Directors of Veterinary Services, Ministers or Permanent Secretaries of Agriculture, decision-makers within the AU, development banks and other players in the donor community. This concern is based on both the composition of the current membership within the various PAAT bodies, as well as the almost total reliance being placed on using essentially scientific and technical outlets like TTI and the PAAT web site as "communications" media. *In addition to the need to reconsider the Membership and functions of the PAAT structures, the Team therefore concludes that greater effort needs to go into tackling the higher level "policy deficit" within T&T affected countries if PAAT is to fully realize its potential. S*uggestions for how this might be achieved are contained within the Team's recommendations (Section 3).

Output 2: Information on policies, resources and activities effectively managed

The main mechanism for achieving this Output is the PAAT - IS which has been hosted and managed by FAO since 2002. The main purpose of PAAT-IS is to guide strategic and technical decisions on T&T interventions in sub-Saharan Africa. The PAAT-IS is accessible via the FAO website and through the PAAT website and consists of four PAAT-related components (i.e. PAAT Information Resources, PAAT Maps, PAAT GIS and RS, and the PAAT Link) with links to the websites of the other organizations represented on the Secretariat i.e. WHO, IAEA and AU/IBAR.

PAAT-IS comprises:

(i) PAAT Information Resources

Tsetse and Trypanosomosis Information: The TTI (formerly Tsetse and Trypanosomosis Information Quarterly, TTIQ) has been published since 1978, initially by the UK's Overseas Development Administration (ODA), and since 2002 by FAO. From 1989 onwards, this

formerly quarterly (TTIQ) and since 2005 biannual (TTI) publication provides titles and abstracts of scientific publications in various fields of tsetse and trypanosomosis research (including those by members of the PAAT Secretariat), together with the names and addresses of authors, and reports on the outcome of relevant meetings (such as meetings of the PAAT-PC and PAG) and on the activities of international organizations and institutions involved in T&T research and control. The TTI constitutes the most complete database of scientific publications in the field of tsetse and non-tsetse transmitted trypanosomosis and is the major source of information for professionals involved in the field of T&T who have no or limited access to up-to-date libraries or more general web-based databases. The TTI is distributed as hard copies in English and French and can be downloaded from the PAAT website. Its production is supported financially by FAO with contributions currently from WHO and IAEA. All groups consulted by the Evaluation Team gave high priority to continuation of this publication by PAAT.

Training manuals: The FAO tsetse and trypanosomosis Training Manuals are probably the most widely distributed manuals for training of field technicians in T&T control. This is certainly so for the volumes published in the mid 1980s and distributed as hard copies. Only three Training Manuals can be downloaded from the PAAT website.

PAAT Technical Documents: Between 1998 and 2009, PAAT published a total of 13 Technical Documents covering various aspects of T&T control (e.g. parasite management, impact, socio-economics, SIT, mapping). Nine publications are part of the PAAT Technical and Scientific Series, one publication is an FAO Guideline and one is a Research Report published in collaboration with DFID. These documents were written by experts in a particular field (some being members of the PAG) and are regarded as internationally agreed guidelines, strategies and criteria on specific fields/subjects relevant to T&T. They are mostly written in English, have been distributed widely (through the TTI distribution network) and are available through the PAAT website. As noted earlier, stakeholders generally gave high marks to these documents, and the Evaluation Team concurs with these views.

Reports: The PAAT-IS makes available the reports of the annual PAAT Committee and the PAAT- PAG meetings. The Evaluation Team's views on these reports are given under 5.10 and 5.13.

(ii)PAAT Maps

Through the PAAT-IS, maps predicting the distribution of the three tsetse subgenera, the distribution of the tsetse species in each subgenus, the national distribution of tsetse in Ethiopia, Kenya, South Africa, Tanzania, and Uganda, and regional tsetse distribution maps (West Africa) are made available. Predictions of tsetse distribution are based on the logistic regression of fly presence data against a range of often remotely sensed predictor variables such as vegetation and climate but also demographics and agro-ecological predictors.

(iii)PAAT GIS and Remote Sensing (PAAT GIS & RS)

Geographic information systems and remote sensing are playing an increasingly important role in the planning, implementation and evaluation of T&T control operations throughout sub-Saharan Africa. To support the application of these tools, PAAT (supported by an IFAD grant) in collaboration with national and international stakeholders has been instrumental in:

the generation of customized land cover datasets for tsetse habitat mapping

Information on land cover and land use is extremely useful for various aspects of T&T intervention such as mapping of tsetse, environmental and economic impact assessment of control strategies and SARD. To harmonize land cover mapping and facilitate the use of land cover maps, the Land Cover Classification System (LCCS) developed by FAO and UNEP, was applied to T&T land cover mapping. This standardized approach enables comparison and integrated analyses of often incompatible databases and could contribute substantially to harmonization at national and regional levels. The outputs of this activity have been summarized in the PAAT Technical and Scientific Series ("Standardizing Land Cover Mapping for Tsetse and Trypanosomosis Decision Making") and have been published in a peer-reviewed journal.

Besides standardization, PAAT-IS aims to improve access and sharing of data. A technical document also published under the PAAT Technical and Scientific Series ("Geospatial Datasets and Analyses for an Environmental Approach to African Trypanosomosis") provides a review of relevant state-of-the-art global geospatial datasets available in the public domain and examples of applying such spatial datasets in T&T control projects.

PAAT-IS and FAO promote the FAO Geonetwork (www.fao.org/geonetwork/)

This is an open and standardized source of information to share geospatial data and metadata on the internet by making its geospatial datasets (e.g. tsetse distribution maps and land cover maps standardized and customized for T&T informed decision-making) more widely available. Training was also provided to selected PAAT partners for using this data platform and facilitating exchange of information among PATTEC projects. However, the use of FAO GeoNetwork by PAAT partners is still limited. Pro-active efforts from FAO/PAAT-IS are on going and will be further expanded so that the PATTEC Office and PATTEC countries can take full advantage of this structured and standardized data-sharing utility.

The production of maps of the distribution of African human trypanosomosis

▪ The Atlas of Human African Trypanosomiasis is a WHO initiative jointly implemented with FAO within the framework of the PAAT. The Atlas brings together all the information obtained through HAT surveillance exercises and maps the prevalence of HAT at village level. Ultimately, the HAT Atlas will constitute an important database, providing a sound basis for the planning, implementation and monitoring of control interventions.

(iv)The PAAT Link (PAAT-L)

The PAAT-L is an electronic mailing list for sharing and disseminating information among the PAAT-L subscribers. The PAAT-L contains the contacts of more than one hundred people but has not been functional for many years.

Conclusions

- *2.1. The PAAT-Information Resources are an important and unique repository of scientific, technical and wider policy-relevant information on various aspects of tsetse and trypanosomosis.* Through the PAAT website much useful information is available to the entire scientific community. Nevertheless, *considerable scope exists to improve the content of the PAAT website, making it both more comprehensive and dynamic and "outward-looking" in terms of knowledge/information exchange between stakeholders and thereby* ***the international resource*** *for all* involved in T&T initiatives. Examples of needed updating/improvements in coverage include:

    - numerous scientific and technical publications on T&T and related land use issues arising from the work of FAO, WHO and IAEA have appeared in some highly reputable and influential scientific and agricultural development journals. These, like contributions appearing under the PAAT Technical and Scientific Series, not only contribute to PAAT's technical objectives but bring international recognition to the staff and agencies concerned. *They are not, however, to be found on the PAAT website*. This is a missed opportunity which should now be grasped to enhance PAAT's credibility and standing among research institutions, universities and development agencies in Africa and elsewhere; and

    - other "missing links" include the absence of linkages to the websites of international institutions outside of those connected directly with the PAAT Secretariat (e.g. ILRI, ICIPE, CIRDES etc) but which are nevertheless conducting valuable work on T&T, links to national T&T centres, and to other sites with, for example, training and computer-based programmes offering advice on strategies and tactics for tsetse or trypanosomosis interventions. One example is the Tsetse Plan (available at http://www.nri.org/tsetse/Plan/index.html) which helps government planners, NGOs and farmer groups to plan and implement tsetse control activities at different geographic scales.

2.2. PAAT's continued involvement in the collation and publication of the TTI on a twice-yearly basis is crucial for scientists working in this field. The TTI is a unique and probably the most complete database of publications in the field of tsetse and trypanosomosis. The PAAT should be commended for this effort and sufficient funds should continue to be allocated to continue supporting this activity.

2.3. The FAO Training Manuals are essential for those implementing T&T interventions at the field level, and while there may be merit in PAAT itself undertaking the task of their

updating or revision, the Evaluation Team concludes that this is best achieved through ongoing projects supplemented, upon request, by information provided directly or through the PAAT Secretariat.

2.4. The guideline documents available under the PAAT Technical and Scientific series constitute important reference documents produced by authorities in the field of tsetse and trypanosomosis. There are, however, several important gaps to be filled. In particular, several countries are already embarking upon and others are now actively considering using the sequential aerosol technique (SAT) against tsetse flies. *They urgently need guidance on using this technique for area-wide tsetse elimination within PATTEC and other projects.* They also need guidance on conducting environmental risk assessments for pesticides under riverine and other situations.

2.5. The geospatial datasets and maps produced and made available by PAAT to the T&T community constitute an important and valuable baseline for planning and implementing T&T interventions. Nevertheless, more awareness needs to be created to ensure optimal use of these datasets and the long-term sustainability of the activities currently conducted at FAO headquarters needs to be secured.

2.6. Concerns have been expressed on the steps taken to ensure that the information/recommendations contained in PAAT guidelines are implemented and produce better outcomes. Indeed PAG itself also noted that PAAT receives very little feedback from intended beneficiaries/stakeholders concerning its outputs, and feedback from others consulted during country visits suggested that greater attention should be given to improving the validity and ensuring the feasibility of guidelines.

2.7. Considering its role as a technical and policy advisory body, PAAT is expected to respond to queries from its user communities and to inform them about new developments in T&T control. As noted earlier, this crucial role is currently achieved through annual meetings of the PAAT -PC and the PAG and, upon their request, the production of technical documents and guidelines available through the PAAT website. *In future, the PAAT-IS should play a much more visible role in supporting these advisory tasks.*

Output 3: Guidance on policy analysis and strategy formulation

The achievements and conclusions with respect to this Output are inextricably connected to those concerning the management of PAAT (i.e. Output 5, see below). The conclusions and recommendations reached on both Outputs should therefore be considered together**.**

PAAT has developed internationally agreed principles/criteria for prioritising areas for intervention against tsetse and trypanosomosis in the context of SARD. It has also developed and promoted the concepts of area-wide integrated pest management (AW-IPM) to guide the planning and implementation of T&T interventions, including the selection of technologies. The importance of adopting this approach for dealing with transboundary issues has likewise been stressed.

PAAT conveyed these principles, criteria and strategies for intervention to Member States of FAO at its Regional Conference for Africa in Cairo (2002) and again at its Conference in 2003 in Rome.

PAAT has laid the foundations for putting these strategic guidelines, criteria and strategies into place at national and regional levels by supporting, quality-assuring the production, and making available through PAAT-IS the comprehensive guidelines, manuals and land/tsetse distribution maps described above. Prominent examples of such decision-support materials include methodologies for cost-benefit analysis, for collection of baseline data on tsetse distribution and on drug management and parasite resistance in interventions against animal trypanosomosis.

In terms of national and inter-country strategy development and project formulation, FAO and IAEA helped identify the Southern Rift Valley of Ethiopia and the Cotton Belt border area between Mali and Burkina Faso as priority areas for tsetse and trypanosomosis control, and in the case of the former, contributed substantially to fund mobilization.

Conclusions

3.1. PAAT's principles, criteria and strategies for intervention against tsetse and trypanosomosis have been made available to T&T affected countries through a variety of hard copy and electronically available documents, and by organising meetings and making conference presentations. Nevertheless, countries are not always receiving consistent policy and technical guidance with respect to both the livestock/agricultural and human health dimensions of the problem from the many organizations and individuals involved in assisting affected countries, indicating the need for continuing efforts towards harmonization on the part of the PAAT Secretariat and individuals within its other structures.

3.2. *The emphasis given by PAAT in recent years to "area-wide" approaches to T&T management and to the technologies (primarily SIT and more recently to SAT) considered necessary for undertaking interventions over large areas needs to be balanced by the provision of updated advice and capacity-building on policies, strategies and technologies appropriate for small-scale/community-based interventions*. Notwithstanding the importance of "area-wide" principles and of related technologies, the Team concludes that the reality on the ground at present is that the depth of knowledge (including e.g. about the vulnerability to reinvasion of areas identified for interventions), and scale of operations and of financial and other resources required to implement these principles and technologies successfully are beyond the means of many affected countries, donors and certainly livestock owners. *PAAT needs to grasp this reality and assist countries in selecting and pursuing other – and possibly more immediately "do-able"- options for T&T interventions in terms of scale, technologies, combinations and strategies, and considering also relative convenience, costs and suitability for farmers and local organizations.*

Output 4: Strengthened training and capacity building programmes

The Evaluation Team noted the activities that were expected to be undertaken by PAAT in support of this output. They included: assessment of current staff resources in sub-Saharan Africa with respect to trypanosomosis control; identifying training needs and collating training opportunities and activities; providing advice on the content of training courses; developing best practices for training in T&T control; promoting regional exchange programmes; and monitoring staffing levels.

Conclusions

4.1. *The essential conclusion of the Team - corroborated strongly by feedback from stakeholders - is that this was (and remains) a major weakness of PAAT* although policies and strategies, guidelines and criteria for informed decision-making in T&T interventions have been produced, published and widely distributed. In a nutshell, PAAT has not addressed training and capacity-building needs with the intensity and commitment required to ensure the appropriate breadth and depth of coverage required by T&T- affected countries.

4.2. *Neither has it established mechanisms to plan, coordinate and quality-assure training/capacity-building activities among centres/institutions providing such services* (notably ICIPE, ILRI, IAEA and FAO itself), all of which e.g. are conducting training/capacity-building on GIS with limited or no prior consultation among them. *It has also been unable to effectively plan and implement training and capacity-building activities with PATTEC*. Apart from the lack of consultative mechanisms, such institutional deficiencies have arisen from a combination of factors including:

- the (false) premise that providing "information" through web pages, documents etc. is synonymous with building "knowledge" and "capacity";
- the institutional requirement within FAO to concentrate Headquarters efforts on "normative" functions combined with the lack of "field presence" and therefore insufficient awareness of and ability to respond to real country/field needs; and
- funding constraints, arising in part from the low priority given to T&T activities within AGA compared with resources allocated to emerging and other transboundary animal diseases (TADs).

Addressing shortcomings in capacity-building "outreach" and of the funding needed to support such activities are therefore two key challenges now facing PAAT.

Output 5: An efficiently and effectively managed PAAT

PAAT was founded on the basis of "concerted action" by its three UN organizations working together with AU/IBAR to form a Secretariat which would collaborate with international R&D centres, national governments and their ministries and institutes and the donor community to find ways of tackling the national and transboundary dimensions of animal and human trypanosomosis in the most effective and sustainable manner. It was also envisaged that the PAAT Secretariat would work together to develop an annual work plan and provide progress reports for approval and consideration by the PAAT-PC, that it would have a management and evaluation system to monitor its effectiveness, and that it would commission an independent evaluation of its effectiveness and efficiency by the end of 1998.

Conclusions

5.1. *From the outset PAAT's noble - and now even more needed roles - as well as its effectiveness and credibility, foundered because of disagreements among some members of its envisaged Secretariat on policies, strategies, roles and responsibilities.* Although inter-Governmental recognition was also afforded to PAAT by the 15th Session of the World Health Assembly in 1997 and individual officers within the PAAT Secretariat now work harmoniously and constructively, differences between FAO, IAEA and the AU (whether through AU/IBAR or AU-PATTEC whose specific roles and responsibilities with respect to T&T remain unclear) have yet to be fully resolved at the institutional level.

5.2. The unwillingness of AU and IAEA (until 2002) to join PAAT formally was not conducive to the pursuit of "joined up" approaches for assisting African countries and the donor community to tackle the problem through the combination of policies, institutions and technologies envisaged by PAAT. *Unquestionably therefore, PAAT's effectiveness, efficiency and reputation were compromised by "internal technical differences" within FAO itself (i.e. between AGA and AGE, the Joint FAO/IAEA Division) and between FAO and IAEA, coupled with failure on both sides to compromise on what to the Evaluation Team was – and relative to the seriousness of subsequent developments (see 5.3-5.6 below) - turned out to be a "storm in a teacup".*

5.3. The logic behind the unwillingness of the IAEA to formally endorse the alliance (although also attending and otherwise supporting PAAT) is not entirely clear, although the failure to refer to "area-wide approaches", "eradication" and the "SIT" in the 1997 PAAT Memorandum appears to be the root cause. Without pursuing this matter further, the Evaluation Team wishes to record its view that this Memorandum was purely an advocacy document aimed at informing the international community about the purpose of PAAT and its structures; it did not advocate any specific approach or technique, mentioning only drugs and vector control, and surveillance and treatment for the human disease.

5.4. Relevant here also, particularly with respect to PAAT's role in developing and disseminating objective and "do-able" policies and options for intervention, is the part played by the IAEA's Department of Technical Cooperation (IAEA-TC) after the successful eradication of *Glossina austeni* from the island of Zanzibar in 1997 (i.e. at the time PAAT was launched). *Based again on wide feedback from stakeholders within and outside PAAT, the Evaluation Team concludes that through a variety of interventions, the IAEA "oversold" the SIT as a readily-available "silver bullet" for eradicating both animal and human trypanosomosis and fostering wider development in sub-Saharan Africa and indirectly (through the PATTEC Plan of Action with its sole reliance on the SIT) encouraged six countries to enter into substantial loans from the AfDB in 2004 for tsetse eradication activities premised on the use of that technique on an "area-wide" scale.*

5.5. *Unfortunately, awareness appeared to be lacking about the present operational viability of the SIT for dealing with tsetse flies before these projects were submitted and approved for funding, or indeed about the substantial planning, financial, logistical and other requirements/constraints to employing this technique within integrated area-wide pest management approaches for dealing with T&T.*

5.6. Concerning the relationship between PAAT and the AU (i.e. with PATTEC), the Evaluation Team expresses strong appreciation of the substantial effort made by both the PAAT Secretariat and the PAAT Chairman to reach agreement with the PATTEC Coordination Office on principles for identifying priority areas for intervention and the respective roles and responsibilities of PAAT and PATTEC in assisting countries and the donor community in developing and implementing "bankable projects" including for training and capacity-building. During its travels to affected countries and in the course of the PAG Meeting in Mombasa, *the Team also noted the excellent working relations between PAAT and the National PATTEC Coordinators of the countries receiving AfDB support.* However, despite a common end objective, the need to work together in harmony, and a joint press release resulting from a "harmonization workshop" in 2002 committing to close collaboration between the PAAT Secretariat and the PATTEC Coordination Office, *the Team was unable to obtain any reliable indicators of improved working relations between PAAT and the PATTEC Coordination Office* (i.e. in terms of developing proposals for funding, consulting on training and capacity-building needs, preparing and disseminating information for policy and technical decision-makers, donors and the public at large, convening joint meetings with actual or potential donors).

5.7. The Team therefore expresses disappointment about the relationship between PAAT and PATTEC *and concludes that while the PAAT-PATTEC "harmonization process" exists "on paper/in theory", it does not function "in practice". At the same time, and notwithstanding the concern expressed among African stakeholders about this state of affairs, the Team noted the genuine wish of all parties to forget the past and move on constructively together for the benefit of their countries and people.*

5.8. The Team further notes that none of the Minutes of the PAAT-PC meetings contained conclusions or recommendations concerning the technical and logistical viability of the PATTEC Plan of Action. Neither was any recommendation made to the PAAT Secretariat for their respective Agencies to engage formally or informally with AfDB, the AU Commissioner or the Directors of Veterinary Services/Ministries of Agriculture concerning planning and implementation of the above mentioned projects and indeed about the state of affairs concerning PAAT-PATTEC "harmonization" through e.g. their respective Division Directors, Head of the FAO Regional Office or the Headquarters-based Technical Department concerned. It is beyond the scope of this evaluation to fully explore the reasons behind the somewhat "laissez-faire" response of the PC to developments within PATTEC or the apparent unwillingness of the PAAT Secretariat to bring issues of a more political nature to higher authorities within their respective Agencies. *The Evaluation Team concludes nevertheless that PAAT's mandate was (and remains) sufficiently robust for it to more vigorously pursue its own agenda and be less subordinate to outside views, while maintaining its strong commitment to collaboration, objectivity, scientific and technical rigour and transparency*

5.9. *The Evaluation Team found no evidence of annual work plans being prepared by the Secretariat or of the PAAT-PC either requesting or approving these. Neither is there a management and evaluation system in place to monitor effectiveness.*

5.10. *These statements of fact must not, however, be interpreted to mean that PAAT was/is poorly managed – from many standpoints and considering the serious institutional challenges that it has faced, PAAT has been well managed. Indeed,* PAAT's *delivery of "normative tangibles" has been first-rate, and all involved should be congratulated for their commitment to "deliver", even if the structures put in place did not follow "to the letter" what are now considered to be "modern management imperatives" and levels of financial and other resources were never sufficient to deliver on its comprehensive and challenging mandate due to other priorities.*

5.11. While recognizing the rather informal nature of the way PAAT goes about its business and the many advantages of retaining this approach, and also being wary of attempts to introduce undue "rigidity", *the Evaluation Team nevertheless concludes that by making a few simple changes (a) to the background of the participants in PAAT structures, (b) to the planning and implementation of its work, and (c) by introducing a simple/light process for monitoring the delivery of its outputs and outcomes, the management of PAAT and the efficiency and effectiveness of its work can be improved.* To provide just two examples:

- the Team noted strong similarities between the agendas for PC and PAG meetings; this suggests the need to improve the "division of labour" between the two; and
- the repetitive nature of some presentations and the great similarities in the attendees at these meetings; this indicates the need to reconsider agendas and bring new talent into the mix.

While understanding and respecting fully the requirement to retain both continuity and flexibility in the operations of what is essentially a "loose alliance" between Agencies contributing to a common goal through a combination of their Agency-specific work and bringing together their collective knowledge, experience and other inputs, *the Team nevertheless concludes that there is a need to reconsider and clarify the respective roles of the Secretariat, the PC and PAG within the "reinvigorated PAAT" that it would now like to see*. This conclusion is reached also on the basis of the conclusions concerning PAAT's management (see later).

5.12. The structure of PAAT - or rather the changes in structure and *modus operandi* that have taken place since PAAT was established in 1977 - is a further issue for consideration. *The disbanding of the FAO Liaison Officers Network*, arising largely from the loss in 2007 of the post of Tsetse and Trypanosomosis Officer based at the FAO Regional Office in Accra upon retirement of the incumbent, and the inability of the two remaining FAO Officers at Headquarters to attend to the duties associated with that post in addition to their other responsibilities, *led to FAO largely losing its "eyes and ears" in the field. In other words, "bottoms up/ country-driven" perspectives became replaced or at least diluted by "top down/ Headquarters-driven" considerations. This has lead to the perception among national stakeholders that although PAAT is generating extremely valuable and much-needed "normative outputs" for technical and policy decision-making, it is not sufficiently engaged in assisting countries to generate "outcomes" at field level. In effect, countries are not being sufficiently helped to convert the "information" contained in PAAT's unique products into*

"knowledge" through parallel training and capacity-building activities (see also 4.1. and 4.2).

5.13. PAAT's *Research and Development* and its *Policy, Planning and Implementation Modules are not functional.* It was envisaged that Working Groups (each of 8-10 persons) would be established within each of these Modules to act as coordinators of technical advisory groups which would develop appropriate recommendations to the Working Groups (in essence to PAAT-PAG) for providing guidance, support and direction to PAAT on specific topics. Lack of funds, inertia in establishing/unwillingness to participate in advisory groups to support the Working Groups have meant that *Working Groups as such do not exist and that the PAAT-PAG currently consists of 15 individuals acting in their personal capacity with no actual "coordinating" function*. The Team notes that few of these individuals attended the Mombasa and even previous PAG meetings and both on the basis of commitment and future needs of the Programme, it concludes that the time is opportune to re-invigorate PAG through new appointments.

5.14. Substantial bureaucracy surrounds the processes of establishing, receiving nominations for and seeking changes in names for inclusion in FAO Statutory Bodies as well as for arranging meetings of such Bodies (in this case, the PAG). On the other hand, the Evaluation Team is aware of the possible negative financial and political consequences of changing the status of the PAG to something requiring less bureaucracy to operationalise. *It therefore concludes that while the continued existence of a technical advisory body within PAAT (as well as a PAAT-PC) remains essential, the modality through which this is secured as well as its membership should be discussed and decided upon by the Agencies forming the Secretariat.*

5.15. *The Team commends FAO's decision to strengthen PAAT by creating a new position of Livestock Officer (Animal Health-PAAT) as of 2010. It believes that this offers significant opportunities for enhancing further the credibility of PAAT through enhanced normative outputs, the provision of technical support to T&T affected countries for planning and implementing integrated field interventions as well as wider animal disease control packages, and for closer and more effective working relationships with PATTEC, AU/IBAR, IAEA, WHO and others.*

3. RECOMMENDATIONS

3.1. Activities of PAAT

- *PAAT should continue to serve the T&T community. In fact, the need has never been greater for "joined up" thinking and action* to provide leadership and strategic direction to an inter-Agency partnership which was founded on a common vision of identifying and mobilising appropriate technologies, institutional arrangements and policies for assisting African countries in their efforts to reduce poverty and hunger, improve human health and enhance the sustainable management of natural resources by eliminating the constraints to SARD caused by T&T.

- *PAAT should therefore now prepare – and communicate to all stakeholders, including the Governing Bodies of their respective Agencies – an advocacy document ("Strategic Framework") that sets out its logic (including its links to broader development goals e.g. CAADP/MDGs), its vision, and its strategic objectives (i.e. how it will achieve that vision).* This document should also define a set of realistic and measurable outcomes, and both the types of modalities and outputs (i.e. the building blocks/ structures) that will be employed and generated to produce these outcomes, including timeframes for achievement. The aims of this document are to announce the arrival of a new/ re-invigorated PAAT – a Programme working through strengthened collective action and results-based management to increase the impact of its work by cultivating stronger and more dynamic partnerships within the T&T community, including with and among funders and users of PAAT's outputs and expertise.

- *PAAT's future activities, outputs and outcomes should be clearly linked to this Strategic Framework*. This is best achieved by preparing biennial and "results–based" Programmes of Work by the joint Secretariat in consultation with PAG and their approval by the PAAT-PC. *Results should be monitored and evaluated regularly by the PAAT-PC and outstanding issues requiring higher management consideration/intervention within the Agencies forming the Secretariat should be raised promptly at the appropriate higher management level(s).*

- *Most importantly, stakeholders' involvement and input are essential before the PAAT Strategy and Biennial Workplans are finalized, and PAAT should obtain the agreement by all members of its Secretariat agencies on these plans prior to their submission for approval to the PAAT-PC.*

- *Implementing the above requires urgent action by staff at the highest level within FAO and possibly the AfDB to secure the recognition of PAAT by the AU and the active participation of both PATTEC and IBAR in its Secretariat and activities*. It is recommended that as an entry point into negotiations, both the AU and AfDB are informed in writing about FAO's intentions to strengthen its commitment to supporting PATTEC by both increasing staff resources to PAAT and locating many of its services within Africa. The rationale for seeking AU support for PAAT is that collective thinking coupled with joint planning and implementation of activities are "win-win" strategies for PATTEC, AU/IBAR, PAAT and most of all the affected countries themselves, ensuring that the T&T problem is addressed continentally using mutually agreed approaches.

- Since FAO operates a Joint Division with the IAEA, *steps also need to be taken by higher management within both FAO and IAEA to ensure that advocacy/public information material produced within the IAEA on technical options for dealing with T&T that have wider policy implications for agricultural development is both objective and consistent with PAAT's position.*

- *PAAT should more actively promote its activities and achievements* through its website, advocacy pamphlets/leaflets and increased field presence through the new FAO staff member functioning as a "coordinating resource person" who acts in the best interests

of all relevant stakeholders i.e. the countries themselves including through their national PATTEC Coordinators, the PATTEC Coordinating Office, IBAR, FAO, IAEA and WHO. Consideration should also be given to preparing an annual PAAT Newsletter (possibly jointly with PATTEC) containing information that is specifically directed towards national policy-makers in T&T affected countries and the international donor community.

3.2. Structure of PAAT

- *The structure of PAAT should be revised* to reflect present day needs and institutional realities, and with clear and distinct definition of structures and roles and complementarity in mind.

The following are recommended as guidelines:

- ***PAAT Committee:*** should provide leadership, strategic direction and fund-raising support to PAAT, being responsible primarily for the coordination of its policies and activities and monitoring achievement of its objectives. *It should be composed of high-level African policy-makers with a range of technical skills including in livestock development and human health, sustainable development and natural resources management*. Representatives of the donor community should also be included (e.g. AfDB, IFAD, DFID, EC). It is suggested that contacts are made with the Secretariat of NEPAD, the Secretariats of the African Regional Economic Communities (e.g. ECOWAS, SADC, EAC) and/or a sub-regionally balanced selection of Ministries of Agriculture and Health to secure appropriate nominations. It is further suggested that this Committee be restricted to ten persons, that it appoints a Chairperson and that it meets once annually, preferably for two days immediately following the annual PAG meeting.

- ***PAG:*** *should continue as the technical body of PAAT*. Its primary roles should be identification of the needs for guidelines and capacity-building/training at national, sub-regional and regional levels, the preparation, coordination and quality-assurance of funding proposals including for training and capacity-building, and providing advice on these and other matters to the PAAT-PC. Its composition in terms of required expertise and number should be determined by the needs of the T&T and wider development communities, while retaining flexibility to allow the inclusion and/or exclusion of experts as deemed necessary. It is recommended, however (a) that one senior technical advisor (STA) is selected by agreement among members of the Secretariat to Chair PAG meetings and consult with both members of the Secretariat and other PAG members on meeting agendas, work plans etc., (b) that PATTEC National Coordinators of AfDB–funded projects as well as representatives of ILRI, ICIPE and possibly CIRAD serve on PAG to promote the flow of information from and to the field, and (c) that PATTEC Coordinators nominate sub-regional PATTEC focal points (two for West and Central Africa and two from East and Southern Africa) to attend PAAT-PC meetings along with the STA.

- *The new PAAT Officer based in Africa must be proactive in supporting PAG both in determining and in following-up on the needs for PAAT assistance at national, sub-regional and regional levels, as well as in transferring PAAT messages to national authorities. With the disbanding of the FAO Liaison Officers Network, attendance at meetings of national PATTEC Coordinators will be essential for feeding national and sub-regional perspectives into the work of PAG.*

- ***PAAT Secretariat:*** should have one representative each from FAO, WHO, IAEA and AU-IBAR and/or AU-PATTEC (clarification of AU's representation should be obtained by FAO from the relevant AU Commissioner). The FAO representative (either a Headquarters-based staff member or the new appointee to be based in Africa) should continue to be the focal contact point for PAAT and assisted by his/her colleague, preparing all correspondence and actions (as necessary) in collaboration with other members of the Secretariat. Members of the Secretariat should attend both PAAT-PC and PAG meetings

- The ***Research and Development and Policy, Planning and Implementation Modules*** should be abolished.

3.3. Outputs of PAAT

- Besides the roles of PAG and the Secretariat in responding to queries from user communities and informing about new developments in T&T control, *the PAAT-IS should play a much more visible role in facilitating information exchange and dialogue. The new PAAT appointee should take primary responsibility for bringing dynamism to the PAAT-L by sharing information gained through field visits, interactions with PATTEC, AU/IBAR, ILRI; ICIPE and others, creating links to other relevant websites, and both organising and moderating e-mail conferences on specific topics*.

- Moreover and as recommended earlier, *the higher level "policy deficit" needs to be addressed through a targeted information campaign* by, for example, clarifying PAAT's objectives and achievements in leaflets and folders and by inclusion of the "policy level" in the PAAT Committee.

- *High priority should be given by PAAT to developing a position paper/guidelines on using the sequential aerosol technique (SAT)* since several countries are now planning to employ this for area-wide tsetse control or the creation of tsetse-free areas. *They also need guidance on conducting environmental risk assessments for pesticides* under riverine and other situations. Similarly, *the PAAT community would benefit from a technical paper updating knowledge about the trypanocidal drug resistance situation and its management.*

- *Greater attention should be given to improving the validity and ensuring the feasibility of implementing guidelines and other decision-support outputs*. The impact of guidelines could be improved by involving end-users in their development and dissemination and ensuring that training is given to support their implementation.

- *The scientific and technical publications arising from the work of FAO, WHO and IAEA and published in scientific journals should be made accessible to the T&T community via the PAAT website*.

- With regard to training in T&T, *PAAT should develop and manage a database containing a list of institutions offering training in T&T control* (and related disciplines) and describing the types of training offered by each of these institutions. This should be made available on the PAAT website. *PAAT should also become pro-active in planning, coordinating and quality assuring training/capacity building activities* – tasks that should be facilitated by the increased presence of PAAT in the field and the role of the PAG in identifying training needs. The training needs assessment already conducted by ICIPE on the basis of site visits and feedback from a number of countries receiving AfDB support and the resultant proposal for developing, strengthening and providing the technical and managerial expertise required to implement the PATTEC projects, as well as the outline of a course on GIS for tsetse control personnel prepared by AGE are excellent starting points which should be expanded upon and followed up. Moreover, PAAT could establish partnerships with training institutions to provide specific types of training. Since training can be very costly, it is important that PAAT investigates ways in which training can be offered (e.g. through web-based distance learning).

- Concerning the website, while primary responsibility for maintaining this should remain within AGA and some recommendations could be fulfilled through consultancy services, *responsibility for providing the information required to better "populate" the website, including for organizing e-mail conferences should rest with the regional officer*. Training should therefore be provided to the officer concerned during his/her briefing at FAO Headquarters, and this should include several sessions with the person responsible for maintaining the FAO Biotechnology website and running e-mail conferences on that subject.

- *The use of FAO's GeoNetwork by PAAT partners is still limited. Pro-active efforts from FAO/PAAT-IS should continue and be expanded further so that the PATTEC Coordination Office and PATTEC countries can take full advantage of this structured and standardized data-sharing utility*.

3.4. Funding of PAAT

PAAT is seriously under-funded for the job at hand, particularly if, as recommended earlier, it needs to become more "operational" and more engaged in training and capacity-building to enhance its relevance and credibility among both policymakers and middle-level technical managers in T&T affected countries. FAO – already the major contributor to PAAT- has now shown its additional commitment. *Other Agencies forming its Secretariat* (e.g. WHO and the IAEA directly or through the relatively small FAO contribution to tsetse work compared with the total allocated to insect pest control activities) *should therefore consider allocating more Regular Programme resources towards furthering its normative aims. Additionally, members of its Secretariat, individually as well as collectively, should strive to secure funding for national, sub-regional and regional training and capacity-building activities through both*

advocacy with individual or groups of countries and by preparing project proposals for donor funding. Success in obtaining funding for TCPs funded by the Agencies concerned and by external donors should be one of the performance indicators used in the future evaluation of PAAT.

3.5. The Location of the Livestock Officer (PAAT) Post and PAAT Support Services

- Having considered a number of options for the country/institutional location of the new post in Africa, *the Team concludes that the best interests of PAAT, FAO and most of all of T&T affected countries would be best served by posting the appointee in the FAO Regional Office, Accra.* This is based on the need (a) to retain PAAT's policy and technical independence from other national, regional and international institutions, (b) to distance PAAT and FAO from current uncertainties within the AU concerning the respective roles of AU-PATTEC and AU/IBAR in planning and implementing T&T activities, (c) to emphasize the roles of PAAT (and FAO) as being to address the challenges and interests of *all T&T affected countries* (as opposed to focusing on countries located in one particular subregion), and emphasizing also the transboundary, multidisciplinary and sustainable agricultural development dimensions of the problem rather than their national and technology-oriented dimensions, and (e) to better promote policy and technical cooperation among the countries of the region.
- .*In reaching this conclusion, the Team cautions about the need to ensure the strongest possible policy and technical supervision and support to the incumbent of this post from AGA, and within that context, in particular about the need to secure the sustainability of the PAAT Information System, including its PAAT-GIS and RS support components that are currently made possible through IFAD funding*. Both are absolute prerequisites if the raised expectations within the PAAT community and T&T affected countries for a more dynamic and outcome-oriented programme of support are to be realized. Regarding the GIS and RS dimensions to PAAT's work, discussions should take place at the earliest possible opportunity with ICIPE, ILRI, the African Regional Centre for Mapping of Resources for Development (RCMRD) and others with a view to preparing costed options for consideration by the PAAT-PC for continuing this work.

TOWARDS AN ATLAS OF TSETSE AND AFRICAN ANIMAL TRYPANOSOMOSIS

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Up-to-date and detailed information on the geographic distribution of African animal trypanosomosis (AAT) and its biological vector, the tsetse fly, is often inaccurate or absent. This knowledge gap must be addressed, most notably to provide an adequate base-line for the development and implementation of tsetse and AAT control programmes that follow the principles of area-wide integrated pest management. A World Health Organization (WHO)

initiative, the Atlas of human African trypanosomiasis (HAT), jointly implemented with the Food and Agriculture Organization of the United Nations (FAO) in the framework of the Programme against African Trypanosomosis (PAAT), is presently developing global maps of sleeping sickness. The initiative has proved both the need and the feasibility of such continent-wide mapping endeavours.

Drawing on the experience gained during the development of the HAT Atlas, FAO set out to explore the possibility of producing an Atlas of tsetse and AAT, to be jointly implemented with the International Atomic Energy Agency (IAEA). The tsetse and AAT Atlas should aim at assembling the most recent and detailed data on the prevalence of the disease and the occurrence of its vector. Accurate geo-positioning of all input data is the pre-requisite that would allow a range of maps at different scales to be developed. The Atlas would provide much needed information to guide technical and strategic decision making in the field of interventions against tsetse and AAT.

Such a global initiative can not be envisaged without fully involving all PAAT partners active in the field, first and foremost the numerous projects currently being implemented or planned under the umbrella of the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC). Close collaboration with all national and international stakeholders involved in field research and interventions will be critical to the quality of the Atlas. PAAT also considers the Atlas as an appropriate platform to scale up support from FAO and IAEA to affected countries, especially in the fields of data collection, data management and data analysis. Concrete steps have already been taken by both UN agencies to build technical capacity at national level for improved information management and sharing. It is believed that further efforts in this direction will contribute to, and be promoted by, an Atlas of tsetse and AAT.

The preliminary assessment carried out by FAO and IAEA indicates that the Atlas is a very useful initiative that is technically achievable. Avenues to secure the necessary human and financial resources are being explored.

SECTION B - ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

15196. **Baral, T. N., 2010.** Immunobiology of African trypanosomes: need of alternative interventions. *Journal of Biomedicine & Biotechnology,* Article 389153.

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Trypanosomiasis is one of the major parasitic diseases for which control is still far from reality. The vaccination approaches using dominant surface proteins have not been successful, mainly due to antigenic variation of the parasite surface coat. On the other hand, the chemotherapeutic drugs in current use for the treatment of this disease are toxic and problems of resistance are increasing. Therefore, alternative approaches in both treatment and vaccination against trypanosomiasis are needed at this time. To be able to design and develop such alternatives, the biology of this parasite and the host response against the pathogen need

to be studied. These two aspects of this disease together with a few examples of alternative approaches are discussed here.

15197. **Brun, R., Blum, J., Chappuis, F. & Burri, C., 2010.** Human African trypanosomiasis. *Lancet,* **375** (9709): 148-159.

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Human African trypanosomiasis (sleeping sickness) occurs in sub-Saharan Africa. It is caused by the protozoan parasite *Trypanosoma brucei*, transmitted by tsetse flies. Almost all cases are due to *Trypanosoma brucei gambiense*, which is indigenous to west and central Africa. Prevalence is strongly dependent on control measures, which are often neglected during periods of political instability, thus leading to resurgence. With fewer than 12 000 cases of this disabling and fatal disease reported per year, trypanosomiasis belongs to the most neglected tropical diseases. The clinical presentation is complex, and diagnosis and treatment difficult. The available drugs are old, complicated to administer, and can cause severe adverse reactions. New diagnostic methods and safe and effective drugs are urgently needed. Vector control, to reduce the number of flies in existing foci, needs to be organized on a pan-African basis. WHO has stated that if national control programmes, international organizations, research institutes, and philanthropic partners engage in concerted action, elimination of this disease might even be possible.

15198. **Deborggraeve, S. & Buscher, P., 2010.** Molecular diagnostics for sleeping sickness: what is the benefit for the patient? *Lancet Infectious Diseases,* **10** (6): 433-439.

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Sleeping sickness, or human African trypanosomiasis, is a vector-borne disease caused by two subspecies of the protozoan parasite *Trypanosoma brucei*, and is geographically restricted to sub-Saharan Africa. Although the disease causes major public-health and socioeconomic problems among affected populations, sleeping sickness is one of the world's most neglected diseases. Within the rapidly evolving field of biotechnology, many molecular diagnostics have been developed to detect the parasite. These range from conventional, high-tech, and low-tech PCR formats (e.g. isothermal nucleic-acid-amplification techniques), to direct visualization of the parasite's nucleic acids by fluorescent probes. Besides reviewing the most important molecular diagnostics available, we discuss their current role in diagnosis and disease control. Although powerful, molecular diagnostics are confined to research settings and do not reach the patient or national control programmes. The current formats are not applicable to field conditions, and simplification, standardization, and proper test evaluation in the target setting should be the main focus for future development.

15199. **Elsheikha, H. M. & Khan, N. A., 2010.** Protozoa traversal of the blood-brain barrier to invade the central nervous system. *FEMS Microbiology Reviews,* **34**(4) 532-553.

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Neuropathogenic protozoa have evolved strategies to breach the blood-brain barrier and invade the central nervous system. These include transcellular, paracellular and the Trojan horse routes but the associated molecular mechanisms remain incompletely understood. Here, we summarize the current understanding of protozoa penetration across the blood-brain barrier, focusing on *Plasmodium, Babesia, Trypanosoma, Toxoplasma, Acanthamoeba* and *Balamuthia.* Advances in understanding the molecular pathways will offer opportunities for the rational development of novel therapeutic interventions.

15200. **Holzmuller, P., Grebaut, P., Cuny, G. & Biron, D. G., 2010.** Tsetse flies, trypanosomes, humans and animals: what is proteomics revealing about their crosstalks? *Expert Review of Proteomics,* **7** (1): 113-126.

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. [philippe.holzmuller@cirad.fr].

Human and animal African trypanosomoses, or sleeping sickness and nagana, are neglected vector-borne parasitic diseases caused by protozoa belonging to the *Trypanosoma* genus. Advances in proteomics offer new tools to better understand host-vector-parasite crosstalks occurring during the complex parasitic developmental cycle, and to determine the outcome of both transmission and infection. In this review, we summarize proteomics studies performed on African trypanosomes and on the interactions with their vector and mammalian hosts. We discuss the contributions and pitfalls of using diverse proteomics tools, and argue about the interest of pathogenoproteomics, both to generate advances in basic research on the best knowledge and understanding of host-vector-pathogen interactions, and to lead to the concrete development of new tools to improve diagnosis and treatment management of trypanosomoses in the near future.

15201. **Landfear, S. M., 2010**. Glucose transporters in parasitic protozoa. *Methods in Molecular Biology,* **637**: 245-262.

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Glucose and related hexoses play central roles in the biochemistry and metabolism of single-cell parasites such as *Leishmania, Trypanosoma*, and *Plasmodium* that are the causative agents of leishmaniasis, African sleeping sickness, and malaria. Glucose transporters and the genes that encode them have been identified in each of these parasites and their functional properties have been scrutinized. These transporters are related in sequence and structure to mammalian facilitative glucose transporters of the SLC2 family, but they are nonetheless quite divergent in sequence. Hexose transporters have been shown to be essential for the viability of the infectious stage of each of these parasites and thus may represent targets for development of novel anti-parasitic drugs. The study of these transporters also illuminates many aspects of the basic biology of *Leishmania*, trypanosomes, and malaria parasites.

15202**. Molyneux, D., Ndung'u, J. & Maudlin, I., 2010.** Controlling sleeping sickness--"when will they ever learn?" *PLoS Neglected Tropical Diseases,* **4** (5): e609.

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The recent announcement that WHO has approved the use of a combination of nifurtimox and eflornithine to treat chronic Gambian sleeping sickness, caused by *Trypanosoma brucei gambiense*, is a welcome step in the seemingly interminable process of searching for less toxic drugs to treat this devastating disease. Arsenical drugs were first used in 1905; melarsoprol remains the drug most frequently used for late stage disease and is a drug for which resistance is now a major problem.

Over the last 50 years the needs of countries afflicted by sleeping sickness and of the foci of infection have changed little, and neither have our priority needs for research and disease management: cheap point-of-care diagnostics and effective, non-toxic, and affordable drugs for late stage, or stage 2 disease. What is standing in the way of attaining these apparently modest research aims? Surprisingly, one problem is the very nature of the trypanosome and its vector the tsetse fly; these beautiful and biologically fascinating creatures continue to attract considerable research funding, resulting in a burgeoning industry; a PubMed search for *Trypanosoma brucei* reveals 2 624 papers published in the last decade producing outputs that, while admittedly elegant, are remote from the needs of patients from afflicted rural populations and are disproportionate to the sums needed to support research to assist disease management. Could it be that, as development economists suspect, “we have here a silent conspiracy of professional interests whose scientific work is justified on the basis of poverty reduction but who would be devastated if they were actually successful in these terms?”. It would be timely now to take a very hard look at the global research agenda within the context of a forgotten hinterland which, up until the 1960s, demonstrated that this disease could be controlled effectively by unsophisticated means - history all too conveniently forgotten by, or perhaps unknown to, most of the current generation of researchers.

The ability of the medical services to translate effective tools and technologies into public health successes when faced with the devastating epidemics of the past was dependent on dedicated teams, skilled staff, and adequate and appropriate financing. In West Africa, epidemics of Gambian sleeping sickness were controlled by the use of chemoprophylactic treatment or “pentamidisation” of populations led by Jamot and military style campaigns; in East and southern Africa where the authorities were equally concerned with the health of livestock, the diagnosis and treatment approach for Rhodesian sleeping sickness was allied to vector control. Targeted, effective, and appropriate research (supported largely by French and British aid) allied to realistic health service delivery options worked, and by the 1960s sleeping sickness was not considered a significant public health problem. The numbers of new cases each year was minimal and controlled effectively in all endemic countries of West and Central Africa through active screening by mobile teams who diagnosed cases by microscopy (gland puncture and lumbar puncture) and treated patients with pentamidine or suramin and melarsoprol as appropriate. Whilst there were relapses, there was also regular follow-up and the observed trend towards increasingly frequent detection of early disease was

a testament to the effectiveness of the system. For *T. b. gambiense*, diagnosis was improved initially by the use of immunofluorescence tests and later, the more practical card agglutination test for trypanosomiasis (CATT) developed in the 1970s. The CATT test is perhaps the sole relevant product deployed at any scale to emerge from the huge amount of research resources devoted to trypanosome antigenic variation. Yet today, the CATT test remains largely underused, due to the cost of the product and packaging (in units of 50), working out at around US$2 per test.

The launch of the Drugs for Neglected Diseases Initiative (DNDi) has focused attention on the need for new drugs for sleeping sickness as well as other kinetoplastid infections (*T. cruzi* and *Leishmania*). The registration of the nifurtimox/eflornithine combination marks progress in improvement of the treatment option for patients with *T. b. gambiense* - albeit at a snail's pace, given that van Nieuwenhoeve did the initial work on eflornithine in 1985 (he also had the vision to suggest the use of nifurtimox for relapse cases). Seventeen years later, adoption of even a small improvement in treatment regimes is a step forward. As the trypanosome biochemist Jim Williamson so cogently remarked "there have been many more reviews of trypanosome chemotherapy than new drugs". However, the challenge of the eflornithine/nifurtimox option, even if this combination therapy is available as an "essential drug," is classic: transport of a weighty product; the difficulties of intravenous administration in rural settings where health facilities are minimal; drug availability and affordability; the intensity of the specialized medical care required for patients; the monitoring of side effects and the potential for relapses requiring regular follow-up: all costly activities where patients are beyond the end of the road. WHO has reported a significant decline in the numbers of new cases over the last five years, indicating that sleeping sickness is coming under control, but we must add a proviso: data on sleeping sickness deaths are subject to gross errors due to under-reporting as the majority of people affected are beyond the reach of health care systems and are not reported in any of the health metrics. However, any apparent improvement in incidence is the result of the deployment of classical approaches as opposed to any new advance in therapy. The agreement by Sanofi-Aventis and Bayer to donate the necessary drugs to WHO to distribute to affected countries has been critical; without this generosity, patients would have no access to life-saving drugs, however unsatisfactory, when national health budgets are so stretched.

The tsetse fly, like the trypanosome, also fascinates scientists but we should be aware that tsetse can be eliminated or their populations dramatically reduced by the simplest of technologies. Sleeping sickness was eliminated from the island of Principe in the early years of the 19th century by use of sticky backpacks that trapped tsetse. Morris reduced the incidence of sleeping sickness in northern Ghana during the second World War by simply removing tsetse habitat. In the 1970s–1980s, scientists in West and southern Africa provided the basis for effective tsetse control using odour baits and impregnated tsetse targets and traps. As there is no record of insecticide resistance evolving in tsetse populations, the use of synthetic pyrethroids poses no risk in terms of the need for alternative insecticides. While traps/targets were effective as part of government funded control schemes, they have been shown to suffer from sustainability problems when left to affected communities to handle, related to the "tragedy of the commons" issues. These common goods problems have been shown to be surmountable by the treatment of cattle with insecticide; costs are dramatically reduced when the area of the animal that is treated with insecticide is restricted, encouraging uptake by individual poor cattle keepers. A public–private partnership (Stamp Out Sleeping Sickness, http://www.stampoutsleepingsickness.com/) set up to prevent the overlap of

Gambian and Rhodesian (acute) forms of sleeping sickness in Uganda has shown that restricted application of insecticide provides benefits not only by removing the main animal reservoir of Rhodesian sleeping sickness but also for animal health. Given that the distribution of sleeping sickness is limited to ancient and recognized foci, such privately funded and locally adopted approaches have more relevance to control of this disease than the continent-wide approach to eliminate tsetse from Africa.

Control of Gambian sleeping sickness depends on the strength of national health systems to provide routine surveillance, effective diagnosis, and drug availability. The classic and successful targeted approach of the dedicated mobile team, however effective in the past, is no longer seen as a priority when services need to be integrated and polyvalent. If the mobile team is no longer a priority, sleeping sickness will continue to be a lingering problem smouldering in the least accessible, poverty-stricken populations and classically in fragile and post-conflict states.

Whilst the optimism of some in the sleeping sickness community applauding the nifurtimox/eflornithine announcement is understandable, it is worth remembering that a recommendation is one thing while implementation at scale, by health services prepared to finance it, is another. Even if the perfect silver bullet emerged and was financed, populations that live far from any functioning health facility are those in real need. For the coming decade, only the tried and tested vertical approaches will work if a sustainable impact on Gambian sleeping sickness - a reduction in incidence - is to be achieved. Research cannot deliver in less than that time scale, and we know the classical approaches - early diagnosis by regular surveillance and treatment - actually work. Although WHO has defined sleeping sickness as a “tool deficient disease,” it can be argued that although the tools available are not ideal, tools of proven efficacy do exist. Now is the time to deploy them at scale.

African trypanosomiasis represents a failure of both science and public health. Two failures of responsibility by these diverse and highly divergent communities is not an enviable legacy when previous generations of committed field workers actually reduced the public health problem to one of almost zero incidence. We hope this provides a context and wake-up call to those who fund research and have an interest in actually making a difference to the thousands suffering and dying from sleeping sickness. Research on the trypanosome is not the same as research on sleeping sickness - the two frequently never meet. Trypanosomes may be attractive biological models for the researcher, but these beautiful creatures offer only a grim reality to those afflicted by an inevitably fatal disease. Today we are able to undertake the most elaborate scientific experimentation on tsetse and trypanosomes, yet we are barely able to manage sleeping sickness during the comfort afforded by the present inter-epidemic period. The huge rise in philanthro-capitalist investments that has been welcome in the past decade now needs to translate into practical solutions for rural peoples to manage this devastating disease. Investments that we have seen in genetics and genomics may reap rewards in years to come, but in the meantime, funds must be provided to sustain effective, if unsexy, control strategies. When the next epidemic comes, and it will in the absence of active surveillance and screening, the tacit knowledge will have been lost and we will have to start all over again. It is time that this reality is moved to the forefront and that we all wake up; we have been caught sleeping. The international health community is regularly challenged to deploy “lessons learnt” through many bitter experiences. We feel empowered as both elder practitioners and students of both tsetse and trypanosomes, with a degree of field experience, to recall the famous words of Pete Seeger so pertinent to sleeping sickness, “when will they ever learn, when will they ever learn?”. Let us abandon the notion that trypanosome and

tsetse research is synonymous with a case of sleeping sickness or a health system trying to control it.

15203. **Ndung'u, J. M., Bieler, S. & Roscigno, G., 2010**. "Piggy-backing" on diagnostic platforms brings hope to neglected diseases: the case of sleeping sickness. *PLoS Neglected Tropical Diseases,* **4** (5): e715.

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Neglected infectious diseases (NIDs) attract little interest from commercial companies that invest in diagnostics and therapeutics, mainly because the people that they affect are amongst the poorest in the world, who cannot afford to pay for them. Many commercial companies shy away from manufacturing diagnostic tests for NIDs because a return on investment is not usually guaranteed. It is therefore not surprising that for a disease such as human African trypanosomiasis (HAT), or sleeping sickness, no diagnostic test has ever been manufactured under full registration by any regulatory agency. Tests that are available today are produced by academic institutions, with no guarantee that good manufacturing practice for *in-vitro* diagnostics (GMP-IVD) is adhered to. The card agglutination test for trypanosomiasis (CATT), developed in 1978, is the primary screening tool used in areas where *Trypanosoma brucei gambiense* is endemic. Detection of antibodies against trypanosomes using CATT is a sensitive indicator of infection. However, in populations undergoing screening, where prevalence of the disease is usually below 2 percent and specificity of the CATT test is around 95 percent, a large number of positive results turn out to be false-positives, and the positive predictive value of the test is not good enough for it to be used on its own to guide treatment. The test is manufactured using whole *T. b. gambiense* organisms recovered from infected laboratory animals in a complex and risky process, has inferior sensitivity in some disease foci, and can be performed only by trained personnel. Furthermore, it is incapable of differentiating between active and cured infections, as antibodies tend to stay in the blood for prolonged periods after patients have been cured. What is worse, no similar test is available for *T. b. rhodesiense* infection.

Until now, no successful attempt has been made to transform the CATT into a single-format lateral flow test (LFT), which would make it more accessible to diagnostics facilities. This could again be because a LFT for HAT provides little promise for a return on investment, especially if it is to be delivered at a price that is affordable to the public sector in endemic countries. Yet for diseases that are comparatively more attractive, such as tuberculosis (TB), HIV, malaria, and avian and swine flu, there has been more commercial interest. In the late 1990s, intense lobbying by endemic countries, the World Health Organization (WHO) and the international community resulted in a paradigm shift, when at the beginning of this decade, the pharmaceutical industry agreed to provide free drugs for HAT, preventing a potentially embarrassing situation. However, this goodwill could not be extended to diagnostics as no company was manufacturing any tests for HAT.

A private foundation in Switzerland, the Foundation for Innovative New Diagnostics (FIND), has devised a novel approach towards development of diagnostic tests for NIDs that is generating a lot of interest in industry. FIND, established in 2003, supports the development of diagnostic tests for diseases of poverty, including TB, HAT, and malaria. The unique management structure of FIND comprises diagnostics programmes that exist as independent vertical business units, supported by expertise that cuts across them. A hallmark

of FIND's style of project management includes the structuring of product development, evaluation, demonstration and implementation into phases that are well defined, with deliverables and milestones that must be met before products can advance in the pipeline and receive further investment. The rigour of FIND's project management has been recognized through certification for ISO 13485:2003 and 9001:2008, standards that are customary for *in vitro* diagnostics (IVD) manufacturing companies, yet are a rare achievement for a non-profit organization.

During the first six years of existence, FIND focused its efforts on a diagnostics development approach that seeks technology platforms that are applicable to more than one disease, and used this knowledge to leverage technology development companies to include NIDs in such platforms. Diagnostic products that have passed through development, evaluation, and demonstration trials are integrated into the public health sectors of target countries in partnerships that ensure their sustainable implementation. This has enabled FIND to create a network of partners spanning the entire diagnostics development pipeline, from discovery to implementation. Leveraging its contribution to the collaborations that are established during the product development process, FIND negotiates access strategies that guarantee sustained availability of high-quality tests at affordable prices for the public and non-profit private healthcare sectors. It does this through a laboratory support programme that provides an excellent opportunity to strengthen capacity for diagnosis of NIDs by ensuring introduction, adaptation, and adoption of the most appropriate diagnostic technologies into an integrated laboratory network.

Two FIND-supported technology platforms that are applicable to more than one disease have completed development and are now undergoing evaluation for HAT. The first, a light-emitting diode (LED)–based fluorescence microscope developed for TB by FIND and Zeiss has become an excellent tool for parasite demonstration in HAT, and only required evaluation studies to prove its worth. Besides TB and HAT, the microscope has great potential for other indications such as malaria and leishmaniasis (it is robust, affordable, uses LED bulbs with a lifespan of more than 10 000 hours, and does not require a dark room. Since the bulbs use very little energy, the microscope can be operated using solar power, making it easy to use in remote rural settings such as those where these diseases occur. The microscope has been successfully evaluated for HAT in laboratories in Uganda and the Democratic Republic of the Congo (DRC), using acridine orange (AO) as the label. Furthermore, the AO staining procedure is faster than Giemsa (3 versus 45 minutes of incubation).

The number of trypanosomes in the blood of HAT patients is usually low (especially *T. b. gambiense*), and various methods are used to concentrate the parasites in order to see them under a microscope. FIND has been working with scientists at Makerere University, Uganda, and has overcome this problem by performing selective lysis of the red cells in blood samples using ammonium chloride or commercial lysis buffers, without affecting the integrity of parasites. When the lysed samples are centrifuged and the sediment is used to prepare smears, the sensitivity of LED fluorescence microscopy is greatly improved. Parasite concentration by lysis of red cells has a number of advantages over standard parasitological methods used to concentrate trypanosomes: it is a simple and fast technique, no cold chain is required, and large volumes of blood (>5 mL) can be lysed to enhance the sensitivity of trypanosome detection. A combination of this method with LED fluorescence microscopy has great potential for inclusion into the HAT diagnostic algorithm. Indeed, clinical evaluation of this method is set to start at several sites in the DRC and Uganda in 2010. Sustainable

manufacture of the LED microscope is guaranteed, because diseases such as TB will provide the market.

The second technology, loop-mediated isothermal amplification (LAMP) of DNA, is a simple molecular method developed by Eiken Chemical in Japan. The LAMP test is a novel strategy for DNA amplification that relies on the auto-cycling strand displacement synthesis of DNA by *Bst* DNA polymerase under isothermal conditions (60–65°C). Since LAMP is carried out at a constant temperature, a simple incubator such as a water bath or heating block is sufficient for DNA amplification. The reaction shows high tolerance to biological products, such that DNA extraction is not necessary. The technique uses a set of six primers that recognise eight sections of target DNA. Simultaneous synthesis of DNA by multiple primers makes LAMP highly sensitive and increases specificity, efficiency, and rapidity. The results of a test can be inspected visually by the addition of the fluorescent dye SYBR Green 1 or calcein (or by measurement of turbidity derived from a precipitate of magnesium pyrophosphate in the reaction mixture).

FIND and its academic partners, Murdoch and Obihiro Universities, have successfully developed a LAMP test for sub-genus *Trypanozoon* using the random insertion mobile element (RIME) sequences and one for *T. b. rhodesiense* based on the serum resistance associated (SRA) gene. Meanwhile, FIND had been working with Eiken on a LAMP test for TB long before they started the HAT programme, and has taken advantage of this relationship with Eiken to include the LAMP test for HAT in this diagnostic platform. Evaluation of manufactured LAMP tests for HAT using blood as the starting material is to be carried out in experimental and clinical settings in 2010. Further studies to determine the feasibility of using saliva or urine as the starting sample will also be carried out.

The LAMP test can be performed by staff with minimal experience in molecular biology. Given its high sensitivity, specificity, speed, and ease of use, LAMP could become a good test for field diagnosis of HAT and confirmation of cure in sub-Saharan Africa, where facilities are limited. FIND is working with the Institute of Primate Research (IPR) in Nairobi, Kenya, to determine the feasibility of using this method to confirm cure after successful treatment, and predict relapses in case of treatment failure. Its application as a test of cure will however depend on the rate at which DNA from dead parasites is cleared from a host after treatment. The test could also be useful for epidemiological studies and disease elimination programmes. It also appears that with a little more effort, LAMP tests for Buruli ulcer, Chagas disease, and leishmaniasis, other NIDs that FIND has taken an interest in, could be included on the same platform, whose commercial development targets diseases such as TB and malaria. This platform has therefore provided a good opportunity to diagnose several diseases from the same sample.

The lateral flow test (LFT) provides yet another platform that is widely used in indications such as pregnancy, malaria, etc. In yet another first for FIND, an LFT for screening for HAT could soon be available. An initiative spearheaded by FIND has been screening candidate antigens for their potential in detecting both *T. b. gambiense* and *T. b. rhodesiense*, to be used for developing a specific and sensitive antibody detection LFT. The test will be developed at minimal additional cost in a new partnership between FIND and Standard Diagnostics, Republic of Korea, a commercial company that has become a global leader in development of IVDs for infectious diseases.

The initiatives described here will result in novel tests for HAT that are more sensitive and specific, and are easier to use than those that are currently available. Application of the tests could lead to an acceleration of the present efforts in surveillance and control of the

disease. Such tools will also be invaluable in setting up better recruitment of patients and confirmation of cure in clinical studies by pharma and other organizations involved in compound development for HAT. While FIND may have devised an innovative approach to solve the problem of technology development by investing in platforms that apply to commercially attractive diseases and to "piggy-back" the NIDs on them, the challenge that remains is to increase the investment in strategies that will make these diagnostics accessible, so that they can easily reach the "neglected people" at little or no cost

15204. **Nussbaum, K., Honek, J., Cadmus, C. M. & Efferth, T., 2010**. Trypanosomatid parasites causing neglected diseases. *Current Medicinal Chemistry*, **17** (15): 1594-1617.

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Parasitic diseases such as Kala azar (visceral leishmaniasis), Chagas disease human (American trypanosomiasis) and African sleeping sickness (African trypanosomiasis) are affecting more than 27 million people worldwide. They are categorized amongst the most important neglected diseases causing approximately 150 000 deaths annually. As no vaccination is available, treatment is solely dependent on chemotherapeutic drugs. This review provides a comprehensive insight into the treatment of Kala azar, Chagas disease and African sleeping sickness. In addition to established drugs, novel small molecule- based therapeutic approaches are discussed. Drugs currently used for the treatment of Kala azar include pentavalent antimonials, amphotericin B, miltefosine, and paromomycin. Liposomal formulations such as AmBisome provide promising alternatives. Furthermore, antiproliferative compounds might open new avenues in Kala azar treatment. Regarding Chagas disease, chemotherapy is based on two drugs, nifurtimox and benznidazole. However, sequencing of *T. cruzi* genome in the year 2005 raises a hope for new drug targets. Proteases, sterols and sialic acids are potential promising drug targets. suramin, pentamidine, melarsoprol and eflornithine are well-established drugs to treat African sleeping sickness. New treatment options include combination therapy of eflornithine and nifurtimox, a Chagas disease therapeutic. However, all approved chemotherapeutic compounds for trypanosomatid diseases suffer from high toxicity. Further, increasing resistance limits their efficacy and compliance.

15205. **Radwanska, M., 2010.** Emerging trends in the diagnosis of human African trypanosomiasis. *Parasitology,* **e-publication ahead of print April 12, 1-10.**

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Human African trypanosomiasis (HAT) or sleeping sickness is caused by protozoan parasites *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. Despite the enormous technological progress in molecular parasitology in recent years, the diagnosis of HAT is still problematic due to the lack of specific tools. To date, there are two realities when it comes to HAT; the first one being the world of modern experimental laboratories, equipped with the latest state-of-the-art technology, and the second being the world of HAT diagnosis, where the latest semi-commercial test was introduced 30 years ago. Hence, it appears that the lack

of progress in HAT diagnosis is not primarily due to a lack of scientific interest or a lack of research funds, but mainly results from the many obstacles encountered in the translation of basic research into field-applicable diagnostics. This review will provide an overview of current diagnostic methods and highlight specific difficulties in solving the shortcomings of these methods. Future perspectives for accurate, robust, affordable diagnostics will be discussed as well.

15206. **Steverding, D., 2010.** The development of drugs for treatment of sleeping sickness: a historical review. *Parasite Vectors,* **3** (1): 15.

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Only four drugs are available for the chemotherapy of human African trypanosomiasis or sleeping sickness: suramin, pentamidine, melarsoprol and eflornithine. The history of the development of these drugs is well known and documented. Suramin, pentamidine and melarsoprol were developed in the first half of the last century by the then recently established methods of medicinal chemistry. Eflornithine, originally developed in the 1970s as an anti-cancer drug, became a treatment of sleeping sickness largely by accident. This review summarizes the developmental processes which led to these chemotherapies from the discovery of the first bioactive lead compounds to the identification of the final drugs.

15207. **Vanhamme, L., 2010**. The human trypanolytic factor: a drug shaped naturally. *Infectious Disorders - Drug Targets*, **10** (4) 266-282.

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African trypanosomes are responsible for sleeping sickness in man and nagana in cattle, which are both tremendous health burdens in Africa. Most African trypanosome species are killed by human serum. This is due to a serum trypanolytic particle specific of some old world monkeys and great apes, an HDL subclass containing two proteins which appeared recently in mammalian evolution, apolipoprotein L1 and haptoglobin related protein. Nevertheless, two African trypanosome species, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are able to infect humans, because they developed resistance to trypanolysis. Resistance to human serum in *Trypanosoma brucei rhodesiense* is due to a single gene called SRA. This mechanism of lysis-resistance is therefore an example of a natural drug-antidote system which evolved during a pathogen-host arms race. The lysis and resistance mechanisms, their molecular components as well as their mode of action are reviewed. Also discussed are how components of the system would be suitable drug targets and how the system could be engineered to generate an effective synthetic drug.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

[See also **33**: 15339].

15208**. Abd-Alla, A. M., Kariithi, H. M., Parker, A. G., Robinson, A. S., Kiflom, M., Bergoin, M. & Vreysen, M. J., 2010.** Dynamics of the salivary gland hypertrophy virus in laboratory colonies of *Glossina pallidipes* (Diptera: Glossinidae). *Virus Research,* **150** (1-2): 103-110.

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Many species of tsetse flies are infected by a virus that causes salivary gland hypertrophy (SGH) and the virus isolated from *Glossina pallidipes* (GpSGHV) has recently been sequenced. Flies with 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 the offspring of symptomatic infected flies, we examined the progeny of tsetse flies reared under different conditions. The results show that the progeny of asymptomatic parents did not develop SGH, while the progeny of symptomatic female flies mated with asymptomatic males developed a high rate of SGH (65 percent in male and 100 percent in females) and these flies were sterile. Stress in the form of high fly density in holding cages (180 flies/cage) and high temperature (30 ^{o}C) in the holding room did not affect the prevalence of the SGH. The virus is excreted in the saliva and there is a strong correlation between the infection status (negative, slight or strong by PCR) and the numbers of virus particles released into the blood on which the flies were fed. On average, around 10^2 and 10^7 virus particles were found in the blood after feeding asymptomatic or symptomatic infected flies respectively. Feeding the flies on new blood at every feed for three generations caused a significant reduction in the virus copy number in these flies when compared with the virus copy number in flies fed under the normal feeding regime. The results of these studies allowed the initiation of colony management protocols that aim to minimize the risk of horizontal transmission and to enable the establishment of colonies with a low virus prevalence or possibly even those that are virus free.

15209. **Caljon, G., De Ridder, K., De Baetselier, P., Coosemans, M. & Van Den Abbeele, J., 2010.** Identification of a tsetse fly salivary protein with dual inhibitory action on human platelet aggregation. *PLoS One,* **5** (3): e9671.

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Tsetse flies (*Glossina* sp.), the African trypanosome vectors, rely on anti-haemostatic compounds for efficient blood feeding. Despite their medical importance, very few salivary

proteins have been characterized and functionally annotated. Here we report on the functional characterization of a 5'nucleotidase-related (5'Nuc) saliva protein of the tsetse fly *Glossina morsitans morsitans*. This protein is encoded by a 1 668 bp cDNA corresponding at the genomic level with a single-copy 4 kb gene that is exclusively transcribed in the tsetse salivary gland tissue. The encoded 5'Nuc protein is a soluble 65 kDa glycosylated compound of tsetse saliva with a dual anti-haemostatic action that relies on its combined apyrase activity and fibrinogen receptor (GPIIb/IIIa) antagonistic properties. Experimental evidence is based on the biochemical and functional characterization of recombinant protein and on the successful silencing of the 5'Nuc translation in the salivary gland by RNA interference (RNAi). Refolding of a 5'Nuc/SUMO-fusion protein yielded an active apyrase enzyme with K(m) and V(max) values of 43+/-4 µM and 684+/-49 nmol Pi/min x mg for ATPase and 49+/-11 µM and 177+/-37 nmol Pi/min x mg for the ADPase activity. In addition, recombinant 5'Nuc was found to bind to GPIIb/IIIa with an apparent K_D of 92+/-25 nM. Consistent with these features, 5'Nuc potently inhibited ADP-induced thrombocyte aggregation and even caused disaggregation of ADP-triggered human platelets. The importance of 5'Nuc for the tsetse fly haematophagy was further illustrated by specific RNAi that reduced the anti-thrombotic activities in saliva by approximately 50 percent resulting in a disturbed blood feeding process. These data show that this 5'nucleotidase-related apyrase exhibits GPIIb/IIIa antagonistic properties and represents a key thromboregulatory compound of tsetse fly saliva.

15210**. Pollock, J. N., 2010**. Bot flies (Insecta: Oestridae, part) and Glossinidae-Hippoboscidae derive from basal Ephydroidea, not Calyptratae. *Journal of Natural History*, **44** (29-32): 1929-1952.

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The ground plan of the bot flies (Oestridae, part) is compared with those of Ephydroidea and Calyptratae. Comparative anatomy suggests that the bot flies are unlikely to have arisen from within Calyptratae and that their derivation from near the base of Ephydroidea is more probable. A large body of evidence is assembled to counter the theory that would place Oestridae within the Tachinidae family-group. It is shown that Hippoboscoidea is unlikely to be monophyletic. The fit of Glossinidae to the suggested ground plan profile of the Ephydroidea/bot flies is shown to be good.

15211. **Snyder, A. K., Deberry, J. W., Runyen-Janecky, L. & Rio, R. V., 2010**. Nutrient provisioning facilitates homeostasis between tsetse fly (Diptera: Glossinidae) symbionts. *Proceedings of the Royal Society B: Biological Sciences,* **277** (1692): 2389-2397.

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Host-associated microbial interactions may involve genome complementation, driving enhanced communal efficiency and stability. The tsetse fly (Diptera: Glossinidae), the obligate vector of African trypanosomes (*Trypanosoma brucei* subspp.), harbours two enteric Gammaproteobacteria symbionts: *Wigglesworthia glossinidia* and *Sodalis glossinidius.* Host coevolution has streamlined the *Wigglesworthia* genome to complement the exclusively

sanguivorous tsetse lifestyle. Comparative genomics reveal that the *Sodalis* genome contains the majority of *Wigglesworthia* genes. This significant genomic overlap calls into question why tsetse maintains the coresidence of both symbionts and, furthermore, how symbiont homeostasis is maintained. One of the few distinctions between the *Wigglesworthia* and *Sodalis* genomes lies in thiamine biosynthesis. While *Wigglesworthia* can synthesize thiamine, *Sodalis* lacks this capability but retains a thiamine ABC transporter (tbpAthiPQ) believed to salvage thiamine. This genetic complementation may represent the early convergence of metabolic pathways that may act to retain *Wigglesworthia* and evade species antagonism. We show that thiamine monophosphate, the specific thiamine derivative putatively synthesized by *Wigglesworthia*, impacts *Sodalis* thiamine transporter expression, proliferation and intracellular localization. A greater understanding of tsetse symbiont interactions may generate alternative control strategies for this significant medical and agricultural pest, while also providing insight into the evolution of microbial associations within hosts.

15212. **Terblanche, J. S. & Chown, S. L., 2010**. Effects of flow rate and temperature on cyclic gas exchange in tsetse flies (Diptera, Glossinidae). *Journal of Insect Physiology,* **56** (5): 513-521.

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Air flow rates may confound the investigation and classification of insect gas exchange patterns. Here we report the effects of flow rates (50, 100, 200, 400 mL/min^{-1}) on gas exchange patterns in wild-caught *Glossina morsitans morsitans* from Zambia. At rest, *G. m. morsitans* generally showed continuous or cyclic gas exchange (CGE) but no evidence of discontinuous gas exchange (DGE). Flow rates had little influence on the ability to detect CGE in tsetse, at least in the present experimental setup and under these laboratory conditions. Importantly, faster flow rates resulted in similar gas exchange patterns to those identified at lower flow rates suggesting that *G. m. morsitans* did not show DGE which had been incorrectly identified as CGE at lower flow rates. While CGE cycle frequency was significantly different among the four flow rates ($P < 0.05$), the direction of effects was inconsistent. Indeed, inter-individual variation in CGE cycle frequency exceeded flow rate treatment variation. Using a laboratory colony of closely related, similar-sized *G. morsitans centralis* we subsequently investigated the effects of temperature, gender and feeding status on CGE pattern variation since these factors can influence insect metabolic rates. At 100 mL/ min^{-1} CGE was typical of *G. m. centralis* at rest, although it was significantly more common in females than in males (57 percent vs. 43 percent of 14 individuals tested per gender). In either sex, temperature (20, 24, 28 and 32 ^{o}C) had little influence on the number of individuals showing CGE. However, increases in metabolic rate with temperature were modulated largely by increases in burst volume and cycle frequency. This is unusual among insects showing CGE or DGE patterns because increases in metabolic rate are usually modulated by increases in frequency, but either no change or a decline in burst volume.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

15213**. Beadell, J. S., Hyseni, C., Abila, P. P., Azabo, R., Enyaru, J. C., Ouma, J. O., Mohammed, Y. O., Okedi, L. M., Aksoy, S. & Caccone, A., 2010**. Phylogeography and population structure of *Glossina fuscipes fuscipes* in Uganda: implications for control of tsetse. *PLoS Neglected Tropical Diseases*, **4** (3): e636.

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Glossina fuscipes fuscipes, a riverine species of tsetse, is the main vector of both human and animal trypanosomiasis in Uganda. Successful implementation of vector control will require establishing an appropriate geographical scale for these activities. Population genetics can help to resolve this issue by characterizing the extent of linkage among apparently isolated groups of tsetse. We conducted genetic analyses on mitochondrial and microsatellite data accumulated from approximately 1 000 individual tsetse captured in Uganda and neighbouring regions of Kenya and Sudan. Phylogeographic analyses suggested that the largest scale genetic structure in *G. f. fuscipes* arose from an historical event that divided two divergent mitochondrial lineages. These lineages are currently partitioned to northern and southern Uganda and co-occur only in a narrow zone of contact extending across central Uganda. Bayesian assignment tests, which provided evidence for admixture between northern and southern flies at the zone of contact and evidence for northerly gene flow across the zone of contact, indicated that this structure may be impermanent. On the other hand, microsatellite structure within the southern lineage indicated that gene flow is currently limited between populations in western and southeastern Uganda. Within regions, the average F_{ST} between populations separated by less than 100 km was less than approximately 0.1. Significant tests of isolation by distance suggested that gene flow is ongoing between neighbouring populations and that island populations are not uniformly more isolated than mainland populations. Despite the presence of population structure arising from historical colonization events, our results have revealed strong signals of current gene flow within regions that should be accounted for when planning tsetse control in Uganda. Populations in southeastern Uganda appeared to receive little gene flow from populations in western or northern Uganda, supporting the feasibility of area wide control in the Lake Victoria region by the Pan African Tsetse and Trypanosomiasis Eradication Campaign.

15214**. Bouyer, J., Ravel, S., Guerrini, L., Dujardin, J. P., Sidibe, I., Vreysen, M. J., Solano, P. & De Meeus, T., 2010.** Population structure of *Glossina palpalis gambiensis* (Diptera: Glossinidae) between river basins in Burkina Faso: consequences for area-wide integrated pest management. *Infection, Genetics & Evolution,* **10** (2): 321-328.

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African animal trypanosomosis is a major obstacle to the development of more efficient and sustainable livestock production systems in West Africa. Riverine tsetse species such as *Glossina palpalis gambiensis* Vanderplank are their major vectors. A wide variety of control tactics is available to manage these vectors, but their elimination will only be sustainable if control is exercised following area-wide integrated pest management (AW-IPM) principles, i.e. the control effort is targeting an entire tsetse population within a circumscribed area. In the present study, genetic variation at microsatellite DNA loci was used to examine the population structure of *G. p. gambiensis* inhabiting two adjacent river basins, i.e. the Comoe and the Mouhoun river basins in Burkina Faso. A remote sensing analysis revealed that the woodland savannah habitats between the river basins have remained unchanged during the last two decades. In addition, genetic variation was studied in two populations that were separated by a man-made lake originating from a dam built in 1991 on the Comoe. Low genetic differentiation was observed between the samples from the Mouhoun and the Comoe river basins and no differentiation was found between the samples separated by the dam. The data presented indicate that the overall genetic differentiation of *G. p. gambiensis* populations inhabiting two adjacent river basins in Burkina Faso is low (F_{ST}=0.016). The results of this study suggest that either *G. p. gambiensis* populations from the Mouhoun are not isolated from those of the Comoe, or that the isolation is too recent to be detected. If elimination of the *G. p. gambiensis* population from the Mouhoun river basin is the selected control strategy, re-invasion from adjacent river basins may need to be prevented by establishing a buffer zone between the Mouhoun and the other river basin(s).

15215. **Courtin, F., Rayaisse, J. B., Tamboura, I., Serdebeogo, O., Koudougou, Z., Solano, P. & Sidibe, I., 2010.** Updating the northern tsetse limit in Burkina Faso (1949-2009): impact of global change. *International Journal of Environmental Research & Public Health,* **7** (4): 1708-1719.

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The northern distribution limit of tsetse flies was updated in Burkina Faso and compared to previous limits to revise the existing map of these vectors of African trypanosomiases dating from several decades ago. From 1949 to 2009, a 25- to 150-km shift has appeared toward the south. Tsetse are now discontinuously distributed in Burkina Faso with a western and an eastern tsetse belt. This range shift can be explained by a combination of decreased rainfall and increased human density. Within a context of international control, this study provides a better understanding of the factors influencing the distribution of tsetse flies.

15216. **Geiger, A., Fardeau, M. L., Falsen, E., Ollivier, B. & Cuny, G., 2010.** *Serratia glossinae* sp. nov., isolated from the midgut of the tsetse fly *Glossina palpalis gambiensis*. *International Journal of Systematic & Evolutionary Microbiology,* **60** (6): 1261-1265.

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We report the isolation of a novel bacterium, strain C1^T, from the midgut of the tsetse fly *Glossina palpalis gambiensis*, one of the vector insects responsible for transmission of the trypanosomes that cause sleeping sickness in sub-Saharan African countries. Strain C1^T is a motile, facultatively anaerobic, rod-like bacterium (0.8-1.0 µm in diameter; 2-6 µm long) that grows as single cells or in chains. Optimum growth occurred at 25-35 ^oC, at pH 6.7-8.4 and in medium containing 5-20 g NaCl L^{-1}. The bacterium hydrolysed urea and used L-lysine, L-ornithine, citrate, pyruvate, D-glucose, D-mannitol, inositol, D-sorbitol, melibiose, amygdalin, L-arabinose, arbutin, aesculin, D-fructose, D-galactose, glycerol, maltose, D-mannose, raffinose, trehalose and D-xylose; it produced acetoin, reduced nitrate to nitrite and was positive for beta-galactosidase and catalase. The DNA G+C content was 53.6 mol percent. It was related phylogenetically to members of the genus *Serratia*, family Enterobacteriaceae, the type strain of *Serratia fonticola* being its closest relative (99 percent similarity between 16S rRNA gene sequences). However, DNA-DNA relatedness between strain C1(T) and *S. fonticola* DSM 4576(T) was only 37.15 percent. Therefore, on the basis of morphological, nutritional, physiological and fatty acid analysis and genetic criteria, strain C1^T is proposed to be assigned to a novel *Serratia* species, *Serratia glossinae* sp. nov. (type strain C1^T =DSM 22080^T =CCUG 57457^T).

15217. **Kone, N., De Meeus, T., Bouyer, J., Ravel, S., Guerrini, L., N'Goran, E. K. & Vial, L., 2010.** Population structuring of the tsetse *Glossina tachinoides* resulting from landscape fragmentation in the Mouhoun River basin, Burkina Faso. *Medical & Veterinary Entomology,* **24** (2): 162-168.

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The impact of landscape fragmentation resulting from human- and climate-mediated factors on the structure of a population of *Glossina tachinoides* Westwood (Diptera: Glossinidae) in the Mouhoun river basin, Burkina Faso, was investigated. Allele frequencies at five microsatellite loci were compared in four populations. The average distance between samples was 72 km. The sampling points traversed an ecological cline in terms of rainfall and riverine forest ecotype, along a river loop that enlarged from upstream to downstream. Microsatellite DNA demonstrated no structuring among the groups studied (F_{ST} = 0.015, P = 0.07), which is contrary to findings pertaining to *Glossina palpalis gambiensis* Vanderplank in the same geographical area. The populations of *G. tachinoides* showed complete panmixia (F_{IS} = 0, P = 0.5 for the whole sample) and no genetic differentiation among populations or global positioning system trap locations. This is in line with the results of dispersal studies which indicated higher diffusion coefficients for *G. tachinoides* than for *G. p. gambiensis*. The impact of these findings is discussed within the framework of control campaigns currently promoted by the Pan African Tsetse and Trypanosomosis Eradication Campaign.

15218. **Lall, G. K., Darby, A. C., Nystedt, B., Macleod, E. T., Bishop, R. P. & Welburn, S. C., 2010.** Amplified fragment length polymorphism (AFLP) analysis of closely related wild and captive tsetse fly (*Glossina morsitans morsitans*) populations. *Parasite Vectors,* **3**: 47.

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Tsetse flies (Diptera: Glossinidae) are vectors of trypanosomes that cause sleeping sickness in humans and nagana in livestock across sub-Saharan Africa. Tsetse control strategies rely on a detailed understanding of the epidemiology and ecology of tsetse together with genetic variation within and among populations. High-resolution nuclear genetic markers are useful tools for elucidation of the genetic basis of phenotypic traits. In this study amplified fragment length polymorphism (AFLP) markers were developed to analyze genetic variation in *Glossina morsitans morsitans* from laboratory and field-collected populations from Zimbabwe. A total of seven hundred and fifty one loci from laboratory and field populations of *G. m. morsitans* from Zimbabwe were genotyped using AFLP with seven primer combinations. Analysis identified 335 polymorphic loci. The two populations could be distinguished by cluster and principal components analysis (PCA) analysis, indicating that AFLP markers can be used to separate genetically similar populations; at the same time differences observed between laboratory and field populations were not very great. Among the techniques investigated, the use of acetone was the most reliable method of preservation of tsetse for subsequent extraction of high molecular weight DNA. An interesting finding was that AFLP also enabled robust within-population discrimination of male and female tsetse

flies due to their different X chromosome DNA complements. It is concluded that AFLP represents a useful additional tool to add to the suite of techniques currently available for the genetic analysis of tsetse populations and represents a useful resource for identification of the genetic basis of important phenotypic traits.

15219**. Sciarretta, A., Tikubet, G., Baumgartner, J., Girma, M. & Trematerra, P., 2010.** Spatial clustering and associations of two savannah tsetse species, *Glossina morsitans submorsitans* and *Glossina pallidipes* (Diptera: Glossinidae), for guiding interventions in an adaptive cattle health management framework. *Bulletin of Entomological Research.* **e-publication ahead of print May 27, 1-10.**

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The paper deals with tsetse (family Glossinidae) control and aims at improving the methodology for precision targeting of interventions in an adaptive pest management system. The spatio-temporal distribution of *Glossina morsitans submorsitans* Newstead, and *Glossina pallidipes* Austen, at Ethiopia's Keto pilot site, is analyzed with the spatial analysis by distance indices (SADIE) methodology that focus on clustering and spatial associations between species and between sexes. Both species displayed an aggregated distribution characterizedd by two main patches in the south and an extended gap in the north. Spatial patterns were positively correlated and stable in most cases, with the exception of the early dry season and the short rainy season when there were differences between the species and sexes. For precision targeting of interventions, the methods presented here are more effective than the previously used geostatistical analyses for identifying and delimiting hot spots on maps, measuring shapes and sizes of patches, and discarding areas with low tsetse density. Because of the improved knowledge on hot spot occurrences, the methods allow a better delimitation of the territory for control operations and a more precise computation of the number of the relatively expensive traps used for monitoring and control purposes.

15220. **Solano, P., Ravel, S. & de Meeus, T., 2010.** How can tsetse population genetics contribute to African trypanosomiasis control? *Trends in Parasitology,* **26** (5): 255-263.

Institut de Recherche pour le Développement (IRD)/Centre International de Recherche pour l'Elevage en zones Subhumides (CIRDES), IRD UMR 177, and CIRDES 01 BP 454 Bobo-Dioulasso 01, Burkina Faso. [solano@ird.bf].

In sub-Saharan Africa, tsetse transmitted trypanosomiases have an enormous impact on human health and economic development. Both the World Health Organization and African countries through the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) have recently asserted their determination to rid the sub-continent of these diseases, and it is increasingly recognized that vector control should play an important role. This review mainly focuses on population genetics of tsetse of the *palpalis* group, the main vectors of sleeping sickness, and reports recent results on tsetse population structure and on measures of gene flow between populations. Implications of these studies for large-scale tsetse control programmes being undertaken in West Africa are important, particularly regarding control strategies (suppression or eradication).

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also: **33:** 15213, 15214, 15217, 15218, 15219, 15220]

15221. **Bekele, J., Asmare, K., Abebe, G., Ayelet, G. & Gelaye, E., 2010.** Evaluation of deltamethrin applications in the control of tsetse and trypanosomosis in the southern Rift Valley areas of Ethiopia. *Veterinary Parasitology,* **168** (3-4): 177-184.

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A study aimed at evaluating the efficacy of deltamethrin (0.4 percent impregnated targets and 1 percent pour-on formulation) in controlling tsetse and trypanosomosis was carried out in two selected 10 km x 10 km Universal Transverse Mercator Grids of the Southern Tsetse Eradication Project (STEP) area in the southern Rift Valley of Ethiopia. The grids selected were H3 (site I) and G5 (site II) in two districts of the Wolaita Zone. The trial was underway from September 2003 to April 2004. The strategy followed to accomplish the trial was a pre-intervention phase (entomology and parasitology) and an intervention phase with insecticide (deltamethrin 0.4 percent)-impregnated odour-baited targets in site I and deltamethrin 1 percent "pour-on" application to cattle in site II. The intervention phase was monitored on a monthly basis. Following the deployment of 460 targets at a density of 4 targets per km^2 in trial site I, the relative abundance of tsetse fly (*Glossina pallidipes*) declined from a pre-intervention mean catch of 1.35 flies/trap/day to 0.05 flies/trap/day at final monitoring (an 88.9 percent overall reduction). Similarly, an 83.25 percent reduction was recorded in the incidence of trypanosomosis in sentinel cattle as it dropped from 10.75 percent (first monitoring) to 1.8 percent (last monitoring). The corresponding measures of packed cell volume (PCV) showed a significant improvement from a mean of 21.8 percent (95 percent confidence interval (CI): 20.7-22.9) at first monitoring to 25.5 percent (95 percent CI: 24.3-26.7) of last monitoring ($P < 0.01$). At site II, the trial was started by spraying deltamethrin 1 percent pour-on to 409 cattle at a rate of 1 mL/10 kg body weight. Pour-on treatment was repeated every month throughout the trial period. A sharp drop in the relative abundance of tsetse fly was revealed soon after. The catch was nil at the fourth monitoring, declining from 0.91 flies/trap/day at pre-intervention ($P < 0.01$). This represents an overall reduction of 94.9 percent. The incidence of trypanosomosis in sentinel cattle also declined from 10 percent (first monitoring) to 0.95 percent (last monitoring) i.e. about 90.5 percent decline. An improvement in the overall mean PCV was seen as it rose from a mean of 24.1 percent (95 percent CI: 22.9-25.3) at first monitoring to 27.2 percent (95 percent CI: 26.2-28.1) at last monitoring which revealed a significant increase ($P < 0.01$) until the third monitoring and maintained a stable state thereafter. This work finally disclosed that a relatively better efficacy was attained by using deltamethrin pour-on formulation than targets in controlling tsetse and trypanosomosis. However, this difference was not statistically significant ($P > 0.05$). It is therefore recommended to continue the current tsetse suppression by using an integrated approach involving both techniques.

15222. **Kotlyar, S., 2010**. Recommendations for control of East African sleeping sickness in Uganda. *Journal of Global Infectious Diseases,* **2** (1): 43-48.

Department of Emergency Medicine, Yale University School of Medicine, New Haven, CT, USA.

East African sleeping sickness, caused by *Trypanosoma brucei rhodesiense*, is prominent in Uganda and poses a serious public health challenge in the region. This publication attempts to provide key components for designing a strategy for a nationwide initiative to provide insecticide-treatment of the animal reservoir to control *T. b. rhodesiense*. The contents of this article will focus on insecticide-based vector control strategies, monitoring and evaluation frameworks required for future initiatives as well as knowledge gaps.

15223. **Maikaje, D. B., Agbede, R. I. S. & Aliu, Y.O., 2009**. Control of bovine trypanosomiasis and its vectors in the Kaura-endemic focus of central Nigeria: A preliminary study. *Nigerian Journal of Parasitology*, **30** (2): 131-137.

Department of Biological Sciences, Nigerian Defence Academy (NDA), P.M.B. 2109 Kaduna, and Faculty of Veterinary Medicine, Ahmadu Bello University (ABU), Zaria, Nigeria. [dbmaik@yahoo.com].

This pilot study was carried out to develop effective field control measures for bovine trypanosomiasis in the Kaura local government endemic area (LGA) of central Nigeria. Natural and experimental trypanosomiasis caused by *T. congolense*, and *T. brucei brucei* commonly detected in cattle in this area appeared curable with therapeutic dosages of Berenil® and Samorin®. One weeks' use of biconical and Nitse tsetse traps effectively reduced the swarming populations of tsetse flies in their habitat. The results showed that uninterrupted mass cattle trypanotherapy and vector trapping for one year will effectively control bovine trypanosomiasis and improve cattle productivity in Kaura LGA.

15224. **Rayaisse, J. B., Tirados, I., Kaba, D., Dewhirst, S. Y., Logan, J. G., Diarrassouba, A., Salou, E., Omolo, M. O., Solano, P., Lehane, M. J., Pickett, J. A., Vale, G. A., Torr, S. J. & Esterhuizen, J., 2010.** Prospects for the development of odour baits to control the tsetse flies *Glossina tachinoides* and *G. palpalis s.l. PLoS Neglected Tropical Diseases,* **4** (3): e632.

Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), Bobo-Dioulasso, Burkina Faso; Natural Resource Institute, University of Greenwich, Chatham, Kent, UK; Institut Pierre Richet, Abidjan, Côte d'Ivoire; Rothamsted Research, Harpenden, UK; International Center for Insect Physiology and Ecology, Nairobi, Kenya; Masinde Muliro University of Science & Technology, Kakamega, Kenya; IRD, UMR 177 IRD/CIRAD, CIRDES, Bobo-Dioulasso, Burkina Faso; and Liverpool School of Tropical Medicine, UK. [m.j.lehane@liv.ac.uk].

Field studies were done of the responses of *Glossina palpalis palpalis* in Côte d'Ivoire, and *G. p. gambiensis* and *G. tachinoides* in Burkina Faso, to odours from humans, cattle and pigs. Responses were measured either by baiting biconical traps or electrocuting black targets with natural host odours. The catch of *G. tachinoides* from traps was significantly enhanced

(approximately 5x) by odour from cattle but not humans. In contrast, catches from electric targets showed inconsistent results. For *G. p. gambiensis* both human and cattle odour increased (>2x) the trap catch significantly but not the catch from electric targets. For *G. p. palpalis*, odours from pigs and humans increased (approximately 5x) the numbers of tsetse attracted to the vicinity of the odour source but had little effect on landing or trap-entry. For *G. tachinoides* a blend of POCA (P = 3-n-propylphenol; O = 1-octen-3-ol; C = 4-methylphenol; A = acetone) alone or synthetic cattle odour (acetone, 1-octen-3-ol, 4-methylphenol and 3-n-propylphenol with carbon dioxide) consistently caught more tsetse than natural cattle odour. For *G. p. gambiensis,* POCA consistently increased catches from both traps and targets. For *G. p. palpalis*, doses of carbon dioxide similar to those produced by a host resulted in similar increases in attraction. Baiting traps with super-normal (approximately 500 mg/h) doses of acetone also consistently produced significant but slight (approximately 1.6x) increases in catches of male flies. The results suggest that odour-baited traps and insecticide-treated targets could assist the AU-Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) in its current efforts to monitor and control *palpalis* group tsetse in West Africa. For all three species, only approximately 50 percent of the flies attracted to the vicinity of the trap were actually caught by it, suggesting that better traps might be developed by an analysis of the visual responses and identification of any semiochemicals involved in short-range interaction.

15225. **Solano, P., Kaba, D., Ravel, S., Dyer, N. A., Sall, B., Vreysen, M. J., Seck, M. T., Darbyshir, H., Gardes, L., Donnelly, M. J., De Meeus, T. & Bouyer, J., 2010.** Population genetics as a tool to select tsetse control strategies: suppression or eradication of *Glossina palpalis gambiensis* in the Niayes of Senegal. *PLoS Neglected Tropical Diseases,* **4** (5): e692.

Institut de Recherche pour le Développement (IRD)/Centre International de Recherche pour l'Elevage en zones Subhumides (CIRDES); UMR 177 IRD-Centre de cooperation Internationale en Recherche Agronomique pour le Développement (CIRAD); and CIRDES 01 BP 454, Bobo-Dioulasso, Burkina Faso. [solano@ird.bf].

The Government of Senegal has initiated the "Projet de lutte contre les glossines dans les Niayes" to remove the trypanosomosis problem from this area in a sustainable way. Due to past failures to sustainably eradicate *Glossina palpalis gambiensis* from the Niayes area, controversies remain as to the best strategy implement, i.e. "eradication" versus "suppression." To inform this debate, we used population genetics to measure genetic differentiation between *G. palpalis gambiensis* from the Niayes and those from the southern tsetse belt (Missira). Three different markers (microsatellite DNA, mitochondrial *CO1* DNA, and geometric morphometrics of the wings) were used on 153 individuals and revealed that the *G. p. gambiensis* populations of the Niayes were genetically isolated from the nearest proximate known population of Missira. The genetic differentiation measured between these two areas (theta = 0.12 using microsatellites) was equivalent to a between-taxa differentiation. We also demonstrated that within the Niayes, the population from Dakar - Hann was isolated from the others and had probably experienced a bottleneck. The information presented in this paper leads to the recommendation that an eradication strategy for the Niayes populations is advisable. This kind of study may be repeated in other habitats

and for other tsetse species to help decision on appropriate tsetse control strategies and find other possible discontinuities in tsetse distribution.

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

[See also **33**: 15214, 15261, 15264, 15265, 15365].

15226. **Enyaru, J. C., Ouma, J. O., Malele, II, Matovu, E. & Masiga, D. K., 2010.** Landmarks in the evolution of technologies for identifying trypanosomes in tsetse flies. *Trends in Parasitology*, **26** (8): 388-394.

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Understanding what the trypanosome pathogens are, their vectors and mode of transmission underpin efforts to control the disease they cause in both humans and livestock. The risk of transmission is estimated by determining what proportion of the vector population is carrying the infectious pathogens. This risk also depends on the infectivity of the trypanosomes to humans and livestock. Most livestock pathogens are not infective to humans, whereas the two sub-species that infect humans also infect livestock. As with other infectious diseases, we can therefore trace the foundation of many continuing disease control programs for trypanosomiasis to the discovery of the pathogens and their vectors more than a century ago. Over this period, methods for detecting and identifying trypanosomes have evolved through various landmark discoveries. This review describes the evolution of methods for identifying African trypanosomes in their tsetse fly vectors.

15227. **Farikou, O., Njiokou, F., Mbida Mbida, J. A., Njitchouang, G. R., Djeunga, H. N., Asonganyi, T., Simarro, P. P., Cuny, G. & Geiger, A., 2010**. Tripartite interactions between tsetse flies, *Sodalis glossinidius* and trypanosomes - an epidemiological approach in two historical human African trypanosomiasis foci in Cameroon. *Infection, Genetics & Evolution,* **10** (1): 115-121.

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Epidemiological surveys were conducted in two historical human African trypanosomiasis foci in South Cameroon, Bipindi and Campo. In each focus, three sampling areas were defined. In Bipindi, only *Glossina palpalis* was identified, whereas four species were identified in Campo, *G. palpalis* being highly predominant (93 percent). For further

analyses, 75 flies were randomly chosen among the flies trapped in each of the six villages. Large and statistically significant differences were recorded between both (1) the prevalence of *Sodalis glossinidius* (tsetse symbiont) and the prevalence of trypanosome infection of the major fly species *G. p. palpalis,* and (2) the respective prevalence of symbiont and infection between the two foci. Despite these differences, the rate of infected flies harbouring the symbiont was very similar (75 percent) in both foci, suggesting that symbionts favour fly infection by trypanosomes. This hypothesis was statistically tested and assessed, showing that *S. glossinidius* is potentially an efficient target for controlling tsetse fly vectorial competence and consequently sleeping sickness

15228**. Farikou, O., Njiokou, F., Simo, G., Asonganyi, T., Cuny, G. & Geiger, A., 2010.** Tsetse fly blood meal modification and trypanosome identification in two sleeping sickness foci in the forest of southern Cameroon. *Acta Tropica*, **116** (1): 81-88.

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The blood meal origins of 222 tsetse flies (213 *Glossina palpalis palpalis*, 7 *Glossina pallicera pallicera*, one *Glossina nigrofusca* and one *Glossina caliginea*) caught in 2008 in two human African trypanosomiasis foci (Bipindi and Campo) of south Cameroon were investigated. 88.7 percent of tsetse fly blood meals were identified using the heteroduplex method and the origin of the remaining blood meals (11.3 percent) was identified by sequencing the cytochrome B gene. Most of the meals were from humans (45.9 percent) and pigs (37.4 percent), 16.7 percent from wild animals. Interestingly, new tsetse fly hosts including turtle (*Trionyx* and *Kinixys*) and snake (*Python sebae*) were identified. Significant differences were recorded between Bipindi where the blood meals from pigs were predominant (66.7 percent vs 23.5 percent from humans) and Campo where blood meals from humans were predominant (62.9 percent vs 22.7 percent from pigs). Comparison with the data recorded in 2004 in the same foci (and with the same molecular approach) demonstrated significant modifications of the feeding patterns: increase in blood meals from pigs in Bipindi (66.7 percent in 2008 vs 44.8 percent in 2004) and in Campo (20.5 percent in 2008 vs 6.8 percent in 2004), decrease in that from humans (significant in Bipindi only). 12.6 percent, 8.1 percent and 2.7 percent of the flies were infected respectively with *Trypanosoma congolense* forest type, *Trypanosoma congolense* savannah type and *Trypanosoma brucei gambiense.* These results demonstrate that tsetse fly feeding patterns can be specific to a given area and can evolve rapidly with time. They show an active circulation of a variety of trypanosomes in sleeping sickness foci of southern Cameroon.

15229. **Gibson, W., Pilkington, J. G. & Pemberton, J. M., 2010**. *Trypanosoma melophagium* from the sheep ked *Melophagus ovinus* on the island of St Kilda. *Parasitology.* **e publication ahead of print June 14.**

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The sheep ked has been largely eradicated in the UK but persists in the feral Soay sheep of St Kilda in the Outer Hebrides. Sheep keds transmit *Trypanosoma melophagium*, but

parasitaemias are typically cryptic and this trypanosome has not been recorded in the St Kilda sheep. Trypanosomes were detected by PCR in preserved keds and were also found in gut smears from live keds; one infected gut was used to establish the trypanosome *in vitro*. Examination of the morphology of bloodstream forms from culture confirmed its identity as *T. melophagium*. Most keds were found to harbour the trypanosome, particularly those collected from lambs. DNA was extracted from preserved keds and from trypanosomes grown *in vitro*. Sequence analysis of the small subunit ribosomal RNA (SSU rRNA) gene and the spliced leader transcript showed the *T. melophagium* sequences to be very similar to those from *T. theileri*. A partial sequence of the ked SSU rRNA gene was also obtained. The close genetic relationship of *T. melophagium* and *T. theileri* suggests that *T. melophagium* represents a lineage of *T. theileri* that adapted to transmission by sheep keds and hence became a specific parasite of sheep.

15230. **Haines, L. R., Lehane, S. M., Pearson, T. W. & Lehane, M. J., 2010.** Tsetse EP protein protects the fly midgut from trypanosome establishment. *PLoS Pathogens,* **6** (3): e1000793.

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African trypanosomes undergo a complex developmental process in their tsetse fly vector before transmission back to a vertebrate host. Typically, 90 percent of fly infections fail, most during initial establishment of the parasite in the fly midgut. The specific mechanism(s) underpinning this failure are unknown. We have previously shown that a *Glossina*-specific, immunoresponsive molecule, tsetse EP protein, is up regulated by the fly in response to gram-negative microbial challenge. Here we show by knockdown using RNA interference that this tsetse EP protein acts as a powerful antagonist of establishment in the fly midgut for both *Trypanosoma brucei brucei* and *T. congolense*. We demonstrate that this phenomenon exists in two species of tsetse, *Glossina morsitans morsitans* and *G. palpalis palpalis*, suggesting tsetse EP protein may be a major determinant of vector competence in all *Glossina* species. Tsetse EP protein levels also decline in response to starvation of the fly, providing a possible explanation for increased susceptibility of starved flies to trypanosome infection. As starvation is a common field event, this fact may be of considerable importance in the epidemiology of African trypanosomiasis.

15231. **Massussi, J. A., Massussi, J. A., Mbida Mbida, J. A., Djieto-Lordon, C., Njiokou, F., Laveissiere, C. & van der Ploeg, J. D., 2010**. Diversity and spatial distribution of vectors and hosts of *T. brucei gambiense* in forest zones of Southern Cameroon: epidemiological implications. *Acta Tropica,* **114** (1): 44-48.

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Host and vector distribution of *Trypanosoma brucei gambiense* was studied in relation to habitat types and seasons. Six (19.35 percent) of the 31 mammal species recorded in Bipindi were reservoir hosts. *Cercopithecus nictitans* was confined to the undisturbed forest and the low intensive shifting cultivation zones, while *Cephalophus monticola, Cephalophus*

dorsalis, Cricetomys gambianus, Atherurus africanus and *Nandinia binotata* occurred in all the habitat types. As for vectors of human African trypanosomiasis (HAT), *Glossina palpalis palpalis*, was the most abundant (99.13 percent) among tsetse fly species. It occurs in all biotopes with its highest density recorded in the village-adjacent forest. The village-adjacent forest is therefore the most risky transmission zone for HAT mainly during the short rainy season when *G. palpalis palpalis'* density is highest (2.91); while, the high and low intensive shifting cultivation zones are the most important contact zones between humans, *G. palpalis palpalis* and wild mammals in all seasons.

15232. **Masumu, J., Akoda, K. & Van den Bossche, P., 2010.** Transmissibility, by *Glossina morsitans morsitans*, of *Trypanosoma congolense* strains during the acute and chronic phases of infection. *Acta Tropica,* **113** (2): 195-198.

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In order to verify whether chronic trypanosomal infections can affect the transmissibility of *Trypanosoma congolense* by tsetse flies, batches of *Glossina morsitans morsitans* were fed on mice infected with the same level of parasitaemia ($10^{8.1}$ trypanosomes/mL of blood) of two cloned low virulent *T. congolense* strains during the acute and the chronic phases of infection. Results showed that the proportions of procyclic infections in flies that were fed during the acute phase (32.6 percent and 45.4 percent for isolates 1 and 2, respectively) were significantly higher ($chi^2 = 4.7$, $P < 0.05$ and $chi^2 = 23.7$, $P < 0.0001$, respectively) compared to the proportions of procyclic infections of flies fed during the chronic phase of infection (18.8 percent and 14.9 percent for isolates 1 and 2, respectively). Similarly the proportions of metacyclic infections in flies fed during the acute phase (32.6 percent and 45.4 percent for isolates 1 and 2, respectively) were significantly higher ($chi^2 = 6.3$, $P < 0.05$ and $chi^2 = 23.7$, $P < 0.0001$, respectively) compared to the proportions of metacyclic infections in flies fed during the chronic phase of infection (16.8 percent and 14.9 percent for isolates 1 and 2, respectively). No significant difference was found in the maturation rate of both strains during the acute phase compared to the chronic phase of infection ($P > 0.05$). The results of this study suggest that *T. congolense* loses part of its transmissiblity by tsetse flies during the chronic phase of infection.

15233. **Njiokou, F., Nimpaye, H., Simo, G., Njitchouang, G. R., Asonganyi, T., Cuny, G. & Herder, S., 2010.** Domestic animals as potential reservoir hosts of *Trypanosoma brucei gambiense* in sleeping sickness foci in Cameroon. *Parasite,* **17** (1): 61-66.

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An explanation of the endemic nature and/or the resurgence of human African trypanosomiasis (HAT) in the historic foci in West and Central Africa may be the existence of an animal reservoir. In some HAT foci, pigs were found infected by *Trypanosoma brucei gambiense* but the implication of the other domestic animals was not quite evaluated. This study aims to determine the prevalence of *T. b. gambiense* in domestic animal species (goat,

sheep, pig and dog) commonly found in the four active HAT foci in Cameroon (Bipindi, Fontem, Campo and Doume). Blood samples were collected from 307 pigs, 264 goats, 267 sheep and 37 dogs and used for parasitological (QBC), immunological (LiTat 1.3 CATT) and molecular (PCR) analyses. The quantitative buffy coat test (QBC) detected trypanosomes in 3.88 percent domestic animals while 22.7 percent were seropositive with LiTat 1.3 CATT tests. Of the 875 animals analyzed, 174 (19.88 percent) harboured *T. brucei* s.l. DNA, found in each of the four types of animal and in the four localities. The infection rate significantly differed among the animal species ($P < 0.0001$) and localities ($P < 0.0001$). The PCR also revealed *T. b. gambiense* group 1 DNA in 27 (3.08 percent) domestic animals. The specific infection rates were as follows: sheep (6.74 percent), goats (3.08 percent), pigs (0.32 percent) and dogs (0 percent). *T. b. gambiense* was found in 8 (3.92 percent) animals from Bipindi, 15 (4.83 percent) from Campo, 4 (2.59 percent) from FontemCenter and none from Doume. The infection rates significantly differed between the localities, and correlated with the intensity of HAT transmission in the foci.

15234. **Njitchouang, G. R., Njiokou, F., Nana Djeunga, H. C., Foundipa Fewou, P., Asonganyi, T., Cuny G. & Simo, G., 2010.** Analysis of the domestic animal reservoir at a microgeographical scale, the Fontem sleeping sickness focus (South-West Cameroon). *Journal of Cell & Animal Biology*, **4** (5): 73-80.

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To better understand the epidemiology of sleeping sickness in two human African trypanosomiasis (HAT) sub foci (central and northern sub foci) of the Fontem focus where diversity in the prevalence of *Trypanosoma bucei gambiense* was reported in domestic animals and man, 397 domestic animals were sampled in eight villages. Parasitological tests revealed trypanosomes in 86 (21.60 percent) animals. The CATT test was positive in 254 (64 percent) animals with the lowest value in dogs. The PCR test revealed *T. b. gambiense* in 11.55 percent of pigs, 3.45 percent of goats and 15.38 percent of sheep. The *T. b. gambiense* infection rates were not significantly different between the two sub foci. However, *T. b. gambiense* was found in animals from all villages of the northern sub focus while only animals from Menji and Nsoko (central sub focus) revealed this infection. The detection of *T. b. gambiense* in animals of the central sub focus was in line with results of medical surveys where HAT patients were detected in the same villages. The absence of patients in the northern sub focus despite the circulation of *T. b. gambiense* in animals from all villages of this sub focus since several years is surprising and needed more investigations.

15235. **Van den Abbeele, J., Caljon, G., De Ridder, K., De Baetselier, P. & Coosemans, M., 2010.** *Trypanosoma brucei* modifies the tsetse salivary composition, altering

the fly feeding behavior that favours parasite transmission. *PLoS Pathogens,* **6** (6): e1000926.

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Tsetse flies are the notorious transmitters of African trypanosomiasis, a disease caused by the *Trypanosoma* parasite that affects humans and livestock on the African continent. Metacyclic infection rates in natural tsetse populations with *Trypanosoma brucei*, including the two human-pathogenic subspecies, are very low, even in epidemic situations. Therefore, the infected fly/host contact frequency is a key determinant of the transmission dynamics. As an obligate blood feeder, tsetse flies rely on their complex salivary potion to inhibit host haemostatic reactions ensuring an efficient feeding. The results of this experimental study suggest that the parasite might promote its transmission through manipulation of the tsetse feeding behaviour by modifying the saliva composition. Indeed, salivary gland *Trypanosoma brucei*-infected flies display a significantly prolonged feeding time, thereby enhancing the likelihood of infecting multiple hosts during the process of a single blood meal cycle. Comparison of the two major anti-haemostatic activities i.e. anti-platelet aggregation and anti-coagulation activity in these flies versus non-infected tsetse flies demonstrates a significant suppression of these activities as a result of the trypanosome-infection status. This effect was mainly related to the parasite-induced reduction in salivary gland gene transcription, resulting in a strong decrease in protein content and related biological activities. Additionally, the anti-thrombin activity and inhibition of thrombin-induced coagulation was even more severely hampered as a result of the trypanosome infection. Indeed, while naive tsetse saliva strongly inhibited human thrombin activity and thrombin-induced blood coagulation, saliva from *T. brucei*-infected flies showed a significantly enhanced thrombinase activity resulting in a far less potent anti-coagulation activity. These data clearly provide evidence for a trypanosome-mediated modification of the tsetse salivary composition that results in a drastically reduced anti-haemostatic potential and a hampered feeding performance which could lead to an increase of the vector/host contact and parasite transmission in field conditions.

15236. **Van den Bossche, P., de La Rocque, S., Hendrickx, G. & Bouyer, J., 2010.** A changing environment and the epidemiology of tsetse-transmitted livestock trypanosomiasis. *Trends in Parasitology,* **26** (5): 236-243.

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The distribution, prevalence and impact of vector-borne diseases are often affected by anthropogenic environmental changes that alter the interactions between the host, the parasite and the vector. In the case of tsetse-transmitted livestock trypanosomiasis these changes are a result of the encroachment of people and their livestock into tsetse-infected wild areas. This has created a sequence of new epidemiological settings that is changing the relative importance of the domestic or sylvatic trypanosome transmission cycles and is causing concomitant changes in the impact of the disease on livestock. These changes in the dynamics of the epidemiology have an important impact on the factors that need to be

considered when developing area-specific strategies for the future management of tsetse-transmitted livestock trypanosomiasis.

15237. **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.

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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.

5. HUMAN TRYPANOSOMOSIS

(a) SURVEILLANCE

[See also **33**: 15197, 15198, 15203, 15205]

15238. **Berrang-Ford, L., Berke, O., Sweeney, S. & Abdelrahman, L., 2010.** Sleeping sickness in southeastern Uganda: a spatio-temporal analysis of disease risk, 1970-2003. *Vector Borne Zoonotic Diseases.* **e publication ahead of print, May 19.**

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Sleeping sickness is a major threat to human health in sub-Saharan Africa. Southeastern Uganda has experienced a number of significant epidemics in the past 100 years, most recently from 1976 to 1989. Recent and continued spread of the disease has highlighted gaps

in the ability of current research to explain and predict the distribution of infection. Vegetation cover and changes in vegetation may be important determinants of transmission and disease risk because of the habitat preferences of the tsetse fly vector. This study examines the determinants of sleeping sickness distribution and incidence in southeastern Uganda from 1970 to 2003, spanning the full epidemic region and cycle, and focusing in particular on vegetation cover and change. Sleeping sickness data were collected from records of the Ugandan Ministry of Health, individual sleeping sickness treatment centres, and interviews with public health officials. Vegetation data were acquired from satellite imagery for four dates spanning the epidemic period, 1973, 1986, 1995, and 2001. Zero-inflated regression models were used to model predictors of disease presence and magnitude. Correlations between disease incidence and the normalized difference vegetation index (NDVI) at the subcounty level were evaluated. Results indicate that sleeping sickness infection is predominantly associated with proximity to water and spatial location, while disease incidence is highest in subcounties with moderate to high NDVI. The vegetation density (NDVI) at which sleeping sickness incidence peaked differed throughout the study period. The optimal vegetation density capable of supporting sleeping sickness transmission may be lower than indicated by data from endemic regions, indicating increased potential for disease spread under suitable conditions.

15239. **Courtin, F., Jamonneau, V., Camara, M., Camara, O., Coulibaly, B., Diarra, A., Solano, P. & Bucheton, B., 2010.** A geographical approach to identify sleeping sickness risk factors in a mangrove ecosystem. *Tropical Medicine & International Health,* **15** (8): 881-889.

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This study was designed to provide a better understanding of sleeping sickness transmission and spread in mangrove areas to optimize its control. In the Forecariah mangrove area, Guinea, 19 sleeping sickness cases and 19 matched controls were followed up in their living areas (at home, in fields and at water points). All occupational sites and pathways were mapped and then placed in their environmental context. The sleeping sickness cases displayed a significantly broader and more diverse spatial occupation than the controls. They covered double the daily walking distances of controls and had on average two more occupational sites, most of which were located in mangrove forests. Activities with a higher transmission risk (rice culture, attendance of pirogue jetties) were identified as well as high-risk areas and pathways. An entomological control strategy targeting transmission risk areas is proposed. Its implementation in a control programme would reduce by 86 percent the efforts needed for a classical vector control programme throughout the area. Medical surveys set up at specific locations, such as pirogue jetties and high-risk paths, should also enable better targeting of the population at highest risk.

15240. **Hasker, E., Mitashi, P., Baelmans, R., Lutumba, P., Jacquet, D., Lejon, V., Kande, V., Declercq, J., Van der Veken, W. & Boelaert, M., 2010.** A new

format of the CATT test for the detection of human African trypanosomiasis, designed for use in peripheral health facilities. *Tropical Medicine & International Health,* **15** (2): 263-267.

Department of Public Health, Epidemiology and Disease Control Unit, Institute of Tropical Medicine, Antwerp, Belgium; Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of Congo; Applied Technology and Production Unit, Institute of Tropical Medicine, Antwerp, Belgium; Department of Parasitology, Institute of Tropical Medicine, Antwerp, Belgium; National Program for Control of Human African Trypanosomiasis, Kinshasa, Democratic Republic of Congo; and Belgian Technical Cooperation, Kinshasa, Democratic Republic of Congo. [ehasker@itg.be].

To test the reproducibility and thermostability of a new format of the card-agglutination test for trypanosomiasis (CATT) test for human African trypanosomiasis (HAT), designed for use at primary health care facility level in endemic countries, 4 217 people from highly endemic villages were screened using the existing format of the CATT test (CATT-R250) on whole blood. All those testing positive (220) and a random sample of negatives (555) were retested in the field with the new format (CATT-D10). Inter-format reproducibility was assessed by calculating kappa. All samples testing positive on whole blood with either method were further evaluated in Belgium by CATT titration of serum by two observers, using both the old and new formats. CATT-D10 test kits were incubated under four temperature regimens (4, 37, 45 ^{o}C and fluctuating) with regular assessments of reactivity over 18 months. Inter-format reproducibility of CATT-D10 vs. CATT-R250 on whole blood performed by laboratory technicians in the field was excellent with kappa values of 0.83-0.89. Both inter- and intra-format reproducibility assessed by CATT titration were excellent, with 96.5-100 percent of all differences observed falling within the limits of +/-1 titration step. After 18 months, reactivity of test kits incubated under all four temperature regimens was still well above the minimum threshold considered acceptable. It is concluded that the CATT-D10 is thermostable and can be used interchangeably with the old format of the CATT test. It is highly suitable for use in peripheral health facilities in HAT-endemic countries.

15241. **Lejon, V., Mumba Ngoyi, D., Ilunga, M., Beelaert, G., Maes, I., Buscher, P. & Fransen, K., 2010.** Low specificities of HIV diagnostic tests caused by *Trypanosoma brucei gambiense* sleeping sickness. *Journal of Clinical Microbiology,* **48** (8): 2836-2839.

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The accuracy of HIV diagnostic tests in tropical infections is poorly documented. Human African trypanosomiasis (HAT) is characterized by a polyclonal B-cell activation, constituting a risk of false positive reactions in diagnostic tests, including HIV tests. A retrospective HIV diagnostic test accuracy study was therefore performed on 360 human

African trypanosomiasis (HAT) patients infected with *T. b. gambiense* before treatment, and 163 patients 2 years after successful treatment in Mbuji Mayi, East Kasai, DR Congo. Sensitivity, specificity and positive predictive value (PPV) of individual tests and algorithms consisting of 3 rapid tests were determined. The sensitivity of all tests was 100 percent (11/11). Low specificity (96.3 percent, 335/348) and PPV (45.8 percent, 11/24) of a classical seroconfirmation strategy (Vironostika ELISA followed by line immunoassay) complicated determination of the HIV status, which had to be determined by PCR. Specificities of rapid diagnostic tests were 39.1 percent for Determine (136/348), 85.3-92.8 percent (297/348-323/348) for VIKIA, Immunoflow, Doublecheck and Bioline, and 96.6-98.3 percent (336/348-342/348) for UniGold, Oraquick and STAT-PAK. Specificity for Vironostika was 67.5 percent (235/348). PPVs ranged between 4.9 and 64.7 percent. Combining 3 different rapid tests resulted in specificities of 98.3-100 percent (342-348/348) and PPVs of 64.7-100 percent (11/17-11/11). In cured HAT patients, specificities were significantly higher for Vironostika, Determine, Unigold and Immunoflow. It is concluded that *T. b. gambiense* infection decreases the specificity of antibody detection tests for HIV diagnosis. Unless tests have been validated for interference with HAT, HIV diagnosis in untreated HAT using classical algorithms should be avoided. Specific, validated combinations of 3 HIV rapid tests can increase specificity.

15242**. Matovu, E., Kuepfer, I., Boobo, A., Kibona, S. & Burri, C., 2010.** Comparative detection of trypanosomal DNA by loop-mediated isothermal amplification and PCR from flinders technology associates cards spotted with patient blood. *Journal of Clinical Microbiology*, **48** (6): 2087-2090.

Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda; Swiss Tropical and Public Health Institute, Pharmaceutical Medicine Unit, Basel, Switzerland; and National Institute for Medical Research, Tabora, Tanzania. [matovue@vetmed.mak.ac.ug].

We analyzed DNA eluted from FTA (Flinders Technology Associates) cards spotted with blood from human African trypanosomiasis (HAT) patients admitted at Lwala Hospital in eastern Uganda and the Kaliua Health Centre in northwestern Tanzania. The aims were to evaluate loop-mediated isothermal amplification (LAMP) for detection of trypanosomal DNA in clinical samples and to characterize the infecting trypanosomes to the subspecies level. LAMP targeting the Trypanozoon conserved random inserted mobile element (RIME-LAMP) and that for the serum resistance-associated (SRA) gene (SRA-LAMP) were performed. For comparison, PCRs for the SRA gene specific for *Trypanosoma brucei rhodesiense* (SRA-PCR) and that to amplify the *Trypanosoma brucei gambiense*-specific surface glycoprotein (TgSGP-PCR) were done. Out of 128 samples analyzed, SRA-PCR was positive in 101 samples (78.9 percent sensitivity; 95 percent confidence interval [CI], 71.1 to 85.1 percent), SRA-LAMP was positive in 120 (93.8 percent; 95 percent CI, 88.2 to 96.8 percent), while RIME-LAMP revealed signals in 122 (95.3 percent; 95 percent CI, 90.2 to 97.8 percent). RIME-LAMP and SRA-LAMP were each significantly more sensitive than SRA-PCR (P values of 0.000 and 0.001, respectively; Fisher's exact test). There was poor agreement between RIME-LAMP and SRA-LAMP and the SRA-PCR, yielding kappa values of 0.31 and 0.40, respectively. Agreement between SRA-LAMP and RIME-LAMP was almost perfect (kappa value, 0.85; 95 percent CI, 0.64 to 1). All the 128 field samples were negative by TgSGP-PCR. Blood spots from three *T. b. gambiense* HAT cases from

northwestern Uganda were positive by TgSGP-PCR and RIME-LAMP. PCR took five times longer to execute than LAMP. LAMP may be useful to monitor emerging HAT foci or to test travellers returning from countries where HAT is endemic. It should be evaluated in a case-control study to determine its utility as a HAT diagnostic.

15243. **Tshimungu, K., Okenge, L. N., Mukeba, J. N. & de Mol, P., 2010**. Re-emergence of human African trypanosomiasis in Kinshasa, Democratic Republic of Congo (DRC). *Médecine et Maladies Infectieuses.* **e Publication January 14.**

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The incidence of human African trypanosomiasis (HAT) or sleeping sickness in Kinshasa has been increasing since 1996. The objectives of this study were first to identify the optimal levels of knowledge, and then to determine the risk factors for HAT in the city of Kinshasa. This case/control study was based on a structured questionnaire. Case patients were detected and treated between 1 January 2004 and 31 December 2005. Each patient was paired with two seronegative controls of the same age and sex, living in the same type of environment. The study included 437 case patients and 874 controls. The optimal level of knowledge defined by the list of elementary notions related to HAT was 44 percent for the case patients and 37.0 percent for controls ($P < 0.0001$). The majority of individuals (86.7 percent) were favourable to passive screening. The patients living in peripheral areas were more at risk than other groups, in rural areas (odds-ratio 12.1; 95 percent IC: 5.7-21.7), and remote areas (odds-ratio 8.9; 95 percent IC: 2.1-38.8). A family history of HAT (odds-ratio 12.9; 95 percent IC: 7.9-20.8), ignoring the transmission route (odds-ratio 11.2; 95 percent IC: 5.8-21.7), and the water supply in natural points (odds-ratio 6.9; 95 percent IC: 2.8-17.2) were also risk factors. The results identified avoidable factors, which could be taken into account, to decrease the incidence of new contamination and the morbidity, and mortality of HAT.

15244**. Vanhecke, C., Guevart, E., Ezzedine, K., Receveur, M. C., Jamonneau, V., Bucheton, B., Camara, M., Vincendeau, P. & Malvy, D., 2010.** Human African trypanosomiasis in mangrove epidemiologic area. Presentation, diagnosis and treatment in Guinea, 2005-2007. *Pathologie Biologie (Paris),* **58** (1): 110-116.

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Gambiense human African trypanosomiasis is still assumed to be endemic in many parts of West Africa, particularly in Guinea coastal area with mangrove swamps. Diagnosis is usually made during active medical screening or by passive initiative. This study set out to describe clinical and epidemiological characteristics of *gambiense* human African trypanosomiasis in the coastal area of Guinea using exhaustive and retrospective analysis of

all patients attending the trypanosomiasis centre in the coastal area of Guinea between January 2005 and December 2007 with a diagnosis of human African trypanosomiasis. A total of 196 patients were recruited for the study. Out of them, 55 percent of the 73 patients diagnosed during active screening were classified as stage 1 (haemolymphatic stage) or early stage 2 (meningoencephalitic stage). Contrarily, 115 of the 120 people diagnosed by the passive procedure were classified late stage 2, which features more specific signs and neurological symptoms, and leads to coma and death. More than 90 percent of all cases presented cervical lymph nodes with identification of trypanosome on direct examination of fluid puncture. Less than one third of the patients were re-examined three months later. It was found that in the coastal area of Guinea with mangrove swamps, direct examination of lymph node fluid puncture seems to be the test contributing most to the diagnosis of human African trypanosomiasis. Hence, associating clinical examination of the cervical lymph nodes area and direct examination of fluid obtained by puncture may allow an early diagnosis of *gambiense* human African trypanosomiasis and favour the implementation of efficient therapeutic strategies.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **33**: 15199].

15245. **Bouteille, B., Mpandzou, G., Cespuglio, R., Ngampo, S., Peeling, R. W., Vincendeau, P. & Buguet, A., 2010**. Cerebrospinal fluid B lymphocyte identification for diagnosis and follow-up in human African trypanosomiasis in the field. *Tropical Medicine & International Health,* **15** (4): 454-461.

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In human African trypanosomiasis (HAT, sleeping sickness), staging of disease and treatment follow-up relies on white cell count in the cerebrospinal fluid (CSF). As B lymphocytes (CD19 positive cells) are not found in the CSF of healthy individuals but occur in neurological disorders such as multiple sclerosis, the B lymphocyte count may be useful for field diagnosis/staging and therapeutic follow-up in HAT. Seventy-one HAT patients were diagnosed and 50 were followed-up 6-24 months after treatment. White cell counts were used for conventional staging (stage 1, < or =5 cells/µL CSF, n = 42; stage 2, > or =20 cells/µL, n = 16) and intermediate stage (6-19 cells/µL, n = 13). Slides containing 1 µL of CSF mixed with Dynabeads CD19 pan B were examined microscopically to detect B cell rosettes (bound to at least four beads). Stage 1 patients exhibited zero (n = 37) or one CSF rosette/µL (n = 5), contrary to most stage 2 patients (14/16: > or =2 rosettes/µL). Intermediate stage patients expressed 0 (n = 9), 1 (n = 3) or 2 (n = 1) rosettes/µL of CSF. During follow-up, rosette counts correlated with white cell count staging but were much easier to read. It is concluded that B cell rosettes being easily detected in the CSF in field conditions may be proposed to replace white cell counts for defining HAT stages 1 and 2 and limit the uncertainty in treatment decision in patients with the intermediate stage.

15246. **Carod-Artal, F. J., 2010**. Trypanosomiasis, cardiomyopathy and the risk of ischemic stroke. *Expert Review of Cardiovascular Therapy*, **8** (5): 717-728.

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American (Chagas disease) and African (sleeping sickness) trypanosomiasis are neglected tropical diseases and are a heavy burden in Latin America and Africa, respectively. Chagas disease is an independent risk factor for stroke. Apical aneurysm, heart failure and cardiac arrhythmias are associated with ischemic stroke in chagasic cardiomyopathy. Not all chagasic patients who suffer an ischemic stroke have a severe cardiomyopathy, and stroke may be the first manifestation of Chagas disease. Cardioembolism affecting the middle cerebral artery is the most common stroke subtype. Risk of recurrence is high and careful evaluation of recurrence risk should be addressed. Repolarization changes, low voltage and prolonged QT interval are common electrocardiography alterations in human African trypanosomiasis, and can be found in more than 70 percent of patients. Epidemiological studies are needed to assess the risk of stroke in African trypanosomiasis perimyocarditis.

15247. **Cherian, P., Junckerstorff, R. K., Rosen, D., Kumarasinghe, P., Morling, A., Tuch, P., Raven, S., Murray, R. J. & Heath, C. H., 2010.** Late-stage human African trypanosomiasis in a Sudanese refugee. *Medical Journal of Australia,* **192** (7): 417-419.

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A 19-year-old Sudanese woman, who had lived for about a decade in Ugandan refugee camps, was referred for investigation of a 12-month history of a generalized rash. Two months later, her condition had deteriorated to include cachexia and drowsiness. Despite initial negative findings on investigation, human African trypanosomiasis (HAT) was suspected, and parasites were found in a double-centrifuged sample of cerebrospinal fluid. Eflornithine, the appropriate drug for treatment of late-stage disease, was obtained through the World Health Organization. This case highlights the diagnostic and therapeutic difficulties in managing late-stage HAT in a non-endemic country.

15248. **Hidron, A., Vogenthaler, N., Santos-Preciado, J. I., Rodriguez-Morales, A. J., Franco-Paredes, C. & Rassi, A., Jr., 2010.** Cardiac involvement with parasitic infections. *Clinical Microbiology Reviews,* **23** (2): 324-349.

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Parasitic infections previously seen only in developing tropical settings can be currently diagnosed worldwide due to travel and population migration. Some parasites may directly or indirectly affect various anatomical structures of the heart, with infections manifested as myocarditis, pericarditis, pancarditis, or pulmonary hypertension. Thus, it has become quite relevant for clinicians in developed settings to consider parasitic infections in the differential diagnosis of myocardial and pericardial disease anywhere around the globe. Chagas disease is by far the most important parasitic infection of the heart and one that it is currently considered a global parasitic infection due to the growing migration of populations from areas where these infections are highly endemic to settings where they are not endemic. Current advances in the treatment of African trypanosomiasis offer hope to prevent not only

the neurological complications but also the frequently identified cardiac manifestations of this life-threatening parasitic infection. The lack of effective vaccines, optimal chemoprophylaxis, or evidence-based pharmacological therapies to control many of the parasitic diseases of the heart, in particular Chagas disease, makes this disease one of the most important public health challenges of our time.

15249. **Kinkela, M. N., Chelo, D., Boula, A., Ebo, O. E. V., Kohagne Tongue, L., Akazong, C. A., Kyebyene, A. & Tietche, F., 2010.** Human African trypanosomiasis: description of two pediatric cases in Yaoundé, Cameroon. *Médecine tropicale,* **70** (1): 73-76.

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During the first decades of the 20th century, about 45 percent of deaths in Cameroon were believed to be due to human African trypanosomiasis. Thanks to the screening and treatment campaigns implemented between 1926 and 1932, a considerable regression of the disease was achieved and, by the 1950s, only a few well-known and delimited foci remained. Today, human African trypanosomiasis is an extremely rare diagnosis, especially in children. The purpose of this report is to describe two cases of neuromeningeal human African trypanosomiasis that were discovered coincidentally in two children, ages 12 and 2 years. The children were from two villages in the center of Cameroon that is not considered as a known endemic focus. These two cases raise difficult questions about the possibility of latent endemic foci of human African trypanosomiasis and of animal-to-human transmission. The outcome was favourable in the first case and fatal in the second.

15250. **Kristensson, K., Nygard, M., Bertini, G. & Bentivoglio, M., 2010.** African trypanosome infections of the nervous system: parasite entry and effects on sleep and synaptic functions. *Progress in Neurobiology,* **91** (2): 152-171.

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The extracellular parasite *Trypanosoma brucei* causes human African trypanosomiasis (HAT), also known as sleeping sickness. Trypanosomes are transmitted by tsetse flies and HAT occurs in foci in sub-Saharan Africa. The disease, which is invariably lethal if untreated, evolves in a first haemo-lymphatic stage, progressing to a second meningo-encephalitic stage when the parasites cross the blood-brain barrier. At first, trypanosomes are restricted to circumventricular organs and choroid plexus in the brain outside the blood-brain barrier, and to dorsal root ganglia. Later, parasites cross the blood-brain barrier at post-capillary venules, through a multi-step process similar to that of lymphocytes. Accumulation of parasites in the brain is regulated by cytokines and chemokines. Trypanosomes can alter neuronal function and the most prominent manifestation is represented by sleep alterations. These are characterized in HAT and experimental rodent infections by disruption of the sleep-wake 24h cycle and internal sleep structure. Trypanosome infections alter also some, but not all, other endogenous biological rhythms. A number of neural pathways and molecules may be involved in such effects. Trypanosomes secrete prostaglandins including

the somnogenic PGD2, and they interact with the host's immune system to cause release of pro-inflammatory cytokines. From the sites of early localization of parasites in the brain and meninges, such molecules could affect adjacent brain areas implicated in sleep-wakefulness regulation, including the suprachiasmatic nucleus and its downstream targets, to cause the changes characteristic of the disease. This raises challenging issues on the effects of cytokines on synaptic functions potentially involved in sleep-wakefulness alterations.

15251. **Liu, A. P., Chou, S., Gomes, L., Ng, T., Salisbury, E. L., Walker, G. L. & Packham, D. R., 2010.** Progressive meningoencephalitis in a Sudanese immigrant. *Medical Journal of Australia,* **192** (7): 413-416.

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A 24-year-old woman presented to hospital in May 2009 after two generalized seizures with focal motor onset involving the right arm and leg. She had a 2-month history of intermittent frontal headaches and twitching of the right hand that did not interfere with usual activities. Her family had noticed she had lost weight. The patient was originally from the Eastern Equatoria province in southern Sudan and spent 12 years in a Ugandan refugee camp before migrating to Australia in 2007 with her family. Her only significant past medical history was malaria. Her family were in good health.

15252. **Vanhollebeke, B. & Pays, E., 2010.** The trypanolytic factor of human serum: many ways to enter the parasite, a single way to kill. *Molecular Microbiology,* **76** (4): 806-814.

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Humans have developed a particular innate immunity system against African trypanosomes, and only two *Trypanosoma brucei* clones (*T. b. gambiense, T. b. rhodesiense*) can resist this defence and cause sleeping sickness. The main players of this immunity are the primate-specific apolipoprotein L-I (apoL1) and haptoglobin-related protein (Hpr). These proteins are both associated with two serum complexes, a minor subfraction of HDLs and an IgM/apolipoprotein A-I (apoA1) complex, respectively, termed trypanosome lytic factor (TLF) 1 and TLF2. Although the two complexes appear to lyse trypanosomes by the same mechanism, they enter the parasite through various modes of uptake. In case of TLF1 one uptake process was characterized. When released in the circulation, haemoglobin (Hb) binds to Hpr, hence to TLF1. In turn the TLF1-Hpr-Hb complex binds to the trypanosome haptoglobin (Hp)-Hb receptor, whose original function is to ensure haem uptake for optimal growth of the parasite. This binding triggers efficient uptake of TLF1 and subsequent trypanosome lysis. While Hpr is involved as TLF ligand, the lytic activity is due to apoL1, a Bcl-2-like pore-forming protein. We discuss the *in vivo* relevance of this uptake pathway in the context of other potentially redundant delivery routes.

(c) TREATMENT

[See also **33**: 15197, 15202, 15203, 15206, 15207, 15275, 15276, 15330].

15253. **Blum, R., Blum, J., Chappuis, F. & Burri, C., 2010**. Human African trypanosomiasis. *The Lancet*, **275** (9709) 148-159.

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Human African trypanosomiasis (sleeping sickness) occurs in sub-Saharan Africa. It is caused by the protozoan parasite *Trypanosoma brucei*, transmitted by tsetse flies. Almost all cases are due to *Trypanosoma brucei gambiense*, which is indigenous to West and Central Africa. Prevalence is strongly dependent on control measures, which are often neglected during periods of political instability, thus leading to resurgence. With fewer than 12 000 cases of this disabling and fatal disease reported per year, trypanosomiasis belongs to the most neglected tropical diseases. The clinical presentation is complex, and diagnosis and treatment difficult. The available drugs are old, complicated to administer, and can cause severe adverse reactions. New diagnostic methods and safe and effective drugs are urgently needed. Vector control to reduce the number of flies in existing foci, needs to be organized on a pan-African basis. WHO has stated that if national control programmes, international organizations, research institutes, and philanthropic partners engage in concerted action, elimination of this disease might even be possible.

15254. **Claessen, F. A. P., Blaauw, G. J., van der Vorst, M. J. D. L., Ang, C. W. & van Agtmael, M. A., 2010**. Tryps after adventurous trips. *Netherlands Journal of Medicine*, **68** (3): 144-145.

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A 30-year-old previously healthy woman was admitted to the general medical ward because of a one-day history of high fever. Six to ten days before, she had visited Tanzania on her honeymoon and went on safari tours in open jeeps in Tarangire, Lake Manyara, Serengeti and Ngorongoro Crater National Parks, successively. She recalled multiple tsetse fly bites. Four days before admission she felt ill and noticed a chancre on her leg.. She was taking atovaquone/ proguanil for malaria prophylaxis and had been vaccinated against yellow fever, hepatitis A and B and typhoid fever. On presentation, she looked ill and was jaundiced, not obtunded, with a temperature of 40 °C, blood pressure 125/70 mmHg and pulse 120 beats/min. There was no nuchal rigidity and on auscultation discrete crackles were heard over the left chest. A thick smear revealed no malaria parasites but trypomastigotes of *Trypanosoma brucei* spp. Her laboratory results revealed pancytopenia (haemoglobin 6.6 mmol/L, leucocytes 2.2 x 10^9/L, thrombocytes 37 x 10^9/L), diffuse intravascular coagulation, metabolic acidosis, elevated bilirubin (212 µmol/L, conjugated fraction 0.66), ASAT (594 U/L) and ALAT (416 U/L), serum creatinine 55 µmol/L and a mild proteinuria. To exclude central nervous system infestation, a lumbar puncture was performed. Cerebrospinal fluid

analysis was normal with no trypomastigotes. The electrocardiogram showed repolarization abnormalities and her chest X-ray was normal. She was treated with suramine intravenously, first with a test dose of 200 mg and then 1000 mg on days 1, 3, 10, 17, 24 and 31. The following day, she developed progressive dyspnoea. Now the chest X-ray showed diffuse changes compatible with acute respiratory distress syndrome (ARDS) and she was transferred to the intensive care unit, where hydrocortisone was given from day 2 to 4 and supportive care. No intubation or vasoactive medication was required. Clinical improvement started on day 3. On day 4 the blood smear was negative for trypomastigotes. The proteinuria disappeared during treatment. However, her serum creatinine gradually increased to 110 µmol/L three months after the start of the treatment, with a creatinine clearance of 79 mL/min. No other side effects of the suramine were noticed (adrenal insufficiency, polyneuropathy). After six months she had fully recovered and the serum creatinine had normalized. This patient presented with acute sleeping sickness or human African trypanosomiasis (HAT) with severe disease and multi-organ involvement four to five days after the first symptoms and after a remarkably short incubation time of less than seven days following visits to game parks in Tanzania. In travellers to endemic areas, blood smears for malaria should also be examined for trypomastigotes. Not all textbooks mention icterus as an early sign of HAT as we observed in this patient. A lumbar puncture in the diagnostic workup is controversial: meningo-encephalitis is unlikely in the first week of illness and false positive results may occur, which could prompt unnecessary treatment with the toxic melarsoprol. Theoretically, accidental contamination of the cerebrospinal fluid after a traumatic lumbar puncture is possible, although proven cases have not been described. The number of tourists returning to Europe yearly with HAT is not known, but presumably very low.Travellers to endemic areas should be made aware of the risk of acquiring trypanosomiasis and minimize exposure to the bite of the vector.

15255. **Dujardin, J. C., Gonzalez-Pacanowska, D., Croft, S. L., Olesen, O. F. & Spath, G. F., 2010.** Collaborative actions in anti-trypanosomatid chemotherapy with partners from disease endemic areas. *Trends in Parasitology*, **26** (8) 395-403.

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The protozoan diseases leishmaniasis, human African trypanosomiasis and Chagas disease are responsible for substantial global morbidity, mortality and economic adversity in tropical and subtropical regions. In most countries, existing strategies for control and treatment are either failing or under serious threat. Environmental changes, drug resistance

and immunosuppression contribute to the emergence and spread of these diseases. In the absence of safe and efficient vaccines, chemotherapy, together with vector control, remains the most important measures to control trypanosomatid diseases. Here, we review current limitations of anti-trypanosomatid chemotherapy and describe new efforts to safeguard existing treatments and to identify novel drug leads through the three multinational and interdisciplinary European Union Framework Programmes for Research and Technological Development (FP7) funded consortia KALADRUG-R, TRYPOBASE, and LEISHDRUG.

15256. **Editorial, 2010**. Killer coma: the evolving story of sleeping sickness treatment. *Lancet,* **375** (9709): 93.

Imagine a disease that starts with a fly's bite and ends in death. The first stage of this disease causes non-specific symptoms such as itching and joint pains. If left untreated, it progresses to the second stage weeks, months, or even years later in which the affected person displays dramatic neurological and psychiatric symptoms before slipping into a fatal coma. This killer disease is endemic to several countries, putting millions of people at risk with around 12 000 people infected every year. Yet there is no field-friendly diagnostic test and, until recently, the most effective treatment for the second stage was almost as dangerous as the disease.
In a Seminar in The Lancet today, Reto Brun and colleagues discuss this very real disease - human African trypanosomiasis, more commonly referred to as sleeping sickness - caused by the protozoan parasite *Trypanosoma brucei* transmitted through the bite of the tsetse fly. The evolving story of the research, development, and implementation of new treatments for this disease, and the many obstacles that have had to be surmounted along the way, has some important lessons for similar action in other neglected tropical diseases. Such diseases, which only affect people in poor countries are not profitable for commercial pharmaceutical companies and need special attention. Although there are two effective drugs to treat stage-one disease, pentamidine and suramin, most people only realize that they are infected when stage-two symptoms appear and so do not seek treatment until then. The most common treatment at this stage is a derivative of arsenic, melarsoprol, which can melt plastic syringes, causes caustic burns, is extremely painful when injected, and kills about 5 percent of patients. More recently, eflornithine, a safer alternative, has been used in the treatment of second-stage disease. Originally developed to treat cancer, this “resurrection drug” (so dubbed because of its dramatic success in rousing people from coma) has had a bumpy ride. Eflornithine was never profitable for the original manufacturer, Hoechst Marion Roussel, who stopped production in 1995. In 2000, WHO began to search for a company that would continue to produce eflornithine. After Bristol-Myers Squibb ran a high-profile advertising campaign for its eflornithine-based women's facial hair remover, media attention to this cosmetic product helped to rouse drug companies' interest in eflornithine. In 2001, and again in 2006, Aventis (now Sanofi-Aventis) made a deal with WHO to continue to produce eflornithine for its use in sleeping sickness. However, despite its “miraculous” qualities, eflornithine also has some downsides - for example, it only works against *T. brucei gambiense* and may lead to resistance if used alone. Jump to 2009, and after The Lancet published a trial (organized by the medical humanitarian organization Médecins Sans Frontières - MSF - and the not-for-profit Drugs for Neglected Diseases initiative - DNDi) of the safest and most effective treatment to date, nifurtimox-eflornithine combination treatment (called NECT), it was hoped that this treatment could be widely implemented. However, nifurtimox (currently used in the management of Chagas disease) is not registered for use in sleeping sickness. WHO is currently asking countries wanting to use the combination treatment to sign disclaimer letters

in which ministries of health will take responsibility for use of this drug, manufactured by Bayer Schering Pharma. DNDi told The Lancet that so far four countries - the Democratic Republic of the Congo, Central African Republic, Chad, and Uganda - have signed the required agreement. The continuing story of implementing effective and practical treatment for sleeping sickness is encouraging. The account of this uphill struggle is a tribute to the determination of all those involved to overcome every problem hampering progress. But does the process really need to be made this complex? Research into new diagnostic methods to help identify those with treatable stage-one disease is urgently needed as is further research into safe, practical, and effective treatments and the implementation of a sustainable and efficient NECT delivery system. Would it make any difference if sleeping sickness was nicknamed "killer coma", or would the tsetse fly need to populate hot, humid savannahs other than those in sub-Saharan Africa for the lives of those infected with *T. brucei* to become more important to the international community?

15257. **Ky, J. M., Zerbo, P., Gnoula, C., Simpore, J., Nikiema, J. B. & Millogo-Rasolodimby, J., 2009**. Medicinal plants used in traditional medicine in the centre east region of Burkina Faso. *Pakistan Journal of Biological Sciences,* **12** (19): 1287-1298.

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The present research focused on the inventory and the use of plants in traditional medicine for the treatment of diseases in this area. The method was based on ethnobotanical surveys with semi-directing interview, conducted from November 2006 to December 2007 among a sample of 50 people aged between 40 and 80 years and very experienced in traditional medicine in the municipalities of Bissiga, Lalgaye and Tenkodogo. We identify 73 phytogenetic species and 175 therapeutic indications used to treat 52 diseases and the principal ones are the gastrointestinal diseases, the malaria, the various fevers, the jaundice, the skin diseases, the respiratory affections, the reproduction diseases, the haemorrhoids and the infantile diseases. In traditional veterinary pharmacopoeia, 18 phytogenetic species are used with 33 therapeutic indications to treat diseases including trypanosomiasis, tuberculosis, diarrheas and wounds. The interest of people of this area for medicinal plants requires special attention to organize the relevant actors to preserve the plant genetic resources.

15258. **Mumba Ngoyi, D., Lejon, V., Pyana, P., Boelaert, M., Ilunga, M., Menten, J., Mulunda, J. P., Van Nieuwenhove, S., Muyembe Tamfum, J. J. & Buscher, P., 2010.** How to shorten patient follow-up after treatment for *Trypanosoma brucei gambiense* sleeping sickness. *Journal of Infectious Diseases,* **201** (3): 453-463.

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Clinical management of human African trypanosomiasis requires patient follow-up of 2 years' duration. At each follow-up visit, cerebrospinal fluid (CSF) is examined for trypanosomes and white blood cells (WBCs). Shortening follow-up would improve patient comfort and facilitate control of human African trypanosomiasis. A prospective study of 360 patients was performed in the Democratic Republic of the Congo. The primary outcomes of the study were cure, relapse, and death. The WBC count, immunoglobulin M level, and

specific antibody levels in CSF samples were evaluated to detect treatment failure. The sensitivity and specificity of shortened follow-up algorithms were calculated. The treatment failure rate was 37 percent. Trypanosomes, a WBC count of > or = 100 cells/µL, and a LATEX/immunoglobulin M titre of 1:16 in CSF before treatment were risk factors for treatment failure, whereas human immunodeficiency virus infection status was not a risk factor. The following algorithm, which had 97.8 percent specificity and 94.4 percent sensitivity, is proposed for shortening the duration of follow-up: at 6 months, patients with trypanosomes or a WBC count of > or = 50 cells/µL in CSF are considered to have treatment failure, whereas patients with a CSF WBC count of > or = 5 cells/µL are considered to be cured and can discontinue follow-up. At 12 months, the remaining patients (those with a WBC count of > or = 6-49 cells/µL) need a test of cure, based on trypanosome presence and WBC count, applying a cutoff value of > or = 20 cells/µL. It is concluded that combining criteria for failure and cure allows follow-up of patients with second-stage human African trypanosomiasis to be shortened to a maximum duration of 12 months.

15259. **Yun, O., Priotto, G., Tong, J., Flevaud, L. & Chappuis, F., 2010**. NECT is next: implementing the new drug combination therapy for *Trypanosoma brucei gambiense* sleeping sickness. *PLoS Neglected Tropical Diseases*, **4** (5): e720.

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In April 2009, a new treatment option, nifurtimox-eflornithine combination therapy (NECT), was added to the WHO Essential Medicines List (EML) for the treatment of second-stage *T. b. gambiense* HAT. NECT was added to the EML based on the high efficacy and good safety profile observed in all studies done to date, against a background of recognized severity of stage 2 disease and toxicity of existing treatments. Surveillance of adverse events was strongly recommended. Compared with eflornithine monotherapy, NECT is easier to administer and requires fewer human and material resources. In the current context, NECT stands as the most promising first-line treatment for second-stage *T. b. gambiense* HAT. Here we describe the developments and challenges in rolling out and implementing NECT in HAT-endemic areas. In randomized, open-label, phase III trials at four centres, NECT was shown to be easier to administer than, and noninferior in efficacy to, eflornithine monotherapy for the treatment of second-stage *T. b. gambiense* HAT. The drug combination was fairly well tolerated: patients treated with NECT had half as many major drug-related adverse events as those treated with eflornithine alone (14 percent versus 29 percent; P = 0.002). The noninferiority in efficacy of NECT versus eflornithine monotherapy (as measured by 10 percent difference in cure rates) was demonstrated by 96.5 percent cure rate for NECT group versus 91.6 percent for eflornithine group in the intention-to-treat patient population, and 97.7 percent versus 91.7 percent in the per-protocol population, both at 18 months follow-up. While eflornithine monotherapy requires 56 intravenous (IV) infusions over 14 days, NECT requires only 14 infusions over 7 days (plus oral nifurtimox 3 times per day for 10 days). NECT's shorter treatment duration and considerably fewer IV infusions make its administration less difficult and cumbersome for both the patients and care providers. The cost of NECT kits (supplies and preparation time; excluding the cost of the drugs, which are donated) is €39 per patient, compared with €107 per patient for eflornithine

monotherapy kits (unpublished data, MSF-Logistique, February 2010). This large cost difference is due to fewer quantities of drugs, injection fluids, and other materials, resulting in less volume and weight to transport (four NECT treatments per kit, compared with two eflornithine monotherapy treatments per kit). Cost differences may be even larger when taking into account indirect expenditures such as shorter lengths of hospital stay, transport of lighter kits to the endemic country's capital and from the capital to the field, and management of fewer adverse events.

When comparing the cost of NECT against melarsoprol, a simple cost comparison would be inappropriate because of melarsoprol's high toxicity and declining effectiveness. A cost-effectiveness study showed that the cost per life saved was similar between melarsoprol and standard eflornithine monotherapy. It is therefore reasonable to assume that NECT's cost per life saved will be lower than that of melarsoprol, though this requires further study.

As a combination of drugs with different modes of action, NECT also has less potential for emergence of parasitic resistance, which is a major drawback of long-term use of monotherapies, as shown with melarsoprol.

The addition of NECT to the WHO EML in April 2009 has paved the way for its rollout and implementation in affected countries. NECT is provided free of charge by WHO through MSF-Logistique, the logistics and supplies division of MSF. Because nifurtimox is not registered for use for HAT, the WHO first requires country MOHs to sign disclaimer letters, in which the MOH takes legal responsibility for the off-label use of the drug. Despite initial fears that this disclaimer letter prerequisite could present an obstacle to NECT use, the MOHs of Central African Republic (CAR), Chad, DRC, Equatorial Guinea, south Sudan, and Uganda have signed the letters at the time of this writing. Country-level acceptance of NECT has therefore been positive, and acceptance by other countries where HAT is present should translate into concrete, rapid improvement in the field. Physician and patient acceptance of NECT is also important and should be followed.

MSF-Logistique assembles and ships the NECT kits from its headquarters in Mérignac, France, near Bordeaux. The kits, designed in collaboration between MSF-Logistique and WHO, include all the drugs, fluids, and medical materials for the treatment protocol. The drugs are donated by the manufacturers. In September 2009, Bayer agreed to donate 400 000 tablets of nifurtimox per year to WHO through 2014. Aventis and later Sanofi-Aventis have donated eflornithine to WHO through two consecutive 5-year agreements since 2001. Kits are being made available free of charge to countries by WHO, with financial support from Sanofi-Aventis covering the costs of materials and transport to the capital of each country. Each 41-kg kit contains four full treatments of NECT. The volume per NECT treatment is reduced by more than half compared to eflornithine monotherapy.

Wide-scale delivery of NECT faces a number of challenges, some specific to NECT, and others related to HAT treatment and control in general. One of the key challenges for NECT implementation is to replace the use of melarsoprol with NECT as first-line treatment for second-stage *T. b. gambiense* HAT. A derivative of arsenic and highly toxic, melarsoprol use is associated with frequent serious adverse events and unacceptably high case-fatality rates. Nevertheless, melarsoprol remains widely used for second-stage *T. b. gambiense* HAT where eflornithine is not available or practical. According to a 2008 assessment of eight provinces in DRC, 50 percent of patients with second-stage HAT were still being treated with melarsoprol (with the other half treated with eflornithine monotherapy). Alarmingly, in one district, Bandundu, which had the heaviest HAT caseload of the provinces surveyed, 96 percent of second-stage HAT patients were still treated with melarsoprol.

Melarsoprol injections are often painful for patients. Severe adverse events are frequently associated with its use, particularly the development of reactive encephalopathy in 5 percent–10 percent of patients, of whom up to 70 percent die. Treatment failure with melarsoprol is also a serious concern in various disease foci in several countries, with reports of relapse rates up to 59 percent. Treatment failures include relapse (or probable relapse), lack of response to treatment, or death. These failures suggest the emergence of parasitic resistance to melarsoprol. Donors, policymakers, and national programmes should now aim for the withdrawal of melarsoprol as first-line treatment for second-stage *T. b. gambiense* HAT with the shortest possible delay. In endemic areas where treatment with melarsoprol is still predominant, NECT protocol change and training should be prioritized. Country-by-country analyses and forecasts will be needed to assess NECT implementation, with comparisons to melarsoprol use. The use of melarsoprol should soon be restricted to treat relapses of *T. b. gambiense* HAT after initial first-line treatment with NECT or eflornithine, and to treat second-stage HAT due to *T. b. rhodesiense*.

Logistical difficulties of getting NECT kits to the field are a concern. The timely transport of treatment kits within endemic countries, from the capital to the hospitals and clinics in the field, remains a common bottleneck. Drug supply and access are perpetual issues for NTD treatment programmes. The donations of nifurtimox and eflornithine from the drug manufacturers are most welcome and must be sustained for NECT to be widely implemented.

Although relatively simpler and safer than the older HAT treatment protocols, the training needs for NECT are still considerable in treatment centers that have not yet used eflornithine. Care providers must be trained in the correct nursing care of IV catheters, precise and time-dependent IV administration of eflornithine, daily oral administration of nifurtimox under surveillance (directly observed treatment [DOT]), monitoring of adverse events, and follow-up. DOT is important to ensure treatment adherence in patients who are often mentally disturbed (due to the neurological effects of stage 2 infection), in a low educational level context, and/or at risk of vomiting the tablets. Less-intense training is needed in places where eflornithine monotherapy has already been introduced, since the NECT protocol is similar but simpler.

Current NTD donor and policy discussions include a strong focus on programme integration into existing primary health care structures. Integration may indeed be ideal for control of NTDs, including for HAT. However, in practice this “one size fits all” strategy may not be feasible for HAT given the complex heterogeneity of its epidemiology and the lack of appropriate diagnostic and treatment tools. Many HAT-endemic areas are in remote, rural areas or in regions of conflict and insecurity, with little or no health infrastructure in which to integrate. In these contexts, obstacles to HAT diagnosis and treatment, including integration into primary health care systems, are therefore expected. One major hurdle lies in the complexity and sophistication of HAT diagnostic algorithms and treatment administration (including NECT), which often exceed the capacities of health centers and district hospitals in resource-constrained settings where HAT is endemic. Another impediment is the physical and logistical difficulties in reaching some of the affected populations. A strong vertical component thus remains necessary for HAT surveillance and case management, particularly in areas where the disease is uncontrolled. Active case finding (including mass screening) for *T. b. gambiense* followed by treatment is a highly recommended control measure in such areas. Access to laboratory testing is necessary for screening and diagnosis, which involves resource-intensive procedures including lumbar punctures. Intervening in conflict zones to

reach patients trapped by violence is a major challenge. Context-appropriate programme approaches that take into account the complex epidemiology of HAT and the precarious situations in which it is found are still necessary.

NECT has a number of limitations as a treatment option for HAT. It is likely less effective against *T. b. rhodesiense* HAT, which badly needs different and better drugs for both stages of the disease. Administration of NECT is relatively complicated, including the requirement of two IV infusions per day for one week. Although this protocol is shorter and simpler than eflornithine monotherapy, and safer than melarsoprol, it is still resource- and training-intensive. Thus, a simpler regimen, preferably based on an oral drug formulation, is desirable. A treatment effective for both disease stages may eliminate the need for painful lumbar punctures and difficult examination of the cerebrospinal fluid, which are currently performed for HAT staging.

R&D for better diagnostic tools for HAT are also needed. The sensitivity of parasite detection tools in body fluids is currently limited. In addition, diagnosis of trypanosomal infection of the central nervous system requires a lumbar puncture, which is painful and difficult to perform, especially in resource-constrained settings. Field-adapted, rapid diagnostic tests for HAT diagnosis and staging must be developed if complete HAT control, including integration into primary health care centers, is to be feasible. The introduction of novel biomarkers, including recently identified markers for disease staging, and the development of field-adapted tests will require the mobilization of research laboratories with adequate funding.

Although there has been recent discourse that the elimination of HAT is feasible, this lofty goal is not likely to be possible in the near future given ongoing constraints, namely the difficulties of implementing complex diagnostic–treatment algorithms in resource-poor areas of high endemicity and persistent security threats. Even if perfect treatment and diagnostic tools were readily available for HAT, certain patient populations would still be difficult or impossible to reach. HAT control in these hotspots should therefore be addressed through targeted programming and access, with robust surveillance and response. International donors and policymakers should be made aware that a “one size fits all” integrated approach may not be suitable for HAT in certain contexts and with the current tools. Dedicated funding for diagnosis and treatment and R&D, as well as allocated national programme funding, must be put forth and sustained. The current paucity of international donors funding HAT control national programmes is highly worrisome. Still and in the future, continued political pressure and will are needed for the prioritization of HAT patient care.

6. ANIMAL TRYPANOSOMOSIS

(a) SURVEY AND DISTRIBUTION

15260. **Abebe, R. & Wolde, A., 2010**. A cross-sectional study of trypanosomosis and its vectors in donkeys and mules in Northwest Ethiopia. *Parasitology Research*, **106** (4): 911-916.

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A preliminary study was conducted in January 2009 in four peasant associations (PAs) selected from two districts in Benishangul Gumuz Regional State, Northwest Ethiopia to

investigate the prevalence and species of trypanosomes infecting donkeys and mules and identify the fly vectors playing a role in the transmission of trypanosomosis. Blood samples were collected from a total of 334 donkeys and 52 mules and examined by dark ground/phase contrast buffy coat technique and Giemsa-stained blood smears. Accordingly, trypanosome species were encountered in 6.3 percent of the examined donkeys (n = 21) while none of the mules examined was positive for trypanosome infection. Trypanosomes and tsetse flies were detected in two of the four PAs surveyed (Tsetsa adurno and Bamadone) with significant (P = 0.004) difference in prevalence. The inability to find trypanosomes in the other two PAs (Ura and Ashura) was most likely due to the absence of appropriate fly vectors. Three species of trypanosomes were detected in donkeys, which in order of predominance were *Trypanosoma congolense* (52.4 percent), *Trypanosoma brucei* (28.6 percent), and *Trypanosoma vivax* (19.05 percent). There was a significant (P = 0.008) difference in mean PCV between trypanosome infected and non-infected donkeys. The body condition score of the donkeys was significantly associated with both prevalence of infection (P = 0.009) and mean packed cell volume (PCV; P < 0.0001). No significant difference was observed between male and female donkeys regarding both prevalence of infection and mean PCV (P > 0.05 for each factor). The entomological surveys revealed the presence of *Glossina morsitans submorsitans* and other biting flies of the family *Stomoxys, Tabanus*, and *Haematopota*. In conclusion, the prevalence of trypanosomosis obtained in the current study is generally low compared to previous studies. As the present study design was a cross-sectional, one that only depicts a momentary picture of the infection status in the herd, a further longitudinal study that makes use of more sensitive techniques and entomological survey is recommended.

15261. **Cordon-Obras, C., Garcia-Estebanez, C., Ndong-Mabale, N., Abaga, S., Ndongo-Asumu, P., Benito, A. & Cano, J., 2010.** Screening of *Trypanosoma brucei gambiense* in domestic livestock and tsetse flies from an insular endemic focus (Luba, Equatorial Guinea). *PLoS Neglected Tropical Diseases,* **4** (6): e704.

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Sleeping sickness is spread over 36 Sub-Saharan African countries. In West and Central Africa, the disease is caused by *Trypanosoma brucei gambiense*, which produces a chronic clinical manifestation. The Luba focus (Bioko Island, Equatorial Guinea) has not reported autochthonous sleeping sickness cases since 1995, but given the complexity of the epidemiological cycle, the elimination of the parasite in the environment is difficult to categorically ensure. The aim of this work is to assess, by a molecular approach (Polymerase Chain Reaction, PCR), the possible permanence of *T. b. gambiense* in the vector (*Glossina* spp.) and domestic fauna in order to improve our understanding of the epidemiological situation of the disease in an isolated focus considered to be under control. The results obtained show the absence of the parasite in peridomestic livestock but its presence, although at very low rate, in the vector. On the other hand, interesting entomological data highlight that an elevated concentration of tsetse flies was observed in two out of the ten villages

considered to be in the focus. These findings demonstrate that even in conditions of apparent control, a complete parasite clearance is difficult to achieve. Further investigations must be focused on animal reservoirs which could allow the parasites to persist without leading to human cases. In Luba, where domestic livestock are scarcer than other foci in mainland Equatorial Guinea, the epidemiological significance of wild fauna should be assessed to establish their role in the maintenance of the infection.

15262. **Efrem, D. B., Yacob, H. T., Hagos, A. T. & Basu, A. K., 2010**. Bovine trypanosomosis in Gimbi district of Western Oromia, Ethiopia. *Animal Biology*, **60**, (2): 123–131

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A study on the epidemiology of bovine trypanosomosis was conducted from September 2006 to April 2007 in six villages of the Gimbi district in west Wollega zone of Ethiopia. The prevalence of the disease, the apparent densities and distribution of tsetse and other biting flies in two seasons, the dry and rainy, were determined. The results of a questionnaire survey from 80 farmers revealed that trypanosomosis was a major health problem affecting animals and impeding agricultural activities. A total of 568 blood samples were collected from randomly selected animals (280 animals in rainy and 288 in dry season) and revealed the presence of *Trypanosoma congolense* Broden, 1904 and *T. vivax* Zieman, 1905 in the area. *Trypanosoma congolense* was the dominant species that accounted for 66.2 percent of the infections. The mean packed cell volume (PCV) concentrations were 22.77 percent (95 percent CI =19.99-21.55) in parasitaemic and 25.25 percent (95 percent CI=24.88-25.61) in aparasitaemic animals with a significant difference ($P < 0.005$). There was a significant ($P < 0.012$) difference in trypanosome infection between age groups of cattle, being higher in adults. The overall prevalence of trypanosomosis was 12.5 percent, while the disease prevalence was higher during the rainy season (15 percent) than the dry season (10.1 percent). In three villages in lowland areas (below 1 600 meter above sea level), a higher prevalence was recorded in the late rainy and dry season respectively. (20.9 percent and 7.9 percent) as compared with 11.8 percent and 8.3 percent in three villages in midland areas (≥ 1 600 meter above sea level). A fly survey was conducted using 80 monoconical pyramidal traps and revealed that two tsetse species, namely *Glossina morsitans sub morsitans* Newstead and *Glossina tachinoides* Westwood were found along with other biting flies (*Tabanus, Haematopota* and *Stomoxys* species). Higher numerical catches of *Glossina* were recorded in the late rainy season and the apparent density was positively correlated ($r = 0.5171$) with the prevalence of infection.

15263. **Gari, F. R., Ashenafi, H., Tola, A., Goddeeris, B. M. & Claes, F., 2010.** Comparative diagnosis of parasitological, serological, and molecular tests in dourine-suspected horses. *Tropical Animal Health & Production.* **Published online 6 June**.

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A study on the comparative sensitivity of parasitological, serological, and molecular tests on 237 horses originating from two dourine-suspected districts of Arsi-Bale highlands of Ethiopia was conducted to determine the prevalence of the disease and degree of agreement of the diagnostic tests. Accordingly, the prevalence of the disease was found to be 4.6 percent, 36.7 percent, and 47.6 percent by parasitological Woo test, RoTat 1.2 and 18S PCR tests, respectively. The seroprevalence of the disease was 27.6 percent in CATT/*Trypanosoma evansi* test. In Ethiopia, it was for the first time that trypanosomes from dourine suspected horses were demonstrated in 4.6 percent of the animals using Woo test. The findings of the present study disclosed that dourine is highly prevalent and one of the major diseases of horses in the area. There was no statistically significant difference ($P > 0.05$) in prevalence of the disease between districts, sexes, and age groups of the animals. However, there was a statistically significant difference ($P < 0.05$) in the prevalence of the disease between emaciated and animals with good body condition. Assessment of the degree of agreement of the diagnostic tests employed revealed low to fair with significantly higher sensitivity by PCR than other tests.

15264. **Munang'andu, H. M., Siamudaala, V., Munyeme, M., Nambota, A., Mutoloki, S. & Matandiko, W., 2010**.*Trypanosoma brucei* infection in asymptomatic greater Kudus (*Tragelaphus strepsiceros*) on a game ranch in Zambia. *Korean Journal of Parasitology,* **48** (1): 67-69.

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Trypomastogotes of *Trypanosoma brucei* were detected from 4 asymptomatic kudus (*Tragelaphus strepsiceros*) on a game ranch located approximately 45 km north east of Lusaka, Zambia. Blood smears examined from 14 wildlife species comprising of the impala (*Aepyceros melampus*), kafue lechwe (*Kobus leche kafuensis*), sable antelope (*Hippotragus niger*), tsessebe (*Damaliscus lunatus*), warthog (*Phacochoerus aethiopicus*), puku (*Kobus vardoni*), zebra (*Equus burchelli*), waterbuck (*Kobus ellipsiprymnus*), bushbuck (*Tragelaphus scriptus*), reedbuck (*Redunca arundinum*), wildebeest (*Connochaetes taurinus*), hartebeest (*Alcephelus lichtensteini*), African buffalo (*Syncerus caffer*), and kudu (*Tragelaphus strepsiceros*) showed that only the kudu had *T. brucei*. Although game ranching has emerged to be a successful *ex situ* conservation strategy aimed at saving the declining wildlife population in the national parks, our findings suggest that it has the potential of aiding the re-distribution of animal diseases. Hence, there is a need for augmenting wildlife conservation with disease control strategies aimed at reducing the risk of disease transmission between wildlife and domestic animals.

15265. **Simukoko, H., Marcotty, T., Vercruysse, J. & Van den Bossche, P., 2010.** Bovine trypanosomiasis risk in an endemic area on the eastern plateau of Zambia. *Research in Veterinary Science.* **In press, corrected proof.**

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The control of bovine trypanosomiasis could be improved by using the available control tools during periods when the incidence of the disease is highest. The present study assessed the monthly risk of bovine trypanosomiasis in 85 sentinel cattle kept on the tsetse-infested eastern plateau of Zambia during a period of 19 consecutive months. To avoid problems associated with persistence of infections because of trypanocidal drug resistance and/or the time lag between sampling and molecular analysis, a survival analysis and the subsequent calculation of risk was used as an indicator of challenge. Results showed that the average monthly risk of infection (92.3 percent due to *Trypanosoma congolense*) was 6 percent. It was significantly higher (7.7 percent) during the beginning of the rainy season (December-February). According to the outcome of the study, bovine trypanosomiasis control in the study area can be improved through increasing control efforts during this period of highest challenge.

15266. **Tadesse, A. & Tsegaye, B., 2010.** Bovine trypanosomosis and its vectors in two districts of Bench Maji zone, South Western Ethiopia. *Tropical Animal Health & Production.* **Published online 26 June.**

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A cross-sectional study was carried out from November 2008 to February 2009 in Guraferda and Sheko districts of Bench Maji Zone, South Western Ethiopia. The objective of the study was to determine the prevalence of bovine trypanosomosis and the density of its vectors. An overall prevalence of trypanosome infection in the study area was 4.4 percent. *Trypanosoma congolense* (36.36 percent) was the dominant trypanosome species followed by *Trypanosoma vivax* (18.18 percent) and *Trypanosoma brucei* (9.09 percent). Mean packed cell volume value of parasitaemic animals (21.8 percent) was significantly ($P < 0.05$) lower than that of aparasitaemic animals (27.7 percent). Biconical and NGU traps were deployed for 72 h, and the result indicated *Glossina pallidipes* followed by *Glossina fuscipes* as the only tsetse fly species caught in the study area along with other biting flies like *Stomoxys* and *Tabanus*. The apparent density of tsetse flies was 2.83 flies $trap^{-1}$ day^{-1}. NGU traps caught more of *G. pallidipes* while biconical traps caught more *G. fuscipes*, and the difference was significant ($P < 0.05$). Although the current study indicated low prevalence of trypanosomosis in the study area, the impacts of trypanosomosis on cattle production and productivity should not be neglected. Therefore, attention should be given to control the disease and also the vectors.

15267. **Thumbi, S. M., Jung'a, J. O., Mosi, R. O. & McOdimba, F. A., 2010**. Spatial distribution of African animal trypanosomiasis in Suba and Teso districts in Western Kenya. *BMC Research Notes,* **3**: 6.

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Studies on the epidemiology of African Animal Trypanosomiasis (AAT) rarely consider the spatial dimension of disease prevalence. This problem is confounded by use of parasitological diagnostic methods of low sensitivity in field surveys. Here we report a study combining highly sensitive and species specific molecular diagnostic methods, and geographical information system (GIS) for spatial analysis of trypanosome infection patterns, to better understand its epidemiology. Blood samples from 44 and 59 animals randomly selected from Teso and Suba districts respectively were screened for trypanosomes using PCR diagnostic assays. Spatial distribution of the positive cases was mapped and average nearest neighbour analysis used to determine the spatial pattern of trypanosome cases detected. Trypanosome prevalence of 41 percent and 29 percent in Suba and Teso districts respectively was observed. *T. vivax* infections were most prevalent in both areas. Higher proportions of *T. brucei* infections (12 percent) were observed in Suba, a known sleeping sickness focus compared with 2 percent in Teso. Average nearest neighbour analysis showed the pattern of trypanosome infections as random. An overlay with tsetse maps showed cases lying outside the tsetse infested areas, mostly being cases of *T. vivax* which is known to be transmitted both biologically by tsetse and mechanically by biting flies. These findings suggest a need to design control strategies that target not just the biological vector tsetse, but also the parasite in cattle in order to clear the possibly mechanically transmitted *T. vivax* infections. There is need to also review the accuracy of available tsetse maps.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **33**: 15260, 15262, 15266].

15268**. Da Silva, A. S., Wolkmer, P., Costa, M. M., Tonin, A. A., Eilers, T. L., Gressler, L. T., Otto, M. A., Zanette, R. A., Santurio, J. M., Lopes, S. T. & Monteiro, S. G., 2010.** Biochemical changes in cats infected with *Trypanosoma evansi*. *Veterinary Parasitology,* **171** (1-2): 48-52.

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This study aimed at evaluating biochemical changes of cats (*Felis catus*) experimentally infected with *Trypanosoma evansi*. Seven animals were infected with 10^8 blood trypomastigotes per animal and six were used as controls. Blood smears were performed daily for 56 days and the hepatic, renal and muscular parameters in blood serum were evaluated at days 0, 7, 21, 35 and 49. The protozoan was found in the bloodstream 24-48 h post-inoculation (PI) and irregular peaks of parasitaemia were observed throughout the experiment. Muscular enzymatic activities (aspartate aminotransferase and creatine kinase)

were increased in infected cats compared to controls. Increased concentrations of total proteins and globulins and decreased levels of albumin and albumin/globulin ratio were observed in infected group versus the controls values ($P < 0.05$). No alteration in serum activity of alanine aminotransferase, gamma-glutamyltransferase, creatinine and urea was observed in both groups.

(c) TRYPANOTOLERANCE

[See also **33:** 15291, 15293, 15294, 15371].

15269. **Behnke, J. M., Chiejina, S. N., Musongong, G. A., Nnadi, P. A., Ngongeh, L. A., Abonyi, F. O. & Fakae, B. B., 2010.** Resistance and resilience of traditionally managed West African Dwarf goats from the savannah zone of northern Nigeria to naturally acquired trypanosome and gastrointestinal nematode infections. *Journal of Helminthology.* **e publication May 12.**

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A survey was conducted of gastrointestinal nematode infections and trypanosomosis in Nigerian West African Dwarf (WAD) goats from the savannah region of the country. Animals were screened at two markets, Gboko and Akpagher, from the beginning of April until the end of September, coinciding with the end of the dry season and the first 5 months of the wet season. Of 1 054 goats that were examined, 80.5 percent carried gastrointestinal (GI) nematodes belonging to the genera *Haemonchus* (61.0 percent), *Oesophagostomum* (21.0 percent) and *Trichostrongylus* (17.9 percent). Faecal egg counts (FEC) increased very slowly but significantly from April to maximum levels in September, and varied marginally between the two market sources. The majority of goats (68.8 and 70.1 percent at the two markets) had low FEC not exceeding 50 eggs/g (epg). FEC did not differ significantly between the sexes or between age classes. Packed cell volume (PCV) also declined significantly with month of the study, but was affected by host sex (a significant month x sex interaction) being generally higher in male animals throughout the period. There was a highly significant negative correlation between $\log_{10}$ (FEC+1) and PCV, when all other factors had been taken into account. Body condition scores (BCS) also declined with month of the study, but there was a marked difference between the two sexes, with male animals generally showing a greater stability of BCS across the months compared with females. Trypanosome infections were found in only 4 percent of the goats and only during the rainy season. Most infections (92.86 percent) were caused by *Trypanosoma brucei* alone although *T. vivax* and *T. congolense* were occasionally detected. Overall, the majority of goats sampled each month maintained generally good body condition (BCS 3.0-5.0), normal or slightly reduced PCV, even when concurrently infected with trypanosomes and GI nematodes. However, four concurrently infected goats showed signs of overt anaemia during periods of peak infection, during the late rainy season, with marked reductions in PCV (<15 percent). Two of the infected goats were also in poor body condition with BCS of < 2.0. There was no evidence of additive or synergistic pathogenic effects of the two parasites. These results are discussed in

the context of the unexpectedly strong resistance and resilience of the savannah WAD ecotype to its native strains of GI nematode and trypanosome parasites.

(d) TREATMENT

[See **33**: 15311].

7. EXPERIMENTAL TRYPANOSOMOSIS

(a) DIAGNOSTICS

[See also **33**: 15198, 15203, 15205, 15263].

15270. **Camara, M., Camara, O., Ilboudo, H., Sakande, H., Kabore, J., N'Dri, L., Jamonneau, V. & Bucheton, B., 2010**. Sleeping sickness diagnosis: use of buffy coats improves the sensitivity of the mini anion exchange centrifugation test. *Tropical Medicine & International Health,* **15** (7): 796-799.

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This study was to evaluate a modification of the mini anion exchange centrifugation test (mAECT) for the diagnosis of *Trypanosoma brucei gambiense* human African trypanosomiasis (HAT). To increase its sensitivity, this test uses 350 µL of buffy coat withdrawn from 5 mL of blood instead of blood. The new protocol was first tested experimentally on serial dilution of trypanosomes and was then further evaluated under field conditions on 57 patients with HAT diagnosed during a medical survey in Guinea. Experimentally, the use of buffy coats improved mAECT sensitivity at least five fold and enabled to consistently detect parasites in blood at a concentration of 10 trypanosomes/mL. During the field evaluation, more patients tested positive by mAECT-bc (96.5 percent) than by mAECT-blood (78.9 percent, $chi^2 = 6.93$, $P = 0.008$) and lymph juice examination (77.2 percent, $chi^2 = 7.67$, $P = 0.005$). Furthermore, the number of parasites per collectors was significantly higher (7.2 vs. 2.6, $P = 0.001$) when buffy coats were used instead of blood. The use of the mAECT-bc protocol enabled a significant improvement of HAT parasitological diagnosis in Guinea, without any additional costs. It would deserve to be tested in other *T. b. gambiense* endemic areas.

15271. **de Clare Bronsvoort, B. M., von Wissmann, B., Fevre, E. M., Handel, I. G., Picozzi, K. & Welburn, S. C., 2010.** No gold standard estimation of the sensitivity and specificity of two molecular diagnostic protocols for *Trypanosoma brucei* spp. in Western Kenya. *PLoS One*, **5** (1): e8628.

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African animal trypanosomiasis is caused by a range of tsetse transmitted protozoan parasites including *Trypanosoma vivax, Trypanosoma congolense* and *Trypansoma brucei*. In Western Kenya and other parts of East Africa two subspecies of *T. brucei, T. b. brucei* and the zoonotic *T. b. rhodesiense*, co-circulate in livestock. A range of polymerase chain reactions (PCR) have been developed as important molecular diagnostic tools for epidemiological investigations of *T. brucei* s.l. in the animal reservoir and of its zoonotic potential. Quantification of the relative performance of different diagnostic PCRs is essential to ensure comparability of studies. This paper describes an evaluation of two diagnostic test systems for *T. brucei* using a *T. brucei* s.l. specific PCR and a single nested PCR targeting the internal transcribed spacer (ITS) regions of trypanosome ribosomal DNA. A Bayesian formulation of the Hui-Walter latent class model was employed to estimate their test performance in the absence of a gold standard test for detecting *T. brucei* s.l. infections in ear-vein blood samples from cattle, pig, sheep and goat populations in Western Kenya, stored on Whatman FTA cards. The results indicate that the system employing the *T. brucei* s.l. specific PCR (Se1 = 0.760) had a higher sensitivity than the ITS-PCR (Se2 = 0.640); both have high specificity (Sp1 = 0.998; Sp2 = 0.997). The true prevalences for livestock populations were estimated (pcattle=0.091, ppigs = 0.066, pgoats = 0.005, psheep = 0.006), taking into account the uncertainties in the specificity and sensitivity of the two test systems. Implications of test performance include the required survey sample size; due to its higher sensitivity and specificity, the *T. brucei* s.l. specific PCR requires a consistently smaller sample size than the ITS-PCR for the detection of *T. brucei* s.l. However the ITS-PCR is able to simultaneously screen samples for other pathogenic trypanosomes and may thus be, overall, a better choice of test in multi-organism studies.

15272. **Manful, T., Mulindwa, J., Frank, F. M., Clayton, C. E. & Matovu, E., 2010.** A search for *Trypanosoma brucei rhodesiense* diagnostic antigens by proteomic screening and targeted cloning. *PLoS One,* **5** (3): e9630.

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The only available diagnostic method for East African trypanosomiasis is light microscopy of blood samples. A simple immunodiagnostic would greatly aid trypanosomiasis control. To find trypanosome proteins that are specifically recognized by sera from human sleeping sickness patients, we have screened the *Trypanosoma brucei brucei* proteome by Western blotting. Using cytosolic, cytoskeletal and glycosomal fractions, we found that the vast majority of abundant trypanosome proteins are not specifically recognized by patient sera. We identified phosphoglycerate kinase (PGKC), heat shock protein (HSP70), and histones H2B and H3 as possible candidate diagnostic antigens. These proteins, plus paraflagellar rod protein 1, rhodesain (a cysteine protease), and an extracellular fragment of the *Trypanosoma brucei* nucleoside transporter TbNT10, were expressed in *E. coli* and tested for reactivity with patient and control sera. Only TbHSP70 was preferentially recognized by

patient sera, but the sensitivity and specificity were insufficient for use of TbHSP70 alone as a diagnostic. Immunoprecipitation using a native protein extract revealed no specifically reacting proteins. It is concluded that no abundant *T. brucei* soluble, glycosomal or cytoskeletal protein is likely to be useful in diagnosis. To find useful diagnostic antigens it will therefore be necessary to use more sophisticated proteomic methods, or to test a very large panel of candidate proteins.

15273**. Mugasa, C. M., Deborggraeve, S., Schoone, G. J., Laurent, T., Leeflang, M. M., Ekangu, R. A., El Safi, S., Saad, A. F., Basiye, F. L., De Doncker, S., Lubega, G. W., Kager, P. A., Buscher, P. & Schallig, H. D., 2010**. Accordance and concordance of PCR and NASBA followed by oligochromatography for the molecular diagnosis of *Trypanosoma brucei* and *Leishmania*. *Tropical Medicine & International Health,* **15** (7): 800-805.

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To evaluate the repeatability and reproducibility of four simplified molecular assays for the diagnosis of *Trypanosoma brucei* spp. or *Leishmania* ssp. in a multicentre ring trial with seven participating laboratories, samples were tested using PCR or NASBA amplification of the parasites' nucleic acids followed by rapid read-out by oligochromatographic dipstick (PCR-OC and NASBA-OC). On purified nucleic acid specimens, the repeatability and reproducibility of the tests were Tryp-PRC-OC, 91.7 percent and 95.5 percent; Tryp-NASBA-OC, 95.8 percent and 100 percent; Leish-PCR-OC, 95.9 percent and 98.1 percent; Leish-NASBA-OC, 92.3 percent and 98.2 percent. On blood specimens spiked with parasites, the repeatability and reproducibility of the tests were Tryp-PRC-OC, 78.4 percent and 86.6 percent; Tryp-NASBA-OC, 81.5 percent and 89.0 percent; Leish-PCR-OC, 87.1 percent and 91.7 percent; Leish-NASBA-OC, 74.8 percent and 86.2 percent. As repeatability and reproducibility of the tests were satisfactory, further phase II and III evaluations in clinical and population specimens from disease endemic countries are justified.

15274. **Sengupta, P. P., Balumahendiran, M., Suryanaryana, V. V., Raghavendra, A. G., Shome, B. R., Gajendragad, M. R. & Prabhudas, K., 2010**. PCR-based diagnosis of surra-targeting VSG gene: experimental studies in small laboratory rodents and buffalo. *Veterinary Parasitology*, **171** (1-2): 22-31.

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Trypanosoma evansi, the causative organism of "surra" expresses its variable surface glycoprotein (VSG) at early, middle and late stages of infection in animals. The variable antigenic nature of VSG caused by switching its expression type favours evasion from the host immune response and leads to chronic and persistent infection. Developing a polymerase chain reaction (PCR)-based diagnostic tool targeting the VSG gene is expected to be highly specific and sensitive for diagnosis of surra. Hence, in the present study, we have designed EXP3F/4R primer pair and amplified the 1.4 kb of VSG gene of *T. evansi* and studied the phylogenetic relationship by *in silico* analysis. The PCR method was standardized using another set of primers, DITRYF/R, and 400 bp were amplified from blood and tissue samples of experimentally infected animals. Applying the PCR method, we were able to detect as low as 0.15 trypanosomes/mL^{-1}. Considering the number of parasites and DNA concentrations, the PCR method has a sensitivity of 0.015 pg/mL^{-1}. The PCR could detect the presence of the parasite as early as 24h post-infection (p.i.) and 72 h p.i., respectively, in experimentally infected rats and buffalo. No amplification was observed with DNA of *Babesia bigemina* and *Theileria annulata*, indicating the primers are specific for *T. evansi*. The PCR method could detect the dog, lion and leopard isolates of *T. evansi*. Similarly, amplifying the DNA from the experimentally infected tissues was also found to be sensitive. Thus, the findings of this study favour the application of PCR over the parasitological methods for the detection of the early and/or chronic stage of surra in domestic and wild animals.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **33**: 15371, 15399, 15401].

15275. **Amin, D. N., Ngoyi, D. M., Nhkwachi, G. M., Palomba, M., Rottenberg, M., Buscher, P., Kristensson, K. & Masocha, W., 2010**. Identification of stage biomarkers for human African trypanosomiasis. *American Journal of Tropical Medicine & Hygiene*, **82** (6): 983-990.

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Human African trypanosomiasis (HAT), caused by infection with sub-species of *Trypanosoma brucei* (*T. b.*), manifests as a haemolymphatic stage followed by an encephalitic stage. The distinction of the two stages needs improvement as drugs used for the late stage are highly toxic. Transcripts encoding 16 secreted proteins differentially expressed in the brains of mice at late stage *T. b. brucei* infection when the early stage drug suramin is no longer effective and different to immunoglobulins, chemokines, and cytokines, were selected by microarray analysis. Lipocalin 2 and secretory leukocyte peptidase inhibitor (SLPI) mRNA showed the highest differential expression in mice. These transcripts were also upregulated in brains from infected rats. Lipocalin 2 was increased in cerebrospinal fluid

(CSF) from rats during late stage *T. b. brucei* infection. Protein levels of lipocalin 2, SLPI, and the chemokine CXCL10 were found increased in CSF from *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* late stage HAT compared to early stage.

15276**. Amrouni, D., Gautier-Sauvigne, S., Meiller, A., Vincendeau, P., Bouteille, B., Buguet, A. & Cespuglio, R., 2010.** Cerebral and peripheral changes occurring in nitric oxide (NO) synthesis in a rat model of sleeping sickness: identification of brain iNOS expressing cells. *PLoS One,* **5** (2): e9211.

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The implication of nitric oxide (NO) in the development of human African trypanosomiasis (HAT) using an animal model, was examined. The manner by which the trypanocidal activity of NO is impaired in the periphery and in the brain of rats infected with *Trypanosoma brucei brucei* (*T. b. brucei*) was analyzed through: (i) the changes occurring in NO concentration in both peripheral (blood) and cerebral compartments; (ii) the activity of nNOS and iNOS enzymes; (iii) identification of the brain cell types in which the NO-pathways are particularly active during the time-course of the infection. NO concentration (direct measures by voltammetry) was determined in central (brain) and peripheral (blood) compartments in healthy and infected animals at various days post-infection: D5, D10, D16 and D22. Opposite changes were observed in the two compartments. NO production increased in the brain (hypothalamus) from D10 (+32 percent) to D16 (+71 percent), but decreased in the blood from D10 (-22 percent) to D16 (-46 percent) and D22 (-60 percent). In parallel with NO measures, cerebral iNOS activity increased and peaked significantly at D16 (up to +700 percent). However, nNOS activity did not vary. Immunohistochemical staining confirmed iNOS activation in several brain regions, particularly in the hypothalamus. In peritoneal macrophages, iNOS activity decreased from D10 (-83 percent) to D16 (-65 percent) and D22 (-74 percent) similarly to circulating NO. The NO changes observed in our rat model were dependent on iNOS activity in both peripheral and central compartments. In the periphery, the NO production decrease may reflect an arginase-mediated synthesis of polyamines necessary to trypanosome growth. In the brain, the increased NO concentration may result from an enhanced activity of iNOS present in neurons and glial cells. It may be regarded as a marker of deleterious inflammatory reactions.

15277. **Vankrunkelsven, A., De Ceulaer, K., Hsu, D., Liu, F-T., De Baetselier, P. & Stiljemans, B., 2010.** Lack of galactin-3 alleviateds trypanosomiasis-associated anaemia of inflammation. *Immunobiology,* **215** (9-10): 833-841.

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A typical pathological feature associated with experimental African trypanosomiasis (*Trypanosoma brucei* infection in mice) is anaemia of chronic disease (ACD), which is due to a sustained type 1 cytokine-mediated inflammation and hyperactivation of M1 macrophages. Galectin-3 (Gal-3) was amply documented to contribute to the onset and persistence of type 1 inflammatory responses and we herein document that this protein is strongly upregulated during *T. brucei* infection. We evaluated the involvement of Gal-3 in trypanosomiasis-associated anaemia using galectin-3 deficient (Gal3(-/-)) mice. *T. brucei* infected Gal3(-/-) mice manifested significant lower levels of anaemia during infection and survived twice as long as wild type mice. Moreover, such mice showed increased levels of serum IL-10 and reduced liver pathology (as evidenced by lower AST/ALT levels). In addition, there was also an increase in gene expression of iron export genes and a reduced expression of genes, which are associated with accumulation of cellular iron. Our data indicate that Gal-3 is involved in the development of inflammation-associated anaemia during African trypanosomiasis, possibly due to a disturbed iron metabolism that in turn may also lead to liver malfunction.

15278. **Bastos, I. M., Motta, F. N., Charneau, S., Santana, J. M., Dubost, L., Augustyns, K. & Grellier, P., 2010.** Prolyl oligopeptidase of *Trypanosoma brucei* hydrolyzes native collagen, peptide hormones and is active in the plasma of infected mice. *Microbes & Infection,* **12** (6): 457-466.

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Proteases play important roles in many biological processes of parasites, including their host interactions. In sleeping sickness, *Trypanosoma brucei* proteases released into the host bloodstream could hydrolyze host factors, such as hormones, contributing to the development of the disease's symptoms. In this study, we present the identification of the *T. brucei* prolyl oligopeptidase gene (poptb) and the characterization of its corresponding enzyme, POP Tb. Secondary structure predictions of POP Tb show a structural composition highly similar to other POPs. Recombinant POP Tb produced in *E. coli* was active and highly sensitive to inhibitors of *Trypanosoma cruzi* POP Tc80. These inhibitors, which prevent *T. cruzi* entry into non-phagocytic cells, arrested growth of the *T. brucei* bloodstream form in a dose-dependent manner. POP Tb hydrolyzes peptide hormones containing Pro or Ala at the P1 position at a slightly alkaline pH, and also cleaves type I collagen *in vitro* and native collagen present in rat mesentery. Furthermore, POP Tb is released into the bloodstream of *T. brucei* infected mice where it remains active. These data suggest that POP Tb might contribute to the pathogenesis of sleeping sickness.

15279. **Bocedi, A., Dawood, K. F., Fabrini, R., Federici, G., Gradoni, L., Pedersen, J. Z. & Ricci, G., 2010.** Trypanothione efficiently intercepts nitric oxide as a harmless iron complex in trypanosomatid parasites. The *FASEB Journal,* **24** (4): 1035-1042.

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Trypanosomatids are protozoan organisms that cause serious diseases, including African sleeping sickness, Chagas disease, and leishmaniasis, affecting about 30 million people in the world. These parasites contain the unusual dithiol trypanothione [T(SH)(2)] instead of glutathione (GSH) as the main intracellular reductant, and they have replaced the otherwise ubiquitous GSH/glutathione reductase redox couple with a T(SH)(2)/trypanothione reductase (TR) system. The reason for the existence of T(SH)(2) in parasitic organisms has remained an enigma. Here, we show that T(SH)(2) is able to intercept nitric oxide and labile iron and form a dinitrosyl-iron complex with at least 600 times higher affinity than GSH. Accumulation of the paramagnetic dinitrosyl-trypanothionyl iron complex *in vivo* was observed in *Trypanosoma brucei* and *Leishmania infantum* exposed to nitric oxide. While the analogous dinitrosyl-diglutathionyl iron complex formed in mammalian cells is a potent irreversible inhibitor of glutathione reductase (IC_{50} = 4 μM), the T(SH)(2) complex does not inactivate TR even at millimolar levels. The peculiar capacity of T(SH)(2) to sequester NO and iron in a harmless stable complex could explain the predominance of this thiol in parasites regularly exposed to NO.

15280. **Costa, M. M., Silva, A. S., Wolkmer, P., Zanette, R. A., Franca, R. T., Monteiro, S. G. & Lopes, S. T., 2010.** Serum proteinogram of cats experimentally infected by *Trypanosoma evansi*. *Preventive Veterinary Medicine,* **95** (3-4) 301-304.

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This study was aimed at evaluating the electrophoretic profile of serum proteins in *Trypanosoma evansi*-infected cats during different periods of infection. Thirteen adult non-breeding female *Felis catus* were separated into two groups. Animals from the infected group (n=7) were inoculated intraperitoneally with a strain of *T. evansi*; whereas, animals from the control group (n=6) received a physiological solution. Blood samples were collected at days 0, 7, 21, and 35 for total protein evaluation and protein fractionation by electrophoresis. Albumin ($P < 0.01$), alpha-2 globulin and gamma globulin ($P < 0.05$) concentrations were statistically different from the seventh day post-inoculation onwards. Beta-globulin levels were increased from day 21 onwards ($P < 0.05$). Alpha-1 globulin fraction did not differ statistically. These results indicate that the infection by *T. evansi* in cats alters the serum protein electrophoretic profile.

15281**. Das, P., Lahiri, A., Lahiri, A. & Chakravortty, D., 2010.** Modulation of the arginase pathway in the context of microbial pathogenesis: a metabolic enzyme moonlighting as an immune modulator. *PLoS Pathogens,* **6** (6): e1000899.

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Arginine is a crucial amino acid that serves to modulate the cellular immune response during infection. Arginine is also a common substrate for both inducible nitric oxide synthase (iNOS) and arginase. The generation of nitric oxide from arginine is responsible for efficient immune response and cytotoxicity of host cells to kill the invading pathogens. On the other hand, the conversion of arginine to ornithine and urea via the arginase pathway can support the growth of bacterial and parasitic pathogens. The competition between iNOS and arginase for arginine can thus contribute to the outcome of several parasitic and bacterial infections. There are two isoforms of vertebrate arginase, both of which catalyze the conversion of arginine to ornithine and urea, but they differ with regard to tissue distribution and subcellular localization. In the case of infection with Mycobacterium*, Leishmania, Trypanosoma, Helicobacter, Schistosoma*, and *Salmonella* spp., arginase isoforms have been shown to modulate the pathology of infection by various means. Despite the existence of a considerable body of evidence about mammalian arginine metabolism and its role in immunology, the critical choice to divert the host arginine pool by pathogenic organisms as a survival strategy is still a mystery in infection biology.

15282**. Emmer, B. T., Daniels, M. D., Taylor, J. M., Epting, C. L. & Engman, D. M., 2010**. Calflagin inhibition prolongs host survival and suppresses parasitaemia in *Trypanosoma brucei* infection. *Eukaryotic Cell,* **9** (6): 934-942.

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African trypanosomes express a family of dually acylated, EF-hand calcium-binding proteins called the calflagins. These proteins associate with lipid raft microdomains in the flagellar membrane, where they putatively function as calcium signalling proteins. Here we show that these proteins bind calcium with high affinity and that their expression is regulated during the life cycle stage of the parasite, with protein levels approximately 10-fold higher in the mammalian bloodstream form than in the insect vector procyclic stage. We also demonstrate a role for the calflagins in mammalian infection, as inhibition of the entire calflagin family by RNA interference dramatically increased host survival and attenuated parasitaemia in a mouse model of sleeping sickness. In contrast to infection with parental wild-type parasites, which demonstrated an unremitting parasitaemia and death within 6 to 10 days, infection with calflagin-depleted parasites demonstrated prolonged survival associated with a sudden decrease in parasitaemia at approximately 8 days postinfection. Subsequent relapsing and remitting waves of parasitaemia thereafter were associated with alternate expression of the variant surface glycoprotein, suggesting that initial clearance was antigen specific. Interestingly, despite the notable *in vivo* phenotype and flagellar localization of the calflagins, *in vitro* analysis of the calflagin-deficient parasites demonstrated normal proliferation, flagellar motility, and morphology. Further analysis of the kinetics of surface antibody clearance also did not demonstrate a deficit in the calflagin-deficient parasites; thus, the molecular basis for the altered course of infection is independent of an effect on parasite cell cycle progression, motility, or degradation of surface-bound antibodies.

15283. **Geiger, A., Hirtz, C., Becue, T., Bellard, E., Centeno, D., Gargani, D., Rossignol, M., Cuny, G. & Peltier, J. B., 2010.** Exocytosis and protein secretion in *Trypanosoma. BMC Microbiology,* **10**: 20.

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Human African trypanosomiasis is a lethal disease caused by the extracellular parasite *Trypanosoma brucei*. The proteins secreted by *T. brucei* inhibit the maturation of dendritic cells and their ability to induce lymphocytic allogenic responses. To better understand the pathogenic process, we combined different approaches to characterize these secreted proteins. Overall, 444 proteins were identified using mass spectrometry, the largest parasite secretome described to date. Functional analysis of these proteins revealed a strong bias toward folding and degradation processes and to a lesser extent toward nucleotide metabolism. These features were shared by different strains of *T. brucei*, but distinguished the secretome from published *T. brucei* whole proteome or glycosome. In addition, several proteins had not been previously described in *Trypanosoma* and some constitute novel potential therapeutic targets or diagnostic markers. Interestingly, a high proportion of these secreted proteins are known to have alternative roles once secreted. Furthermore, bioinformatic analysis showed that a significant proportion of proteins in the secretome lack transit peptide and are probably not secreted through the classical sorting pathway. Membrane vesicles from secretion buffer and infested rat serum were purified on sucrose gradient and electron microscopy pictures have shown 50- to 100-nm vesicles budding from the coated plasma membrane. Mass spectrometry confirmed the presence of *Trypanosoma* proteins in these microvesicles, showing that an active exocytosis might occur beyond the flagellar pocket. This study brings out several unexpected features of the secreted proteins and opens novel perspectives concerning the survival strategy of *Trypanosoma* as well as possible ways to control the disease. In addition, concordant lines of evidence support the original hypothesis of the involvement of microvesicle-like bodies in the survival strategy allowing *Trypanosoma* to exchange proteins at least between parasites and/or to manipulate the host immune system.

15284. **Harrington, J. M., Widener, J., Stephens, N., Johnson, T., Francia, M., Capewell, P., Macleod, A. & Hajduk, S. L., 2010.** The plasma membrane of bloodstream form African trypanosomes confers susceptibility and specificity to killing by hydrophobic peptides. *Journal of Biological Chemistry.* **In press, corrected proof July 13.**

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Trypanosoma brucei is the causative agent of both a veterinary wasting disease and human African trypanosomiasis, or sleeping sickness. The cellular membrane of the developmental stage found within the mammalian host, the bloodstream form (BSF), is

highly dynamic, exhibiting rapid rates of endocytosis and lateral flow of GPI-anchored proteins. Here we show that the cell membrane of these organisms is a target for killing by small hydrophobic peptides that increase the rigidity of lipid bilayers. Specifically, we have derived trypanocidal peptides that are based upon the hydrophobic N-terminal signal sequences of human apolipoproteins. These peptides selectively partition into the plasma membrane of BSF trypanosomes resulting in an increase in the rigidity of the bilayer, dramatic changes in cell motility and subsequent cell death. No killing of the developmental stage found within the insect midgut, the procyclic form, was observed. Additionally the peptides exhibit no toxicity towards mammalian cell lines nor do they induce hemolysis. Studies with model liposomes indicate that bilayer fluidity dictates the susceptibility of membranes to manipulation by hydrophobic peptides. We suggest that the composition of the BSF trypanosome cell membrane confers a high degree of fluidity and unique susceptibility to killing by hydrophobic peptides and is therefore a target for the development of trypanocidal drugs.

15285. **Inverso, J. A., Uphoff, T. S., Johnson, S. C., Paulnock, D. M. & Mansfield, J. M., 2010.** Biological variation among African trypanosomes: I. Clonal expression of virulence is not linked to the variant surface glycoprotein or the variant surface glycoprotein gene telomeric expression site. *DNA Cell Biology,* **29** (5): 215-227.

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The potential association of variant surface glycoprotein (VSG) gene expression with clonal expression of virulence in African trypanosomes was addressed. Two populations of clonally related trypanosomes, which differ dramatically in virulence for the infected host, but display the same apparent VSG surface coat phenotype, were characterized with respect to the VSG genes expressed as well as the chromosome telomeric expression sites (ES) utilized for VSG gene transcription. The VSG gene sequences expressed by clones LouTat 1 and LouTat 1A of *Trypanosoma brucei rhodesiense* were identical, and gene expression in both clones occurred precisely by the same gene conversion events (duplication and transposition), which generated an expression-linked copy (ELC) of the VSG gene. The ELC was present on the same genomic restriction fragments in both populations and resided in the telomere of a 330-kb chromosome; a single basic copy of the LouTat 1/1A VSG gene, present in all variants of the LouTat 1 serodeme, was located at an internal site of a 1.5-Mb chromosome. Restriction endonuclease mapping of the ES telomere revealed that the VSG ELC of clones LouTat 1 and 1A resides in the same site. Therefore, these findings provide evidence that the VSG gene ES and, potentially, any cotranscribed ES-associated genes do not play a role in the clonal regulation of virulence because trypanosome clones LouTat 1 and 1A, which differ markedly in their virulence properties, both express identical VSG genes from the same chromosome telomeric ES.

15286. **Jia, Y., Zhao, X., Zou, J. & Suo, X., 2010**. *Trypanosoma evansi*: identification and characterization of a variant surface glycoprotein lacking cysteine residues in its C-terminal domain. *Experimental Parasitology*. **In press, corrected proof.**

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African trypanosomes are flagellated unicellular parasites which proliferate extracellularly in the mammalian host blood-stream and tissue spaces. They evade the hosts' antibody-mediated lyses by sequentially changing their variant surface glycoprotein (VSG). VSG tightly coats the entire parasite body, serving as a physical barrier. In *Trypanosoma brucei* and the closely related species *Trypanosoma evansi* and *Trypanosoma equiperdum*, each VSG polypeptide can be divided into N- and C- terminal domains, based on cysteine distribution and sequence homology. N-terminal domain, the basis of antigenic variation, is hypervariable and contains all the exposed epitopes; the C-terminal domain is relatively conserved and a full set of 4 or 8 cysteines were generally observed. We cloned two genes from two distinct variants of *T. evansi*, utilizing RT-PCR with VSG-specific primers. One contained a VSG type A N-terminal domain followed a C-terminal domain lacking cysteine residues. To confirm that this gene is expressed as a functional VSG, the expression and localization of the corresponding gene product were characterized using western blotting and immunofluorescent staining of living trypanosomes. Expression analysis showed that this protein was highly expressed, variant-specific, and had a ubiquitous cellular surface localization. All these results indicated that it was expressed as a functional VSG. Our finding showed that cysteine residues in VSG C-terminal domain were not essential; the conserved C-terminal domain generally in *T. brucei* like VSGs would possibly evolve for regulating the VSG expression.

15287. **Koning, N., van Eijk, M., Pouwels, W., Brouwer, M. S., Voehringer, D., Huitinga, I., Hoek, R. M., Raes, G. & Hamann, J., 2010.** Expression of the inhibitory CD200 receptor is associated with alternative macrophage activation. *Journal of Innate Immunity,* **2** (2): 195-200.

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Classical macrophage activation is inhibited by the CD200 receptor (CD200R). Here, we show that CD200R expression was specifically induced on human *in vitro* polarized macrophages of the alternatively activated M2a subtype, generated by incubation with IL-4 or IL-13. In mice, peritoneal M2 macrophages, elicited during infection with the parasites *Taenia crassiceps* or *Trypanosoma brucei brucei*, expressed increased CD200R levels compared to those derived from uninfected mice. However, *in vitro* stimulation of mouse peritoneal macrophages and *T. crassiceps* infection in IL-4-/- and IL-4R-/- mice showed that, in contrast to humans, induction of CD200R in mice was not IL-4 or IL-13 dependent. Our data identify CD200R as a suitable marker for alternatively activated macrophages in humans and corroborate observations of distinct species- and/or site-specific mechanisms regulating macrophage polarization in mouse and man.

15288. **Lanca, A. S., de Sousa, K. P., Atouguia, J., Prazeres, D. M., Monteiro, G. A. & Silva, M. S., 2010.** *Trypanosoma brucei*: immunization with plasmid DNA encoding invariant surface glycoprotein gene is able to induce partial protection in experimental African trypanosomiasis. *Experimental Parasitology*. **e publication ahead of print June 18.**

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Trypanosoma brucei is the aetiological agent responsible for African trypanosomiasis, an infectious pathology which represents a serious problem of public health and economic losses in sub-Saharan Africa. As one of the foremost neglected illnesses, few resources have been available for the development of vaccines or new drugs, in spite of the current therapeutical drugs showing little efficiency and high toxicity. Hence, it is obviously important to widen effective therapeutics and preventive strategies against African trypanosomiasis. In this work, we use the DNA vaccine model to evaluate immunization effectiveness in mice challenged with *Trypanosoma brucei brucei*. We demonstrate that Balb/C mice immunized intramuscularly with a single dose of a DNA plasmid encoding a bloodstream stage specific invariant surface glycoprotein (ISG) are partially protected from a lethal dose of *Trypanosoma brucei brucei*. Interestingly, the surviving animals show high levels of IgG2a anti-trypanosomal antibodies, suggesting that the Th1 response profile seems important for the induced mechanisms of immune protection.

15289**. Lundkvist, G. B., Sellix, M. T., Nygard, M., Davis, E., Straume, M., Kristensson, K. & Block, G. D., 2010.** Clock gene expression during chronic inflammation induced by infection with *Trypanosoma brucei brucei* in rats. *Journal of Biological Rhythms,* **25** (2): 92-102.

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African sleeping sickness is characterized by alterations in rhythmic functions. It is not known if the disease affects the expression of clock genes, which are the molecular basis for rhythm generation. We used a chronic rat model of experimental sleeping sickness, caused by the extracellular parasite *Trypanosoma brucei brucei* (*Tb brucei*), to study the effects on clock gene expression. In tissue explants of pituitary glands from Period1-luciferase (Per1-luc) transgenic rats infected with *Tb brucei*, the period of Per1-luc expression was significantly shorter. In explants containing the suprachiasmatic nuclei (SCN), the Per1-luc rhythms were flat in 21 percent of the tissues. We also examined the relative expression of Per1, Clock, and Bmal1 mRNA in the SCN, pineal gland, and spleen from control and infected rats using qPCR. Both Clock and Bmal1 mRNA expression was reduced in the

pineal gland and spleen following *Tb brucei* infection. Infected rats were periodic both in core body temperature and in locomotor activity; however, early after infection, we observed a significant decline in the amplitude of the locomotor activity rhythm. In addition, both activity and body temperature rhythms exhibited decreased regularity and "robustness." In conclusion, although experimental trypanosome infection has previously been shown to cause functional disturbances in SCN neurons, only 21 percent of the SCN explants had disturbed Per1-luc rhythms. However, our data show that the infection overall alters molecular clock function in peripheral clocks including the pituitary gland, pineal gland, and spleen.

15290**. Magez, S., Caljon, G., Tran, T., Stijlemans, B. & Radwanska, M., 2010.** Current status of vaccination against African trypanosomiasis. *Parasitology.* **e Publication ahead of print May 11.**

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Anti-trypanosomiasis vaccination still remains the best theoretical option in the fight against a disease that is continuously hovering between its wildlife reservoir and its reservoir in man and livestock. While antigenic variation of the parasite surface coat has been considered the major obstacle in the development of a functional vaccine, recent research into the biology of B cells has indicated that the problems might go further than that. This paper reviews past and current attempts to design both anti-trypanosome vaccines, as well as vaccines directed towards the inhibition of infection-associated pathology.

15291. **Morrison, L. J., McLellan, S., Sweeney, L., Chan, C. N., MacLeod, A., Tait, A. & Turner, C. M., 2010**. Role for parasite genetic diversity in differential host responses to *Trypanosoma brucei* infection. *Infection & Immunity,* **78** (3): 1096-1108.

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The postgenomic era has revolutionized approaches to defining host-pathogen interactions and the investigation of the influence of genetic variation in either protagonist upon infection outcome. We analyzed the pathology induced by infection with two genetically distinct *Trypanosoma brucei* strains and found that pathogenesis is partly strain specific, involving distinct host mechanisms. Infections of BALB/c mice with one strain (927) resulted in more severe anaemia and greater erythropoietin production compared to infections with the second strain (247), which, contrastingly, produced greater splenomegaly and reticulocytosis. Plasma interleukin-10 (IL-10) and gamma interferon levels were significantly higher in strain 927-infected mice, whereas IL-12 was higher in strain 247-infected mice. To define mechanisms underlying these differences, expression microarray analysis of host genes in the spleen at day 10 postinfection was undertaken. Rank product

analysis (RPA) showed that 40 percent of the significantly differentially expressed genes were specific to infection with one or the other trypanosome strain. RPA and pathway analysis identified LXR/RXR signalling, IL-10 signalling, and alternative macrophage activation as the most significantly differentially activated host processes. These data suggest that innate immune response modulation is a key determinant in trypanosome infections, the pattern of which can vary, dependent upon the trypanosome strain. This strongly suggests that a parasite genetic component is responsible for causing disease in the host. Our understanding of trypanosome infections is largely based on studies involving single parasite strains, and our results suggest that an integrated host-parasite approach is required for future studies on trypanosome pathogenesis. Furthermore, it is necessary to incorporate parasite variation into both experimental systems and models of pathogenesis.

15292. **Otto, M. A., da Silva, A. S., Gressler, L. T., Farret, M. H., Tavares, K. C., Zanette, R. A., Miletti, L. C. & Monteiro, S. G., 2010.** Susceptibility of *Trypanosoma evansi* to human blood and plasma in infected mice. *Veterinary Parasitology,* **168** (1-2): 1-4.

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Around 1900 Laveran and Mesnil discovered that African trypanosomes do not survive in the blood of some primates and humans. The nature of the trypanolytic factor present in these sera has been the focus of a long-standing debate between different groups. The aim of this study was to investigate the susceptibility of *T. evansi* isolates to therapy using human blood and plasma in experimentally infected mice. Forty-eight 2-month-old female mice (*Mus musculus*) were divided into six groups of eight animals per group (A, B, C, D, E and F). Plasma was obtained after blood collection in order to perform therapy. Animals from group A (positive control) were inoculated with *T. evansi* and treated with 0.2mL of saline solution. Animals from groups B and C were infected with the flagellate and received a curative treatment with 0.2mL of human blood (group B) and 0.2mL of human plasma (group C), 24h after infection. Animals from groups D and E received a prophylactic treatment with 0.2mL of human blood and 0.2mL of human plasma, respectively, 24h prior to the infection. Animals from group F (negative control) were not infected and received 0.2mL of saline solution. The four treatments (B, C, D and E) increased animals' longevity when compared to group A. Prepatency period was longer in groups D (15 days) and E (37.7 days) under prophylactic immunotherapy. Moreover, no parasites were found in most of the animals 60 days post-inoculation (PI). Besides the longer longevity, treatments were capable of curing 50 percent of mice of group B, 37.5 percent of group C, 37.5 percent of group D and 25 percent of the animals from group E.

15293. **Stijlemans, B., Vankrunkelsven, A., Brys, L., Raes, G., Magez, S. & De Baetselier, P., 2010.** Scrutinizing the mechanisms underlying the induction of anaemia of inflammation through GPI-mediated modulation of macrophage activation in a model of African trypanosomiasis. *Microbes & Infection,* **12** (5): 389-399.

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In animal trypanosomiasis the severity of infection is reflected by the degree of anaemia which resembles anaemia of inflammation, involving a skewed iron homeostasis leading to iron accumulation within the reticuloendothelial system. Myeloid cells (M cells) have been implicated in the induction and maintenance of this type of anaemia and modulation of M cells through the main trypanosome-derived glycosylphosphatidylinositol (GPI)-anchor could attenuate both anaemia and trypano-susceptibility in *Trypanosoma brucei*-infected mice. Herein the GPI-based treatment, allowing a straightforward comparison between trypanotolerance and susceptibility in *T. brucei*-infected C57Bl/6 mice, was further adopted to scrutinize mechanisms/pathways underlying trypanosome-elicited anaemia. Hereby, the following interlinkable observations were made in GPI-based treated (GBT) *T. brucei*-infected mice: (i) a reduced inflammatory cytokine production and increased IL-10 production associated with alleviation of anaemia and restoration of serum iron levels, (ii) a shift in increased liver expression of iron storage towards iron export genes, (iii) increased erythropoiesis in the bone marrow and extramedullar sites (spleen) probably reflecting a normalized iron homeostasis and availability. Collectively, our results demonstrate that reprogramming macrophages towards an anti-inflammatory state alleviates anaemia of inflammation by normalizing iron homeostasis and restoring erythropoiesis.

15294. **Stijlemans, B., Vankrunkelsven, A., Caljon, G., Bockstal, V., Guilliams, M., Bosschaerts, T., Beschin, A., Raes, G., Magez, S. & De Baetselier, P., 2010.** The central role of macrophages in trypanosomiasis-associated anaemia: rationale for therapeutical approaches. *Endocrine, Metabolic & Immune Disorders - Drug Targets,* **10** (1): 71-82.

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Bovine African trypanosomiasis causes severe economical problems on the African continent and one of the most prominent immunopathological parameters associated with this parasitic infection is anaemia. In this report we review the current knowledge of the mechanisms underlying trypanosomiasis-associated anaemia. In the first instance, the central role of macrophages and particularly their activation state in determining the outcome of the disease (i.e. trypanosusceptibility versus trypanotolerance) will be discussed. In essence, while persistence of classically activated macrophages (M1) contributes to anaemia development, switching towards alternatively activated macrophages (M2) alleviates pathology including anaemia. Secondly, while parasite-derived glycolipids such as the glycosylphosphatidylinositol (GPI) induce M1, host-derived IL-10 blocks M1-mediated inflammation, promotes M2 development and prevents anaemia development. In this context, strategies aimed at inducing the M1 to M2 switch, such as GPI-based treatment, adenoviral delivery of IL-10 and induction of IL-10 producing regulatory T cells will be discussed. Finally, the crucial role of iron homeostasis in trypanosomiasis-associated anaemia development will be documented to stress the analogy with anaemia of chronic disease (ACD), hereby providing new insight that might contribute to the treatment of ACD.

(c) CHEMOTHERAPEUTICS

[See also **33**: 15294,].

15295. **Bakunov, S. A., Bakunova, S. M., Wenzler, T., Ghebru, M., Werbovetz, K. A., Brun, R. & Tidwell, R. R., 2010.** Synthesis and antiprotozoal activity of cationic 1,4-diphenyl-1H-1,2,3-triazoles. *Journal of Medicinal Chemistry,* **53** (1): 254-272.

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Novel dicationic triazoles 1-60 were synthesized by the Pinner method from the corresponding dinitriles, prepared via the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC). The type and the placement of cationic moieties as well as the nature of aromatic substituents influenced *in vitro* antiprotozoal activities of compounds 1-60 against *Trypanosoma brucei rhodesiense, Plasmodium falciparum*, and *Leishmania donovani* and their cytotoxicity for mammalian cells. Eight congeners displayed antitrypanosomal IC_{50} values below 10 nM. Thirty-nine dications were more potent against *P. falciparum* than pentamidine (IC_{50} = 58 nM), and eight analogues were more active than artemisinin (IC_{50} = 6 nM). Diimidazoline 60 exhibited antiplasmodial IC_{50} value of 0.6 nM. Seven congeners administered at 4 x 5 mg/kg by the intraperitoneal route cured at least three out of four animals in the acute mouse model of African trypanosomiasis. At 4 x 1 mg/kg, diamidine 46 displayed better antitrypanosomal efficacy than melarsoprol, curing all infected mice.

15296. **Bawm, S., Tiwananthagorn, S., Lin, K. S., Hirota, J., Irie, T., Htun, L. L., Maw, N. N., Myaing, T. T., Phay, N., Miyazaki, S., Sakurai, T., Oku, Y., Matsuura, H. & Katakura, K., 2010.** Evaluation of Myanmar medicinal plant extracts for antitrypanosomal and cytotoxic activities. *Journal of Veterinary Medical Science,* **72** (4): 525-528.

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Current chemotherapeutic options for African trypanosomiasis in humans and livestock are very limited. In the present study, a total of 71 medicinal plant specimens from 60 plant species collected in Myanmar were screened for antitrypanosomal activity against trypomastigotes of *Trypanosoma evansi* and cytotoxicity against MRC-5 cells *in vitro*. The methanol extract of dried rootbark of *Vitis repens* showed the highest antitrypanosomal activity with an IC_{50} value of 8.6 +/- 1.5 µg/mL and the highest selectivity index of 24.4. The extracts of *Brucea javanica, Vitex arborea, Eucalyptus globulus* and *Jatropha podagrica* had also remarkable activity with IC_{50} values and selectivity indices in the range of 27.2-52.6 µg/mL and 11.4-15.1 respectively.

15297. **Berg, M., Kohl, L., Van der Veken, P., Joossens, J., Al-Salabi, M. I., Castagna, V., Giannese, F., Cos, P., Versees, W., Steyaert, J., Grellier, P., Haemers, A., Degano, M., Maes, L., de Koning, H. P. & Augustyns, K., 2010.** Evaluation of

nucleoside hydrolase inhibitors for treatment of African trypanosomiasis. *Antimicrobial Agents & Chemotherapy,* **54** (5): 1900-1908.

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In this paper, we present the biochemical and biological evaluation of N-arylmethyl-substituted iminoribitol derivatives as potential chemotherapeutic agents against trypanosomiasis. Previously, a library of 52 compounds was designed and synthesized as potent and selective inhibitors of *Trypanosoma vivax* inosine-adenosine-guanosine nucleoside hydrolase (IAG-NH). However, when the compounds were tested against bloodstream-form *Trypanosoma brucei brucei*, only one inhibitor, N-(9-deaza-adenin-9-yl)methyl-1,4-dideoxy-1,4-imino-d-ribitol (UAMC-00363), displayed significant activity (mean 50 percent inhibitory concentration [IC_{50}] +/- standard error, 0.49 +/- 0.31 µM). Validation in an *in vivo* model of African trypanosomiasis showed promising results for this compound. Several experiments were performed to investigate why only UAMC-00363 showed antiparasitic activity. First, the compound library was screened against *T. b. brucei* IAG-NH and inosine-guanosine nucleoside hydrolase (IG-NH) to confirm the previously demonstrated inhibitory effects of the compounds on *T. vivax* IAG-NH. Second, to verify the uptake of these compounds by *T. b. brucei*, their affinities for the nucleoside P1 and nucleoside/nucleobase P2 transporters of *T. b. brucei* were tested. Only UAMC-00363 displayed significant affinity for the P2 transporter. It was also shown that UAMC-00363 is concentrated in the cell via at least one additional transporter, since P2 knockout mutants of *T. b. brucei* displayed no resistance to the compound. Consequently, no cross-resistance to the diamidine or the melaminophenyl arsenical classes of trypanocides is expected. Third, three enzymes of the purine salvage pathway of procyclic *T. b. brucei* (IAG-NH, IG-NH, and methylthioadenosine phosphorylase [MTAP]) were investigated using RNA interference. The findings from all these studies showed that it is probably not sufficient to target only the nucleoside hydrolase activity to block the purine salvage pathway of *T. b. brucei* and that, therefore, it is possible that UAMC-00363 acts on an additional target.

15298**. Berg, M., Van der Veken, P., Goeminne, A., Haemers, A. & Augustyns, K., 2010.** Inhibitors of the purine salvage pathway: a valuable approach for antiprotozoal chemotherapy? *Current Medicinal Chemistry,* **17** (23): 2456-2481.

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For many years, the purine salvage pathway of parasitic protozoa has been regarded as an attractive chemotherapeutic target. Parasitic protozoa lack *de novo* synthesis and rely entirely on the purine salvage pathway to meet their purine demands. Because of the great phylogenetic difference between parasite and host, there are often sufficient distinctions that can be exploited to design specific inhibitors for the parasitic enzymes. As a result, this pathway has been thoroughly investigated over the last twenty years. It is only quite recently that the genome studies of *Trypanosoma, Leishmania* and *Plasmodium* have been published. Based on these genomic data however, the existence of by-pass mechanisms by other enzymes and transporter systems could be suggested. Taking into account such a proposition,

the question might arise as to whether inhibition of a single salvage enzyme will be able or not to cause parasite death or growth arrest. In this paper, the key enzymes in the purine salvage pathways of relevant pathogenic species from the genera *Trypanosoma, Leishmania* and *Plasmodium* are reviewed. Their potential as drug targets is critically evaluated and where possible, correlated to literature data on antiparasitic activity of their inhibitors. While many studies over the past ten years have yielded contradictory results, this review attempts to clarify these findings by discussing the latest elements of progress in the field. Additionally, as part of a broader discussion on substrate analogue types of inhibitors, special attention is paid to iminoribitol derivatives, serving as transition state analogues of nucleoside-processing enzymes and comprising the most potent inhibitors reported for purine salvage enzymes. More specifically, the development of three generations of immucillins and a newer series of N-(arylmethyl-) substituted iminoribitol derivatives will be discussed. Finally, this review also covers subversive substrates of salvage enzymes: compounds that are transformed by enzymatic activity into cytotoxic agents. Although not by directly intervening in the process of purine recovery, the subversive substrate approach might deliver antiprotozoal compounds that rely on salvage enzymes for their activity.

15299**. Branowska, D., Farahat, A. A., Kumar, A., Wenzler, T., Brun, R., Liu, Y., Wilson, W. D. & Boykin, D. W., 2010.** Synthesis and antiprotozoal activity of 2,5-bis[amidinoaryl]thiazoles. *Bioorganic & Medicinal Chemistry,* **18** (10): 3551-3558.

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Seven novel diamidino 2,5-bis(aryl)thiazoles (5a-g) were synthesized and evaluated against *Trypanosoma brucei rhodensiense* (*T. b. r.*) and *Plasmodium falciparum* (*P. f.*). The diamidines were obtained directly from the corresponding bis-nitriles (4a-g) by the action of lithium bis(trimethylsilyl)amide. The bis-nitriles 4a-f were synthesized in four steps starting with the Stille coupling of 2-tributyltinthiazole with the appropriate cyanoaryl halide. The bis-nitrile 5g was obtained by the palladium facilitated coupling of the mixed tin-silyl reagent 2-trimethylsilyl-5-trimethyltinthiazole with 2-bromo-5-cyanopyridine. The amidoxime potential prodrugs 6a-e, 6g were obtained by the reaction of hydroxylamine with the bis-nitriles. O-methylation of the amidoximes gave the corresponding N-methoxyamidines 7a-c, 7e, 7g. The diamidines showed strong DNA binding affinity as reflected by DeltaT_m measurements. Four of the diamidines 5a, 5b, 5d and 5e were highly active *in vitro* against *P. f.* giving IC_{50} values between 1.1 and 2.5nM. The same four diamidines showed IC_{50} values between 4 and 6nM against *T. b. r.* The selectivity indices ranged from 233 to 9175. One diamidine 5a produced one of four cures at an ip dose of 4x5mg/kg in the STIB900 mouse model for acute African trypanosomiasis. The amidoxime and N-methoxyamidine of 5a were the only produgs to provide cures (1/4 cures) in the same mouse model on oral dosage at 4x25mg/kg.

15300. **Caceres, A. J., Michels, P. A. & Hannaert, V., 2010.** Genetic validation of aldolase and glyceraldehyde-3-phosphate dehydrogenase as drug targets in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **169** (1): 50-54.

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Aldolase (ALD) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of *Trypanosoma brucei* are considered to be promising targets for chemotherapeutic treatment of African sleeping sickness, because glycolysis is the single source of ATP for the parasite when living in the human bloodstream. Moreover, these enzymes appeared to possess distinct kinetic and structural properties that have already been exploited for the discovery of effective and selective inhibitors with trypanocidal activity. Here we present an experimental, quantitative assessment of the importance of these enzymes for the glycolytic pathway. This was achieved by decreasing the concentrations of ALD and GAPDH by RNA interference. The effects of these knockdowns on parasite growth, levels of various enzymes and transcripts, enzyme activities and glucose consumption were studied. A partial depletion of ALD and GAPDH was already sufficient to rapidly kill the trypanosomes. An effect was also observed on the activity of some other glycolytic enzymes.

15301. **Chavda, S., Babu, B., Yanow, S. K., Jardim, A., Spithill, T. W., Kiakos, K., Kluza, J., Hartley, J. A. & Lee, M., 2010.** A novel achiral seco-cyclopropylpyrido[e]indolone (CPyI) analogue of CC-1065 and the duocarmycins: synthesis, DNA interactions, *in vivo* anticancer and anti-parasitic evaluation. *Bioorganic & Medicinal Chemistry,* **18** (14): 5016-5024

Division of Natural Sciences and Department of Chemistry, Hope College, 35 East 12th Street, Holland, MI 49423, USA. [lee@hope.edu].

15302. **Chen, C. K., Leung, S. S., Guilbert, C., Jacobson, M. P., McKerrow, J. H. & Podust, L. M., 2010.** Structural characterization of CYP51 from *Trypanosoma cruzi* and *Trypanosoma brucei* bound to the antifungal drugs posaconazole and fluconazole. *PLoS Neglected Tropical Diseases,* **4** (4): e651.

Department of Pharmaceutical Chemistry, University of California, San Francisco, California, USA. [larissa.podust@ucsf.edu].

15303**. Cross, G. A., 2010.** Drug discovery: fat-free proteins kill parasites. *Nature,* **464** (7289): 689-690.

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The addition of a fatty acid to certain proteins is vital for the survival of protozoa that cause sleeping sickness and of their mammalian hosts. Compounds that target this process in the protozoa are now reported.

15304. **Davis, R. A., Demirkiran, O., Sykes, M. L., Avery, V. M., Suraweera, L., Fechner, G. A. & Quinn, R. J., 2010**. 7',8'-Dihydroobolactone, a trypanocidal alpha-pyrone from the rainforest tree *Cryptocarya obovata*. *Bioorganic & Medicinal Chemistry Letters,* **20** (14): 4057-4059.

Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia.

Mass-directed isolation of the CH_2Cl_2/MeOH extract from the leaves of *Cryptocarya obovata* resulted in the purification of a new trypanocidal alpha-pyrone, 7',8'-dihydroobolactone (1). The chemical structure of 1 was determined by 1D/2D NMR, MS and CD data analysis. 7',8'-Dihydroobolactone was shown to inhibit *Trypanosoma brucei brucei* with an IC_{50} of 2.8 µM.

15305. **Debierre-Grockiego, F., 2010**. Glycolipids are potential targets for protozoan parasite diseases. *Trends in Parasitology,* **26** (8): 404-411.

UMR Université-INRA 0483, UFR Sciences Pharmaceutiques, Immunologie Parasitaire, Vaccinologie et Biothérapies anti-infectieuses, 31 Avenue Monge, F-37200 Tours, France. [francoise.debierre@univ-tours.fr].

Induction of sterilizing immunity by vaccination is extremely difficult because of the evasion mechanisms developed by parasites, and identification of new targets for therapy is therefore important. Glycosylphosphatidylinositols (GPIs) of parasites are glycolipids that participate in pathogenicity of parasitic diseases. Studies of *Plasmodium falciparum* and *Trypanosoma brucei* indicate that GPIs are good candidates for developing vaccines against malaria and sleeping sickness, respectively. By contrast, fatty acids isolated from *P. falciparum* and *Toxoplasma gondii* can inhibit the production of inflammatory cytokines induced by the GPIs in macrophages. GPIs are considered to be toxins that, if present in large amounts, induce irreversible damages to the host, and treatment with fatty acids could reduce this effect.

15306. **Durrant, J. D., Urbaniak, M. D., Ferguson, M. A. & McCammon, J. A., 2010.** Computer-aided identification of *Trypanosoma brucei* uridine diphosphate galactose 4'-epimerase inhibitors: toward the development of novel therapies for African sleeping sickness. *Journal of Medicinal Chemistry,* **53** (13): 5025-5032.

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Trypanosoma brucei, the causative agent of human African trypanosomiasis, affects tens of thousands of sub-Saharan Africans. As current therapeutics are inadequate due to toxic side effects, drug resistance, and limited effectiveness, novel therapies are urgently needed. UDP-galactose 4'-epimerase (TbGalE), an enzyme of the Leloir pathway of galactose metabolism, is one promising *T. brucei* drug target. Here we use the relaxed complex scheme, an advanced computer-docking methodology that accounts for full protein flexibility, to identify inhibitors of TbGalE. An initial hit rate of 62 percent was obtained at 100 µM, ultimately leading to the identification of 14 low-micromolar inhibitors. Thirteen of these inhibitors belong to a distinct series with a conserved binding motif that may prove useful in future drug design and optimization.

15307. **Fernandez, L. S., Sykes, M. L., Andrews, K. T. & Avery, V. M., 2010.** Antiparasitic activity of alkaloids from plant species of Papua New Guinea and Australia. *International Journal of Antimicrobial Agents,* **36** (3): 275-279.

Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, Australia; and Griffith Medical Research College, A Joint Program of Griffith University and the Queensland Institute of Medical Research, QIMR, Herston, QLD 4006, Australia. [v.avery@griffith.edu.au].

New drugs are needed to help overcome the increasing problem of drug resistance in parasites that cause diseases such as malaria and trypanosomiasis. In this study, alkaloid compounds isolated from extracts of the plants *Flindersia amboinensis, Stephania zippeliana* and *Voacanga papuana* from Papua New Guinea and *Flindersia acuminata* from Australia were examined for their antiparasitic activity against *Plasmodium falciparum* strains and *Trypanosoma brucei brucei* as well as their cytotoxicity against the mammalian cell lines HEK 293 and HeLa. The most active compound, dimethylisoborreverine (DMIB), showed sub micromolar activity, with 50 percent inhibitory concentration (IC_{50}) values between 20nM and 810nM both against drug-sensitive and drug-resistant *P. falciparum* strains, along with moderate selectivity against *T. b. brucei* and mammalian cells. Stage specificity studies revealed that *P. falciparum* trophozoite-stage parasites were more susceptible to DMIB than ring- or schizont-stage parasites. DMIB-treated trophozoites showed changes in food vacuole morphology, with an apparent reduction in haemozoin formation that does not appear to be inhibited via the direct binding of haem. These findings suggest a potential for indole alkaloids from *Flindersia* spp. as new antiparasitic agents.

15308. **Fotie, J., Kaiser, M., Delfin, D. A., Manley, J., Reid, C. S., Paris, J. M., Wenzler, T., Maes, L., Mahasenan, K. V., Li, C. & Werbovetz, K. A., 2010.** Antitrypanosomal activity of 1,2-dihydroquinolin-6-ols and their ester derivatives. *Journal of Medicinal Chemistry,* **53** (3): 966-982.

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The current chemotherapy for second stage human African trypanosomiasis is unsatisfactory. A synthetic optimization study based on the lead antitrypanosomal compound 1,2-dihydro-2,2,4-trimethylquinolin-6-yl 3,5-dimethoxybenzoate (TDR20364, 1a) was undertaken in an attempt to discover new trypanocides with potent *in vivo* activity. While 6-ether derivatives were less active than the lead compound, several N1-substituted derivatives displayed nanomolar IC_{50} values against *T. b. rhodesiense* STIB900 *in vitro*, with selectivity indexes up to >18 000. 1-Benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate (10a) displayed an IC_{50} value of 0.014 μM against these parasites and a selectivity index of 1 700. Intraperitoneal administration of 10a at 50 mg/kg/day for 4 days caused a promising prolongation of lifespan in *T. b. brucei* STIB795-infected mice (>14 days vs 7.75 days for untreated controls). Reactive oxygen species were produced when *T. b. brucei* were exposed to 10a *in vitro*, implicating oxidative stress in the trypanocidal mode of action of these 1,2-dihydroquinoline derivatives.

15309. **Frearson, J. A., Brand, S., McElroy, S. P., Cleghorn, L. A., Smid, O., Stojanovski, L., Price, H. P., Guther, M. L., Torrie, L. S., Robinson, D. A., Hallyburton, I., Mpamhanga, C. P., Brannigan, J. A., Wilkinson, A. J., Hodgkinson, M., Hui, R., Qiu, W., Raimi, O. G., van Aalten, D. M., Brenk, R., Gilbert, I. H., Read, K. D., Fairlamb, A. H., Ferguson, M. A., Smith, D. F. & Wyatt, P. G., 2010**. N-myristoyltransferase inhibitors as new leads to treat sleeping sickness. *Nature*, **464** (7289): 728-732.

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African sleeping sickness or human African trypanosomiasis, caused by *Trypanosoma brucei* spp., is responsible for approximately 30 000 deaths each year. Available treatments for this disease are poor, with unacceptable efficacy and safety profiles, particularly in the late stage of the disease when the parasite has infected the central nervous system. Here we report the validation of a molecular target and the discovery of associated lead compounds with the potential to address this lack of suitable treatments. Inhibition of this target-*T. brucei* N-myristoyltransferase-leads to rapid killing of trypanosomes both *in vitro* and *in vivo* and cures trypanosomiasis in mice. These high-affinity inhibitors bind into the peptide substrate pocket of the enzyme and inhibit protein N-myristoylation in trypanosomes. The compounds identified have promising pharmaceutical properties and represent an opportunity to develop oral drugs to treat this devastating disease. Our studies validate *T. brucei* N-myristoyltransferase as a promising therapeutic target for human African trypanosomiasis.

15310. **Goldsmith, R. B., Gray, D. R., Yan, Z., Generaux, C. N., Tidwell, R. R. & Reisner, H. M., 2010.** Application of monoclonal antibodies to measure metabolism of an anti-trypanosomal compound *in vitro* and *in vivo*. *Journal of Clinical Laboratory Analysis,* **24** (3): 187-194.

Department of Pathology and Laboratory Medicine, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599-7525, USA.

Human African trypanosomiasis (HAT), also called African sleeping sickness, is a neglected tropical parasitic disease indigenous to sub-Saharan Africa. Diamidine compounds, including pentamidine and CPD-0801, are potent anti-trypanosomal molecules. The latter is a potential drug in the development at the UNC based Consortium for Parasitic Drug Development. An orally bioavailable prodrug of CPD-0801, DB868, is metabolized primarily in the liver to the active form. A monoclonal antibody developed against a pentamidine derivative has shown significant reactivity with CPD-0801 (EC_{50} 65.1 nM), but not with the prodrug (EC_{50} > 18 000 nM). An inhibitory enzyme-linked immunosorbent assay (IELISA) has been used to quantitatively monitor prodrug metabolism by detecting the production of the active compound over time in a sandwich culture rat hepatocyte system and in rats. These results were compared with the results of the standard LC/MS/MS assay. Spearman coefficients of 0.96 and 0.933 (*in vitro* and *in vivo*, respectively) indicate a high correlation between these two measurement methods. This novel IELISA provides a facile, inexpensive, and accurate method for drug detection that may aid in elucidating the mechanisms of action and toxicity of existing and future diamidine compounds.

15311. **Hagos, A., Goddeeris, B. M., Yilkal, K., Alemu, T., Fikru, R., Yacob, H. T., Feseha, G. & Claes, F., 2010.** Efficacy of Cymelarsan® and Diminasan® against *Trypanosoma equiperdum* infections in mice and horses. *Veterinary Parasitology,* 171 (3-4): 200-206.

Addis Ababa University, Faculty of Veterinary Medicine, Department of Pathology and Parasitology, P.O. Box 34, Debre Zeit, Ethiopia; and Katholieke Universiteit Leuven, Faculty of Bioscience Engineering, Department Biosystems, Division Gene Technology, Kasteelpark Arenberg 30, B-3001 Leuven, Belgium. [fclaes@itg.be].

Trypanocidal sensitivity studies were conducted to assess the efficacy of diminazene diaceturate (Diminasan®) and bis (aminoethylthio) 4-melaminophenylarsine dihydrochloride (Cymelarsan®) against *Trypanosoma equiperdum* (isolated from two mares with chronic cases of dourine) 713/943 and 834/940 Dodola strains in experimentally infected mice and horses. Diminasan® at doses from 3.5mg/kg to 28mg/kg and Cymelarsan® at doses of 0.25mg/kg and 0.5mg/kg body weight failed to cure any of the mice, indicating a clear dose dependent relationship in the mean time of relapse observed in mice. Indeed, mice treated with lower doses relapsed after a shorter time than mice treated with higher doses. However, mice treated with Cymelarsan® at doses of 1.0mg/kg and 2.0mg/kg body weight were cured and no parasitaemia was observed for 60 days. The efficacy of Cymelarsan® was also tested in horses. Two groups of horses containing two animals each were infected with *T. equiperdum* 834/940 Dodola strain and treated with Cymelarsan® at a dose rate of 0.25mg/kg and 0.5mg/kg, respectively. Cymelarsan® at 0.25mg/kg and 0.5mg/kg body weight cleared parasitaemia within 24h post treatment and none of the animals were found to show relapse throughout the 320 days of observation. The sensitivity of the particular trypanosome strain to Cymelarsan® was also supported by the relative improvement in the mean PCV levels of horses following treatment. A statistically significant difference ($P < 0.01$) in the mean PCV levels of horses treated with Cymelarsan® was observed between day 20 at peak parasitaemia and days 40 as well as 60 of observation. The mean PCV levels of horses in the control group progressively decreased within the first 60 days of post infection. Two of the horses in the control group developed chronic form of dourine manifested by genital as well as nervous signs with progressive loss of body condition within 320 days post infection. The efficacy of Cymelarsan® against the chronic form of dourine was confirmed after treatment of one of the control horses with Cymelarsan® at a dose rate of 0.25mg/kg body weight at day 282 post infection. It was noted that the treated horse showed an improved overall body condition and clinical signs such as incoordination of hind legs, weakness and ventral oedema disappeared within 10 days of treatment. Thus, Cymelarsan® was found to be quite effective in curing horses with acute as well as the chronic form of dourine. The results obtained from the present study will be important for designing effective control measures against dourine.

15312. **Hwang, J. Y., Smithson, D., Connelly, M., Maier, J., Zhu, F. & Guy, K. R., 2010.** Discovery of halo-nitrobenzamides with potential application against human African trypanosomiasis. *Bioorganic & Medicinal Chemistry Letters,* **20** (1): 149-152.

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A series of halo-nitrobenzamide were synthesized and evaluated for their ability to block proliferation of *Trypanosoma brucei brucei*. A number of these compounds had significant activity against the parasite, particularly 2-chloro-N-(4-chlorophenyl)-5-nitrobenzamide 17 which exhibited low micromolar inhibitory potency against *T. brucei* and selectivity towards both malaria and mammalian cells.

15313. **Jones, D. C., Ariza, A., Chow, W. H., Oza, S. L. & Fairlamb, A. H., 2010.** Comparative structural, kinetic and inhibitor studies of *Trypanosoma brucei* trypanothione reductase with *T. cruzi*. *Molecular & Biochemical Parasitology,* **169** (1): 12-19.

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As part of a drug discovery programme to discover new treatments for human African trypanosomiasis, recombinant trypanothione reductase from *Trypanosoma brucei* has been expressed, purified and characterized. The crystal structure was solved by molecular replacement to a resolution of 2.3A and found to be nearly identical to the *T. cruzi* enzyme (root mean square deviation 0.6A over 482 Calpha atoms). Kinetically, the K_m for trypanothione disulphide for the *T. brucei* enzyme was 4.4-fold lower than for *T. cruzi* measured by either direct (NADPH oxidation) or DTNB-coupled assay. The K(m) for NADPH for the *T. brucei* enzyme was found to be 0.77µM using an NADPH-regenerating system coupled to reduction of DTNB. Both enzymes were assayed for inhibition at their respective S=K_m values for trypanothione disulphide using a range of chemotypes, including CNS-active drugs such as clomipramine, trifluoperazine, thioridazine and citalopram. The relative IC_{50} values for the two enzymes were found to vary by no more than 3-fold. Thus trypanothione reductases from these species are highly similar in all aspects, indicating that they may be used interchangeably for structure-based inhibitor design and high-throughput screening.

15314. **Kerr, I. D., Wu, P., Marion-Tsukamaki, R., Mackey, Z. B. & Brinen, L. S., 2010.** Crystal structures of TbCatB and rhodesain, potential chemotherapeutic targets and major cysteine proteases of *Trypanosoma brucei*. *PLoS Neglected Tropical Diseases,* **4** (6): e701.

Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, California, USA.. [brinen@cmp.ucsf.edu].

15315. **Kido, Y., Sakamoto, K., Nakamura, K., Harada, M., Suzuki, T., Yabu, Y., Saimoto, H., Yamakura, F., Ohmori, D., Moore, A., Harada, S. & Kita, K., 2010**. Purification and kinetic characterization of recombinant alternative oxidase from *Trypanosoma brucei brucei*. *Biochimica et Biophysica Acta,* **1797** (4): 443-450.

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

15316. **Kido, Y., Shiba, T., Inaoka, D. K., Sakamoto, K., Nara, T., Aoki, T., Honma, T., Tanaka, A., Inoue, M., Matsuoka, S., Moore, A., Harada, S. & Kita, K., 2010.** Crystallization and preliminary crystallographic analysis of cyanide-insensitive alternative oxidase from *Trypanosoma brucei brucei*. *Acta Crystallographica Section F Structural Biology & Crystalization Communications,* **66** (Pt 3): 275-278.

Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan.

15317. **Klee, N., Wong, P. E., Baragana, B., Mazouni, F. E., Phillips, M. A., Barrett, M. P. & Gilbert, I. H., 2010.** Selective delivery of 2-hydroxy APA to *Trypanosoma brucei* using the melamine motif. *Bioorganic and Medicinal Chemistry Letters,* **20** (15): 4364-4366.

Division of Biological Chemistry and Drug Discovery, College of Life Science, University of Dundee, Sir James Black Centre, Dundee DD1 5EH, UK; Division of Infection and Immunity and Wellcome Trust Centre for Molecular Parasitology, Glasgow Biomedical Research Centre, University of Glasgow G12 8TA, UK; and UT Southwestern Medical Center at Dallas 6001 Forest Park, Dallas, TX 75390-9041, USA. [i.h.gilbert@dundee.ac.uk].

Trypanosoma brucei, the parasite that causes human African trypanosomiasis, is auxotrophic for purines and has specialist nucleoside transporters to import these metabolites. In particular, the P2 aminopurine transporter can also selectively accumulate melamine derivatives. In this Letter, we report the coupling of the melamine moiety to 2-hydroxy APA, a potent ornithine decarboxylase inhibitor, with the aim of selectively delivering this compound to the parasite. The best compound described here shows an increased *in vitro* trypanocidal activity compared with the parent.

15318. **Lepesheva, G. I., Park, H. W., Hargrove, T. Y., Vanhollebeke, B., Wawrzak, Z., Harp, J. M., Sundaramoorthy, M., Nes, W. D., Pays, E., Chaudhuri, M., Villalta, F. & Waterman, M. R., 2010.** Crystal structures of *Trypanosoma brucei* sterol 14alpha-demethylase and implications for selective treatment of human infections. *Journal of Biological Chemistry,* **285** (3): 1773-1780.

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15319. **Mott, B. T., Ferreira, R. S., Simeonov, A., Jadhav, A., Ang, K. K., Leister, W., Shen, M., Silveira, J. T., Doyle, P. S., Arkin, M. R., McKerrow, J. H., Inglese, J., Austin, C. P., Thomas, C. J., Shoichet, B. K. & Maloney, D. J., 2010.** Identification and optimization of inhibitors of trypanosomal cysteine proteases: cruzain, rhodesain, and TbCatB. *Journal of Medicinal Chemistry,* **53** (1): 52-60.

NIH Chemical Genomics Center, National Human Genome Research Institute, National Institutes of Health, 9800 Medical Center Drive, MSC 3370 Bethesda, Maryland 20892-3370, USA. [maloneyd@mail.nih.gov].

Trypanosoma cruzi and *Trypanosoma brucei* are parasites that cause Chagas disease and African sleeping sickness, respectively. Both parasites rely on essential cysteine proteases for survival: cruzain for *T. cruzi* and TbCatB/rhodesain for *T. brucei*. A recent quantitative high-throughput screen of cruzain identified triazine nitriles, which are known inhibitors of other cysteine proteases, as reversible inhibitors of the enzyme. Structural modifications detailed herein, including core scaffold modification from triazine to purine, improved the *in vitro* potency against both cruzain and rhodesain by 350-fold, while also gaining activity against *T. brucei* parasites. Selected compounds were screened against a panel of human cysteine and serine proteases to determine selectivity, and a cocrystal was obtained of our most potent analogue bound to cruzain.

15320. **Ngantchou, I., Nyasse, B., Denier, C., Blonski, C., Hannaert, V. & Schneider, B., 2010.** Antitrypanosomal alkaloids from *Polyalthia suaveolens* (Annonaceae): their effects on three selected glycolytic enzymes of *Trypanosoma brucei. Bioorganic & Medicinal Chemistry Letters,* **20** (12): 3495-3498.

Department of Organic Chemistry, Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon. [nwete@yahoo.fr].

In continuation of our study on medicinal plants of Cameroon, stem barks of *Polyalthia suaveolens* were phytochemically studied. This investigation yielded a new indolosesquiterpene alkaloid, named polysin (1) and four hitherto known alkaloids (2-5). Polysin (1) appeared as a competitive reversible inhibitor (K_i = 10 µM) of phosphofructo kinase (PFK) of *Trypanosoma brucei* with respect to fructose-6-phosphate (K_i/K_M = 0.05) and could be used in the design of new trypanocidal drugs. The other isolated compounds (2-5) also exhibited interesting inhibitory effects on selected glycolytic enzymes (PFK, glyceraldehyde-3-phosphate dehydrogenase and aldolase).

15321. **Nour, A. M., Khalid, S. A., Kaiser, M., Brun, R., Abdalla, W. E. & Schmidt, T. J., 2010.** The antiprotozoal activity of methylated flavonoids from *Ageratum conyzoides L. Journal of Ethnopharmacology,* **129** (1): 127-130.

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The dichloromethane extract prepared from aerial parts of *Ageratum conyzoides* L. (Asteraceae), a plant commonly used in folk medicine for a number of illnesses including sleeping sickness, was recently found to exhibit a prominent activity (IC_{50}=0.78 µg/mL) against bloodstream forms of *Trypanosoma brucei rhodesiense*, the aetiologic agent of human African trypanosomiasis (East African sleeping sickness). This extract also exhibited noticeable activities against *Leishmania donovani* (Kala-Azar, IC_{50} = 3.4 µg/mL) as well as *Plasmodium falciparum* (IC_{50} = 8.0 µg/mL). In the current study, we sought potentially active

constituents of *Ageratum conyzoides*. Extracts prepared with solvents of different polarity were tested for activity against the above mentioned parasites as well as against *Trypanosoma cruzi* (Chagas disease) and for cytotoxicity using established protocols. The dicholoromethane extract showed the highest level of activity and was chosen for phytochemical studies aimed at the isolation of potential active constituents. Five highly methoxylated flavonoids along with the chromene derivative encecalol methyl ether were isolated. All isolated compounds were previously reported from *Ageratum conyzoides*. While the chromene turned out to be inactive against the tested parasites, the flavonoids showed activity against the protozoan pathogens, some in the lower µM range. However, none of these isolated compounds was as active as the crude extract. This is the first report on antiprotozoal activity of this plant species and some of its constituents. The chemical principle accounting for the high activity of the crude extract, however, remains to be identified.

15322**. Oldfield, E., 2010.** Targeting isoprenoid biosynthesis for drug discovery: bench to bedside. *Accounts of Chemical Research***. e publication June 18**.

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15323. **Otoguro, K., Ishiyama, A., Iwatsuki, M., Namatame, M., Nishihara-Tukashima, A., Nakashima, T., Shibahara, S., Kondo, S., Yamada, H. & Omura, S., 2010**. *In vitro* and *in vivo* anti-*Trypanosoma brucei* activities of phenazinomycin and related compounds. *Journal of Antibiotics (Tokyo)*. **Advance online publication 30 June**.

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During the course of our screening programme to discover new antitrypanosomal compounds, we have evaluated isolates from soil microorganisms as well as compounds from the antibiotic libraries of the Kitasato Institute for Life Sciences and Bioscience Associates. We have previously reported on various microbial metabolites exhibiting potent anti-*Trypanosoma brucei* properties, which are defined as antitrypanosomal properties.

15324. **Regalado, E. L., Tasdemir, D., Kaiser, M., Cachet, N., Amade, P. & Thomas, O. P., 2010**. Antiprotozoal steroidal saponins from the marine sponge *Pandaros acanthifolium*. *Journal of Natural Products*. **Web publication July 8.**

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The chemical composition of the Caribbean sponge *Pandaros acanthifolium* was reinvestigated and led to the isolation of 12 new steroidal glycosides, namely, pandarosides E-J (1-6) and their methyl esters (7-12). Their structures were determined on the basis of extensive spectroscopic analyses, including two-dimensional NMR and HRESIMS data. Like the previously isolated pandarosides A-D (13-16), the new compounds 1-12 share an unusual oxidized D-ring and a cis C/D ring junction. The absolute configurations of the aglycones were assigned by interpretation of CD spectra, whereas the absolute configurations of the monosaccharide units were determined by chiral GC analyses of the acid methanolysates. The majority of the metabolites showed *in vitro* activity against three or four parasitic protozoa. Particularly active were the compounds 3 (pandaroside G) and its methyl ester (9), which potently inhibited the growth of *Trypanosoma brucei rhodesiense* (IC_{50} values 0.78 and 0.038 μM, respectively) and *Leishmania donovani* (IC_{50} values of 1.3 and 0.051 μM, respectively).

15325**. Rodrigues, C., Batista, A. A., Ellena, J., Castellano, E. E., Benitez, D., Cerecetto, H., Gonzalez, M., Teixeira, L. R. & Beraldo, H., 2010.** Coordination of nitro-thiosemicarbazones to ruthenium (II) as a strategy for anti-trypanosomal activity improvement. *European Journal of Medicinal Chemistry,* **45** (7): 2847-2853.

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15326. **Rodriguez-Soca, Y., Munteanu, C. R., Dorado, J., Pazos, A., Prado-Prado, F. J. & Gonzalez-Diaz, H., 2010.** Trypano-PPI: a web server for prediction of unique targets in the trypanosome proteome by using electrostatic parameters of protein-protein interactions. *Journal of Proteome Research,* **9** (2): 1182-1190.

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Trypanosoma brucei causes African trypanosomiasis in humans (HAT or African sleeping sickness) and nagana in cattle. The disease threatens over 60 million people and uncounted numbers of cattle in 36 countries of sub-Saharan Africa and has a devastating impact on human health and the economy. On the other hand, *Trypanosoma cruzi* is responsible in South America for Chagas disease, which can cause acute illness and death, especially in young children. In this context, the discovery of novel drug targets in the trypanosome proteome is a major focus for the scientific community. Recently, many researchers have spent important efforts on the study of protein-protein interactions (PPIs) in pathogenic trypanosome species concluding that the low sequence identities between some parasite proteins and their human host render these PPIs as highly promising drug targets. To the best of our knowledge, there are no general models to predict unique PPIs in trypanosomes (TPPIs). On the other hand, the 3D structure of an increasing number of trypanosome proteins is reported in databases. In this regard, the introduction of a new model to predict TPPIs from the 3D structure of proteins involved in PPI is very important. For this purpose, we introduced new protein-protein complex invariants based on the Markov average electrostatic potential for amino acids located in different regions (R_i) of i^{th} protein and

placed at a distance k one from each other. We calculated more than 30 different types of parameters for 7 866 pairs of proteins (1 023 TPPIs and 6 823 non-TPPIs) from more than 20 organisms, including parasites and human or cattle hosts. We found a very simple linear model that predicts more than 90 percent of TPPIs and non-TPPIs both in training and independent test subsets using only two parameters. We also tested nonlinear ANN models for comparison purposes but the linear model gives the best results. We implemented this predictor in the web server named TrypanoPPI which is freely available to the public at http://miaja.tic.udc.es/Bio-AIMS/TrypanoPPI.php. This is the first model that predicts how unique a protein-protein complex in trypanosome proteome is with respect to other parasites and hosts, opening new opportunities for antitrypanosome drug target discovery.

15327. **Ruda, G. F., Campbell, G., Alibu, V. P., Barrett, M. P., Brenk, R. & Gilbert, I. H., 2010.** Virtual fragment screening for novel inhibitors of 6-phosphogluconate dehydrogenase. *Bioorganic & Medicinal Chemistry,* **18** (14): 5056-5062.

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The enzyme 6-phosphogluconate dehydrogenase is a potential drug target for the parasitic protozoan *Trypanosoma brucei*, the causative organism of human African trypanosomiasis. This enzyme has a polar active site to accommodate the phosphate, hydroxyl and carboxylate groups of the substrate, 6-phosphogluconate. A virtual fragment screen was undertaken of the enzyme to discover starting points for the development of inhibitors which are likely to have appropriate physicochemical properties for an orally bioavailable compound. A virtual screening library was developed, consisting of compounds with functional groups that could mimic the phosphate group of the substrate, but which have a higher pK_a. Following docking, hits were clustered and appropriate compounds purchased and assayed against the enzyme. Three fragments were identified that had IC_{50} values in the low µ molar range and good ligand efficiencies. Based on these initial hits, analogues were procured and further active compounds were identified. Some of the fragments identified represent potential starting points for a medicinal chemistry programme to develop potent drug-like inhibitors of the enzyme.

15328. **Sharlow, E. R., Lyda, T. A., Dodson, H. C., Mustata, G., Morris, M. T., Leimgruber, S. S., Lee, K. H., Kashiwada, Y., Close, D., Lazo, J. S. & Morris, J. 2010.** A target-based high throughput screen yields *Trypanosoma brucei* hexokinase small molecule inhibitors with antiparasitic activity. *PLoS Neglected Tropical Diseases,* **4** (4): e659.

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The parasitic protozoan *Trypanosoma brucei* utilizes glycolysis exclusively for ATP production during infection of the mammalian host. The first step in this metabolic pathway is mediated by hexokinase (TbHK), an enzyme essential to the parasite that transfers the gamma-phospho of ATP to a hexose. Here we describe the identification and confirmation of novel small molecule inhibitors of bacterially expressed TbHK1, one of two TbHKs

expressed by *T. brucei*, using a high throughput screening assay. Exploiting optimized high throughput screening assay procedures, we interrogated 220 233 unique compounds and identified 239 active compounds from which ten small molecules were further characterized. Computation chemical cluster analyses indicated that six compounds were structurally related while the remaining four compounds were classified as unrelated or singletons. All ten compounds were approximately 20-17 000-fold more potent than lonidamine, a previously identified TbHK1 inhibitor. Seven compounds inhibited *T. brucei* blood stage form parasite growth (0.03 < or = EC_{50} < 3 µM) with parasite specificity of the compounds being demonstrated using insect stage *T. brucei* parasites, *Leishmania* promastigotes, and mammalian cell lines. Analysis of two structurally related compounds, ebselen and SID 17387000, revealed that both were mixed inhibitors of TbHK1 with respect to ATP. Additionally, both compounds inhibited parasite lysate-derived HK activity. None of the compounds displayed structural similarity to known hexokinase inhibitors or human African trypanosomiasis therapeutics. The novel chemotypes identified here could represent leads for future therapeutic development against the African trypanosome.

15329. **Smithson, D. C., Lee, J., Shelat, A. A., Phillips, M. A. & Guy, R. K., 2010.** Discovery of potent and selective inhibitors of *Trypanosoma brucei* ornithine decarboxylase. *Journal of Biological Chemistry,* **285** (22): 16771-16781.

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Human African trypanosomiasis, caused by the eukaryotic parasite *Trypanosoma brucei*, is a serious health problem in much of central Africa. The only validated molecular target for treatment of human African trypanosomiasis is ornithine decarboxylase (ODC), which catalyzes the first step in polyamine metabolism. Here, we describe the use of an enzymatic high throughput screen of 316 114 unique molecules to identify potent and selective inhibitors of ODC. This screen identified four novel families of ODC inhibitors, including the first inhibitors selective for the parasitic enzyme. These compounds display unique binding modes, suggesting the presence of allosteric regulatory sites on the enzyme. Docking of a subset of these inhibitors, coupled with mutagenesis, also supports the existence of these allosteric sites.

15330. **Sokolova, A. Y., Wyllie, S., Patterson, S., Oza, S. L., Read, K. D. & Fairlamb, A. H., 2010.** Cross-resistance to nitro drugs and implications for treatment of human African trypanosomiasis. *Antimicrobial Agents & Chemotherapy,* **54** (7): 2893-2900.

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The success of nifurtimox-eflornithine combination therapy (NECT) for the treatment of human African trypanosomiasis (HAT) has renewed interest in the potential of nitro drugs as chemotherapeutics. In order to study the implications of the more widespread use of nitro drugs against these parasites, we examined the *in vivo* and *in vitro* resistance potentials of nifurtimox and fexinidazole and its metabolites. Following selection *in vitro* by exposure to

increasing concentrations of nifurtimox, *Trypanosoma brucei brucei* nifurtimox-resistant clones designated NfxR1 and NfxR2 were generated. Both cell lines were found to be 8-fold less sensitive to nifurtimox than parental cells and demonstrated cross-resistance to a number of other nitro drugs, most notably the clinical trial candidate fexinidazole (approximately 27-fold more resistant than parental cells). Studies on mice confirmed that the generation of nifurtimox resistance in these parasites did not compromise virulence, and NfxR1 remained resistant to both nifurtimox and fexinidazole *in vivo*. In the case of fexinidazole, drug metabolism and pharmacokinetic studies indicate that the parent drug is rapidly metabolized to the sulfoxide and sulfone form of this compound. These metabolites retained trypanocidal activity but were less effective in nifurtimox-resistant lines. Significantly, trypanosomes selected for resistance to fexinidazole were 10-fold more resistant to nifurtimox than parental cells. This reciprocal cross-resistance has important implications for the therapeutic use of nifurtimox in a clinical setting and highlights a potential danger in the use of fexinidazole as a monotherapy.

15331**. Spavieri, J., Allmendinger, A., Kaiser, M., Casey, R., Hingley-Wilson, S., Lalvani, A., Guiry, M. D., Blunden, G. & Tasdemir, D., 2010.** Antimycobacterial, antiprotozoal and cytotoxic potential of twenty-one brown algae (Phaeophyceae) from British and Irish waters. *Phytotherapy Research***. e publication ahead of print June 17**.

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In the continuation of our research on seaweeds, crude extracts of 21 brown algae collected from the south coast of England and the west coast of Ireland were screened for *in vitro* trypanocidal, leishmanicidal and antimycobacterial activities. Mammalian stages of a small set of parasitic protozoa; i.e. *Trypanosoma brucei rhodesiense, T. cruzi* and *Leishmania donovani*, and the tubercle bacillus *Mycobacterium tuberculosis* were used as test organisms. The extracts were also evaluated for selectivity by testing on a mammalian cell line (L6 cells). Only four extracts were moderately active against *T. cruzi*, whereas all algal extracts showed significant activity against *T. brucei rhodesiense*, with *Halidrys siliquosa* and *Bifurcaria bifurcata* (Sargassaceae) being the most potent (IC_{50} values 1.2 and 1.9 µg/mL). All algal extracts also displayed leishmanicidal activity, with *H. siliquosa* and *B. bifurcata* again being the most active (IC_{50} values 6.4 and 8.6 µg/mL). When tested against *M. tuberculosis*, only the *B. bifurcata* extract was found to have some antitubercular potential (MIC value 64.0 µg/mL). Only three seaweed extracts, i.e. *H. siliquosa, B. bifurcata* and *Cystoseira tamariscifolia* showed some cytotoxicity. To our knowledge, this is the first study on the antiprotozoal and antimycobacterial activity of brown algae from British and Irish waters.

15332. **Tang, S. C. & Shapiro, T. A., 2010.** Newly identified antibacterial compounds are topoisomerase poisons in African trypanosomes. *Antimicrobial Agents & Chemotherapy,* **54** (2): 620-626.

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Human African trypanosomiasis, caused by the *Trypanosoma brucei* protozoan parasite, is fatal when left untreated. Current therapies are antiquated, and there is a need for new pharmacologic agents against *T. brucei* targets that have no human orthologue. Trypanosomes have a single mitochondrion with a unique mitochondrial DNA, known as kinetoplast DNA (kDNA), a topologically complex network that contains thousands of interlocking circular DNAs, termed minicircles (approximately 1 kb) and maxicircles (approximately 23 kb). Replication of kDNA depends on topoisomerases, enzymes that catalyze reactions that change DNA topology. *T. brucei* has an unusual type IA topoisomerase that is dedicated to kDNA metabolism. This enzyme has no orthologue in humans, and RNA interference (RNAi) studies have shown that it is essential for parasite survival, making it an ideal drug target. In a large chemical library screen, two compounds were recently identified as poisons of bacterial topoisomerase IA. We found that these compounds are trypanocidal in the low μ molar range and that they promote the formation of linearized minicircles covalently bound to protein on the 5' end, consistent with the poisoning of mitochondrial topoisomerase IA. Surprisingly, however, band depletion studies showed that it is topoisomerase IImt, and not topoisomerase IAmt, that is trapped. Both compounds are planar aromatic polycyclic structures that intercalate into and unwind DNA. These findings reinforce the utility of topoisomerase IImt as a target for development of new drugs for African sleeping sickness.

15333. **Tulloch, L. B., Martini, V. P., Iulek, J., Huggan, J. K., Lee, J. H., Gibson, C. L., Smith, T. K., Suckling, C. J. & Hunter, W. N., 2010.** Structure-based design of pteridine reductase inhibitors targeting African sleeping sickness and the leishmaniases. *Journal of Medicinal Chemistry,* **53** (1): 221-229.

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Pteridine reductase (PTR1) is a target for drug development against *Trypanosoma* and *Leishmania* species, parasites that cause serious tropical diseases and for which therapies are inadequate. We adopted a structure-based approach to the design of novel PTR1 inhibitors based on three molecular scaffolds. A series of compounds, most newly synthesized, were identified as inhibitors with PTR1-species specific properties explained by structural differences between the *T. brucei* and *L. major* enzymes. The most potent inhibitors target *T. brucei* PTR1, and two compounds displayed antiparasite activity against the bloodstream form of the parasite. PTR1 contributes to antifolate drug resistance by providing a molecular bypass of dihydrofolate reductase (DHFR) inhibition. Therefore, combining PTR1 and DHFR inhibitors might improve therapeutic efficacy. We tested two new compounds with known DHFR inhibitors. A synergistic effect was observed for one particular combination highlighting the potential of such an approach for treatment of African sleeping sickness.

15334. **Watts, K. R., Ratnam, J., Ang, K. H., Tenney, K., Compton, J. E., McKerrow, J. & Crews, P., 2010**. Assessing the trypanocidal potential of natural and semi-

synthetic diketopiperazines from two deep water marine-derived fungi. *Bioorganic & Medicinal Chemistry,* **18** (7): 2566-2574.

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Human African trypanosomiasis (HAT, commonly known as African sleeping sickness) is categorized as a neglected disease, as it afflicts 50 000 people annually in sub-saharan Africa, and there are few formal programmes in the world focused on drug discovery approaches for this disease. In this study, we examined the crude extracts of two fungal strains (*Aspergillus fumigatus* and *Nectria inventa*) isolated from deep water sediment which provided >99 percent growth inhibition at 1µg/mL of *Trypanosoma brucei*, the causative parasite of HAT. A collection of fifteen natural products was supplemented with six semi-synthetic derivatives and one commercially available compound. Twelve of the compounds, each containing a diketopiperazine core, showed excellent activity against *T. brucei* (IC_{50} = 0.002-40 µM), with selectivity over mammalian cells as great as 20-fold. The trypanocidal diketopiperazines were also tested against two cysteine protease targets Rhodesain and TbCatB, where five compounds showed inhibition activity at concentrations less than 20 µM. A preliminary activity pattern is described and analyzed.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

15335**. Tavares, K. C., Da Silva, A. S., Wolkmer, P., Monteiro, S. G. & Miletti, L. C., 2010.** Cryopreservation of *Trypanosoma evansi* after DEAE-cellulose purification: Evaluation of infective parameters. *Research in Veterinary Science*. **e- publication ahead of print June 7.**

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Cryopreservation is a method of keeping parasites alive in a laboratory. However, this technique may also damage the parasite. Alternatively, parasites may be maintained by *in vitro* culture. Unfortunately, for *Trypanosoma evansi* no effective medium that is able to maintain the parasite for more than 4 months has been described. In this study, we examined the effect of purifying trypomastigote through DEAE-cellulose chromatography before and after cryopreservation, by analyzing the pre-patent period, longevity, parasitaemia, and count of viable parasites. Our results showed a three-times increase in the concentration of viable trypomastigotes in DEAE-purified cryopreserved parasites as compared to non-DEAE-purified cryopreserved parasites. This indicates that DEAE-cellulose chromatography followed by cryopreservation is an effective method for the storage and preservation of *T. evansi*, with the advantage that the stocked parasites will be ready to use in molecular biology procedures.

(b) TAXONOMY; CHARACTERIZATION OF ISOLATES

[See also **33**: 15337, 15350, 15352, 15358, 15359, 15382].

15336. **Adams, E. R., Hamilton, P. B. & Gibson, W. C., 2010.** African trypanosomes: celebrating diversity. *Trends in Parasitology,* **26** (7): 324-328.

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Recent advances in molecular identification techniques and phylogenetic analysis have revealed the presence of previously unidentified tsetse-transmitted trypanosomes in Africa. This is surprising in a comparatively well-known group of pathogens that includes the causative agents of human and animal trypanosomiasis. Despite levels of genetic divergence that warrant taxonomic recognition, only one of these new trypanosomes has been named as a new species; the increased diversity is largely ignored or regarded as an inconvenient complication. Yet, some of these trypanosomes have demonstrated pathogenicity, whereas others are closely related to known pathogens, and might share this trait. We should first acknowledge that these novel trypanosomes exist and then take steps to investigate their host range, pathogenicity to livestock and response to chemotherapy.

(c) LIFE CYCLE, MORPHOLOGY, BIOCHEMICAL AND MOLECULAR STUDIES

[See also **33**: 15300, 15306, 15311, 15315, 15328].

15337. **Adams, E. R., Hamilton, P. B., Rodrigues, A. C., Malele, II, Delespaux, V., Teixeira, M. M. & Gibson, W., 2010.** New *Trypanosoma (Duttonella) vivax* genotypes from tsetse flies in East Africa. *Parasitology,* **137** (4): 641-650.

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Salivarian trypanosomes pose a substantial threat to livestock, but their full diversity is not known. To survey trypanosomes carried by tsetse in Tanzania, DNA samples from infected proboscides of *Glossina pallidipes* and *G. swynnertoni* were identified using fluorescent fragment length barcoding (FFLB), which discriminates species by size polymorphisms in multiple regions of the ribosomal RNA locus. FFLB identified the trypanosomes in 65 of 105 (61.9 percent) infected proboscides, revealing 9 mixed infections. Of 7 different FFLB profiles, 2 were similar but not identical to reference West African *Trypanosoma vivax*; 5 other profiles belonged to known species also identified in fly midguts. Phylogenetic analysis of the glycosomal glyceraldehyde phosphate dehydrogenase gene revealed that the Tanzanian *T. vivax* samples fell into 2 distinct groups, both outside the main clade of African and South American *T. vivax*. These new *T. vivax* genotypes were common and widespread in tsetse in Tanzania. The *T. brucei*-like trypanosome previously described from tsetse midguts was also found in 2 proboscides, demonstrating a salivarian transmission route. Investigation of mammalian host range and pathogenicity will reveal the importance of

these new trypanosomes for the epidemiology and control of animal trypanosomiasis in East Africa.

15338. **Aeby, E., Ullu, E., Yepiskoposyan, H., Schimanski, B., Roditi, I., Muhlemann, O. & Schneider, A., 2010.** tRNASec is transcribed by RNA polymerase II in *Trypanosoma brucei* but not in humans. *Nucleic Acids Research*. **Published online May 5.**

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Nuclear-encoded tRNAs are universally transcribed by RNA polymerase III (Pol-III) and contain intragenic promoters. Transcription of vertebrate tRNA(Sec) however requires extragenic promoters similar to Pol-III transcribed U6 snRNA. Here, we present a comparative analysis of tRNA(Sec) transcription in humans and the parasitic protozoa *Trypanosoma brucei*, two evolutionary highly diverged eukaryotes. RNAi-mediated ablation of Pol-II and Pol-III as well as oligo-dT induced transcription termination show that the human tRNA(Sec) is a Pol-III transcript. In *T. brucei* protein-coding genes are polycistronically transcribed by Pol-II and processed by trans-splicing and polyadenylation. tRNA genes are generally clustered in between polycistrons. However, the trypanosomal tRNA(Sec) genes are embedded within a polycistron. Their transcription is sensitive to alpha-amanitin and RNAi-mediated ablation of Pol-II, but not of Pol-III. Ectopic expression of the tRNA(Sec) outside but not inside a polycistron requires an added external promoter. These experiments demonstrate that trypanosomal tRNA(Sec), in contrast to its human counterpart, is transcribed by Pol-II. Synteny analysis shows that in trypanosomatids the tRNA(Sec) gene can be found in two different polycistrons, suggesting that it has evolved twice independently. Moreover, intron-encoded tRNAs are present in a number of eukaryotic genomes indicating that Pol-II transcription of tRNAs may not be restricted to trypanosomatids.

15339. **Alves-Silva, J., Ribeiro, J. M., Van Den Abbeele, J., Attardo, G., Hao, Z., Haines, L. R., Soares, M. B., Berriman, M., Aksoy, S. & Lehane, M. J., 2010.** An insight into the sialome of *Glossina morsitans morsitans*. *BMC Genomics,* **11**: 213.

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Blood feeding evolved independently in worms, arthropods and mammals. Among the adaptations to this peculiar diet, these animals developed an armament of salivary molecules that disarm their host's anti-bleeding defences (haemostasis), inflammatory and immune reactions. Recent sialotranscriptome analyses (from the Greek sialo = saliva) of blood feeding insects and ticks have revealed that the saliva contains hundreds of polypeptides, many unique to their genus or family. Adult tsetse flies feed exclusively on vertebrate blood and are important vectors of human and animal diseases. Thus far, only limited information exists regarding the *Glossina* sialome, or any other fly belonging to the Hippoboscidae. As part of

the effort to sequence the genome of *Glossina morsitans morsitans*, several organ specific, high quality normalized cDNA libraries have been constructed, from which over 20 000 ESTs from an adult salivary gland library were sequenced. These ESTs have been assembled using previously described ESTs from the fat body and midgut libraries of the same fly, thus totaling 62 251 ESTs, which have been assembled into 16 743 clusters (8506 of which had one or more EST from the salivary gland library). Coding sequences were obtained for 2 509 novel proteins, 1 792 of which had at least one EST expressed in the salivary glands. Despite library normalization, 59 transcripts were overrepresented in the salivary library indicating high levels of expression. This work presents a detailed analysis of the salivary protein families identified. Protein expression was confirmed by 2D gel electrophoresis, enzymatic digestion and mass spectrometry. Concurrently, an initial attempt to determine the immunogenic properties of selected salivary proteins was undertaken. The sialome of *G. m. morsitans* contains over 250 proteins that are possibly associated with blood feeding. This set includes alleles of previously described gene products, reveals new evidence that several salivary proteins are multigenic and identifies at least seven new polypeptide families unique to *Glossina*. Most of these proteins have no known function and thus provide a discovery platform for the identification of novel pharmacologically active compounds, innovative vector-based vaccine targets, and immunological markers of vector exposure.

15340. **Atyame Nten, C. M., Sommerer, N., Rofidal, V., Hirtz, C., Rossignol, M., Cuny, G., Peltier, J. B. & Geiger, A., 2010.** Excreted/secreted proteins from trypanosome procyclic strains. *Journal of Biomedical Biotechnology,* **2010**: 212817.

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Trypanosoma secretome was shown to be involved in parasite virulence and is suspected of interfering in parasite life-cycle steps such as establishment in the *Glossina* midgut, metacyclogenesis. Therefore, we attempted to identify the proteins secreted by procyclic strains of *T. brucei gambiense* and *T. brucei brucei*, responsible for human and animal trypanosomiasis, respectively. Using mass spectrometry, 427 and 483 nonredundant proteins were characterized in *T. brucei brucei* and *T. brucei gambiense* secretomes, respectively; 35 percent and 42 percent of the corresponding secretome proteins were specifically secreted by *T. brucei brucei* and *T. brucei gambiense*, respectively, while 279 proteins were common to both subspecies. The proteins were assigned to 12 functional classes. Special attention was paid to the most abundant proteases (14 families) because of their potential implication in the infection process and nutrient supply. The presence of proteins usually secreted via an exosome pathway suggests that this type of process is involved in trypanosome ESP secretion. The overall results provide leads for further research to develop novel tools for blocking trypanosome transmission.

15341. **Bodyl, A., Mackiewicz, P. & Milanowski, R., 2010.** Did trypanosomatid parasites contain a eukaryotic alga-derived plastid in their evolutionary past? *Journal of Parasitology,* **96** (2): 465-475.

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The Trypanosomatidae is closely related to euglenids that harbour plastids acquired from a green alga via secondary endosymbiosis. This discovery led to the idea that trypanosomatid parasites contained a green alga-derived plastid in their evolutionary past, an evolutionary scenario that was criticized based on the rarity of plant/plastid/cyanobacterium-like genes in the completely sequenced genomes of *Trypanosoma* and *Leishmania* species. Because it is difficult to identify such genes, however, their apparent rarity does not preclude a previous plastid endosymbiosis in the Trypanosomatidae. The genome of the plastid-less apicomplexan *Cryptosporidium parvum* preserves only a handful of plant/plastid/cyanobacterium-like genes, suggesting massive loss of plastid genes after elimination of its plastid. Additional support for such wholesale gene loss comes from fucoxanthin-containing dinoflagellates. Trypanosomatid nuclear genomes contain cyanobacterium-, green plant-, and haptophyte alga-derived genes, suggesting that they could have possessed a plastid in their evolutionary past; however, these genes also could represent examples of more typical horizontal gene transfer that did not accompany a plastid endosymbiosis. Thus, the presence of host cell genes that were adapted for use in the plastid would be much stronger evidence for a past plastid endosymbiosis in the Trypanosomatidae. Good examples of such genes are those encoding superoxide dismutases (SODs). Trypanosomatid parasites possess 4 iron-containing SODs, with 2 of them, SODA and SODC, targeted to the mitochondrion. In contrast to SODAs with classical single-domain mitochondrial targeting signals, SODCs carry bipartite pre-sequences composed of a signal peptide, followed by a transit peptide. Interestingly, these N-terminal extensions show striking similarities in length, hydropathy profiles, amino acid composition, and targeting properties to pre-sequences of proteins targeted to eukaryotic alga-derived plastids of euglenids and dinoflagellates. In turn, phylogenetic analyses indicate that SODCs originated from a mitochondrion-targeted SOD via gene duplication and were inherited vertically in the trypanosomatid lineage. These data represent a new kind of evidence for a past plastid endosymbiosis in the Trypanosomatidae, but the nature of this plastid remains unclear. It is usually assumed that the trypanosomatid plastid shared a common origin with that of euglenids, but Delta 4 desaturase phylogenies suggest that it could have originated via an independent, tertiary endosymbiosis involving a haptophyte alga. It is also possible that ancestors of the Trypanosomatidae initially possessed a primary plastid that later was replaced by a secondary or tertiary plastid.

15342**. Brenndorfer, M. & Boshart, M., 2010.** Selection of reference genes for mRNA quantification in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **172** (1): 52-55.

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Internal normalization is an established procedure that is necessary for accurate and reliable quantification of differentially regulated mRNAs. The profound changes of gene expression in parasitic life cycles pose a particular challenge to selection of appropriate reference genes for normalization, most importantly when using quantitative real time PCR (qPCR). Here we use the ranking algorithm implemented in the geNorm application to identify suitable *Trypanosoma brucei* reference genes for comparisons between the bloodstream and procyclic developmental stages and for analysis of mRNA induction by

environmental conditions. For these conditions, the TERT gene is a good choice for valid normalization of qPCR and is clearly superior to some other reference genes reported in the literature. For comparison of other conditions, the ranking algorithm is recommended to verify a reliable and valid normalization that is instrumental to quantitative analysis of gene expression.

15343. **Butikofer, P., Greganova, E., Liu, Y. C., Edwards, I. J., Lehane, M. J. & Acosta-Serrano, A., 2010**. Lipid remodelling of glycosylphosphatidylinositol (GPI) glycoconjugates in procyclic-form trypanosomes: biosynthesis and processing of GPIs revisited. *Biochemical Journal,* **428** (3): 409-418.

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The African trypanosome, *Trypanosoma brucei*, has been used as a model to study the biosynthesis of GPI (glycosylphosphatidylinositol) anchors. In mammalian (bloodstream)-form parasites, diacyl-type GPI precursors are remodelled in their lipid moieties before attachment to variant surface glycoproteins. In contrast, the GPI precursors of insect (procyclic)-form parasites, consisting of lyso-(acyl)PI (inositol-acylated acyl-lyso-phosphatidylinositol) species, remain unaltered before protein attachment. By using a combination of metabolic labelling, cell-free assays and complementary MS analyses, we show in the present study that GPI-anchored glycoconjugates in *T. congolense* procyclic forms initially receive tri-acylated GPI precursors, which are subsequently de-acylated either at the glycerol backbone or on the inositol ring. Chemical and enzymatic treatments of ^{3}H myristate-labelled lipids in combination with ESI-MS/MS (electrospray ionization-tandem MS) and MALDI-QIT-TOF-MS3 (matrix-assisted laser-desorption ionization-quadrupole ion trap-time-of-flight MS) analyses indicate that the structure of the lipid moieties of steady-state GPI lipids from *T. congolense* procyclic forms consists of a mixture of lyso-(acyl)PI, diacyl-PI and diacyl-(acyl)PI species. Interestingly, some of these species are myristoylated at the sn-2 position. To our knowledge, this is the first demonstration of lipid remodelling at the level of protein- or polysaccharide-linked GPI anchors in procyclic-form trypanosomes.

15344. **Chou, S., Jensen, B. C., Parsons, M., Alber, T. & Grundner, C., 2010.** The *Trypanosoma brucei* life cycle switch TbPTP1 is structurally conserved and dephosphorylates the nucleolar protein, NOPP44/46. *Journal of Biological Chemistry*. **In press, corrected proof**.

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Trypanosoma brucei adapts to changing environments as it cycles through arrested and proliferating stages in the human and tsetse fly hosts. Changes in protein tyrosine phosphorylation of several proteins, including NOPP44/46, accompany *T. brucei* development. Moreover, inactivation of *T. brucei* protein tyrosine phosphatase 1 (TbPTP1) triggers differentiation of bloodstream stumpy forms into tsetse procyclic forms through unknown downstream effects. Here, we link these events by showing that NOPP44/46 is a major substrate of TbPTP1. TbPTP1 substrate-trapping mutants selectively enrich NOPP44/46 from procyclic stage cell lysates, and TbPTP1 efficiently and selectively dephosphorylates NOPP44/46 *in vitro*. To provide insights into the mechanism of

NOPP44/46 recognition, we determined the crystal structure of TbPTP1. The TbPTP1 structure, the first of a kinetoplastid PTP, emphasizes the conservation of the protein tyrosine phosphatase (PTP) fold, extending to one of the most diverged eukaryotes. The structure reveals surfaces that may mediate substrate specificity and affords a template for the design of selective inhibitors to interfere with *T. brucei* transmission.

15345**. Cliffe, L. J., Siegel, T. N., Marshall, M., Cross, G. A. & Sabatini, R., 2010.** Two thymidine hydroxylases differentially regulate the formation of glucosylated DNA at regions flanking polymerase II polycistronic transcription units throughout the genome of *Trypanosoma brucei*. *Nucleic Acids Research,* **38** (12): 3923-3935.

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Base J is a hypermodified DNA base localized primarily to telomeric regions of the genome of *Trypanosoma brucei*. We have previously characterized two thymidine-hydroxylases (TH), JBP1 and JBP2, which regulate J-biosynthesis. JBP2 is a chromatin re-modelling protein that induces *de novo* J-synthesis, allowing JBP1, a J-DNA binding protein, to stimulate additional J-synthesis. Here, we show that both JBP2 and JBP1 are capable of stimulating *de novo* J-synthesis. We localized the JBP1- and JBP2-stimulated J by anti-J immunoprecipitation and high-throughput sequencing. This genome-wide analysis revealed an enrichment of base J at regions flanking polymerase II polycistronic transcription units (Pol II PTUs) throughout the *T. brucei* genome. Chromosome-internal J deposition is primarily mediated by JBP1, whereas JBP2 stimulated J deposition at the telomeric regions. However, the maintenance of J at JBP1-specific regions is dependent on JBP2 SWI/SNF and TH activity. That similar regions of *Leishmania major* also contain base J highlights the functional importance of the modified base at Pol II PTUs within members of the kinetoplastid family. The regulation of J synthesis/localization by two THs and potential biological function of J in regulating kinetoplastid gene expression are discussed.

15346. **de Jesus, T. C., Tonelli, R. R., Nardelli, S. C., Augusto, L. D., Motta, M. C., Girard-Dias, W., Miranda, K., Ulrich, P., Jimenez, V., Barquilla, A., Navarro, M., Docampo, R. & Schenkman, S., 2010.** Tor-like 1 kinase is involved in the control of polyphosphate levels and acidocalcisome maintenance in *Trypanosoma brucei*. *Journal of Biological Chemistry*. **In press, corrected proof**.

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Target of rapamycin (TOR) kinases are highly conserved protein kinases that integrate signals from nutrients and growth factors to coordinate cell growth and cell cycle progression. It has been previously described that two TOR kinases control cell growth in the protozoan parasite *Trypanosoma brucei*, the causative agent of African trypanosomiasis. Here we studied an unusual TOR-like protein named TbTOR-like 1, containing a PDZ domain and found exclusively in kinetoplastids. TbTOR-like 1 localizes to unique cytosolic granules. After hyperosmotic stress the localization of the protein shifts to the cell periphery, differently from other organelle markers. Ablation of TbTOR-like 1 causes a progressive inhibition of cell proliferation, producing parasites accumulating in S/G2 phase of the cell cycle. TbTOR-like 1 knocked down cells have an increased area occupied by acidic vacuoles,

known as acidocalcisomes, and are enriched in polyphosphate and pyrophosphate. These results suggest that TbTOR-like 1 might be involved in the control of acidocalcisome and polyphosphate metabolism in *T. brucei.*

15347. **de Sousa, K. P., Atouguia, J. & Silva, M. S., 2010.** Partial biochemical characterization of a metalloproteinase from the bloodstream forms of *Trypanosoma brucei brucei* parasites. *Protein Journal,* **29** (4): 283-289.

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Metalloproteinases (MMP) belong to the family of cation dependent endopeptidases that degrade matrices at physiological pH and cleave extracellular matrix proteins. They play an important role in diverse physiological and pathological processes; not only their diverse types of MMP differ in structure and functionally, but also their enzymatic activity is regulated at multiple levels. Trying to shed some light over the processes that govern the pathology of African trypanosomiasis, the aim of the present study was to examine the proteolytic activity of the crude trypanosome protein extract obtained from the bloodstream forms of *Trypanosoma brucei brucei* parasites. We hereby report the partial biochemical characterization of a neutral *Trypanosoma brucei*-metalloproteinase that displays marked proteolytic activities on gelatin and casein, with a molecular mass of approximately 40 kDa, whose activity is strongly dependent of pH and temperature. Furthermore, we show that this activity can be inhibited by classical MMP inhibitors such as EDTA, EGTA, phenantroline, and also by tetracycline and derivatives. This study has a relevant role in the search for new therapeutical targets, for the use of metalloproteinases inhibitors as treatment strategies, or as enhancement to trypanocidal drugs used in the treatment of the disease.

15348**. Denton, H., Fyffe, S. & Smith, T. K., 2010.** GDP-mannose pyrophosphorylase is essential in the bloodstream form of *Trypanosoma brucei*. *Biochemical Journal,* **425** (3): 603-614.

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A putative GDP-Man PP (guanidine diphosphomannose pyrophosphorylase) gene from *Trypanosoma brucei* (TbGDP-Man PP) was identified in the genome and subsequently cloned, sequenced and recombinantly expressed, and shown to be a catalytically active dimer. Kinetic analysis revealed a V_{max} of 0.34 µM/min/ mg of protein and K_m values of 67 µM and 12 µM for GTP and mannose 1-phosphate respectively. Further kinetic studies showed GDP-Man was a potent product feedback inhibitor. RNAi (RNA interference) of the cytosolic TbGDP-Man PP showed that mRNA levels were reduced to ~20 percent of wild-type levels, causing the cells to die after 3-4 days, demonstrating that TbGDP-Man PP is essential in the bloodstream form of *T. brucei* and thus a potential drug target. The RNAi-induced parasites have a greatly reduced capability to form GDP-Man, leading ultimately to a reduction in their ability to synthesize their essential GPI (glycosylphosphatidylinositol) anchors. The RNAi-induced parasites also showed aberrant N-glycosylation of their major cell-surface

glycoprotein, variant surface glycoprotein, with loss of the high-mannose Man9GlcNAc2 N-glycosylation at Asn428 and formation of complex N-glycans at Asn263.

15349. **Erben, E. D., Valguarnera, E., Nardelli, S., Chung, J., Daum, S., Potenza, M., Schenkman, S. & Tellez-Inon, M. T., 2010.** Identification of an atypical peptidyl-prolyl cis/trans isomerase from trypanosomatids. *Biochimica et Biophysica Acta,* **1803** (9): 1028-1037.

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15350. **Fisher, P., Noyes, H., Kemp, S., Stevens, R. & Brass, A., 2009**. A systematic strategy for the discovery of candidate genes responsible for phenotypic variation. *Methods in Molecular Biology,* **573**: 329-345.

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It is increasingly common to combine genome-wide expression data with quantitative trait mapping data to aid in the search for sequence polymorphisms responsible for phenotypic variation. By joining these complex but different data types at the level of the biological pathway, we can take advantage of existing biological knowledge to systematically identify possible mechanisms of genotype-phenotype interaction. With the development of web services and workflows, this process can be made rapid and systematic. Our methodology was applied to a case of resistance to African trypanosomiasis in mice. Workflows developed in this investigation, including a guide to loading and executing them with example data, are available at http://www.myexperiment.org/users/43/workflows.

15351. **Fisk, J. C., Zurita-Lopez, C., Sayegh, J., Tomasello, D. L., Clarke, S. G. & Read, L. K., 2010.** TbPRMT6 is a type I protein arginine methyltransferase that contributes to cytokinesis in *Trypanosoma brucei*. *Eukaryotic Cell,* **9** (6): 866-877.

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Arginine methylation is a widespread posttranslational modification of proteins catalyzed by a family of protein arginine methyltransferases (PRMTs). In *Saccharomyces cerevisiae* and mammals, this modification affects multiple cellular processes, such as chromatin remodelling leading to transcriptional regulation, RNA processing, DNA repair, and cell signalling. The protozoan parasite *Trypanosoma brucei* possesses five putative PRMTs in its genome. This is a large number of PRMTs relative to other unicellular eukaryotes, suggesting an important role for arginine methylation in trypanosomes. Here, we present the *in vitro* and *in vivo* characterization of a *T. brucei* enzyme homologous to human PRMT6, which we term TbPRMT6. Like human PRMT6, TbPRMT6 is a type I PRMT, catalyzing the production of monomethylarginine and asymmetric dimethylarginine residues. In *in vitro* methylation assays, TbPRMT6 utilizes bovine histones as a substrate, but it does not methylate several *T. brucei* glycine/arginine-rich proteins. As such, it exhibits a relatively

narrow substrate specificity compared to other *T. brucei* PRMTs. Knockdown of TbPRMT6 in both procyclic form and bloodstream form *T. brucei* leads to a modest but reproducible effect on parasite growth in culture. Moreover, upon TbPRMT6 depletion, both PF and BF exhibit aberrant morphologies indicating defects in cell division, and these defects differ in the two life cycle stages. Mass spectrometry of TbPRMT6-associated proteins reveals histones, components of the nuclear pore complex, and flagellar proteins that may represent TbPRMT6 substrates contributing to the observed growth and morphological defects.

15352. **Gibson, W., Nemetschke, L. & Ndung'u, J., 2010.** Conserved sequence of the TgsGP gene in Group 1 *Trypanosoma brucei gambiense*. *Infection, Genetics & Evolution,* **10** (4): 453-458.

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The trypanosome responsible for the majority of cases of human trypanosomiasis in Africa is Group 1 *Trypanosoma brucei gambiense*. Currently the most reliable test for the parasite is based on a single gene, which encodes a 47kDa receptor-like *T. b. gambiense*-specific glycoprotein, TgsGP, expressed in the flagellar pocket of bloodstream forms. Although TgsGP has been demonstrated in *T. b. gambiense* throughout its geographic range, similar genes have been demonstrated in other *T. brucei* sspp. isolates, and there are no data on the extent of sequence variation in TgsGP. Here we have carried out a comparison of TgsGP sequences in a range of Group 1 *T. b. gambiense* isolates and compared the gene to homologues in other *T. brucei* sspp. in order to provide information to support the use of this gene as the key identification target for Group 1 *T. b. gambiense*. We demonstrate that the sequence of TgsGP is well conserved in Group 1 *T. b. gambiense* across the endemic range of Gambian human trypanosomiasis and confirm that this gene is a suitable target for specific detection of this parasite. The TgsGp-like genes in some isolates of *T. b. brucei, T. b. rhodesiense* and Group 2 *T. b. gambiense* are closely similar to VSG Tb10.v4.0178, which may be the ancestral gene from which TgsGP was derived.

15353. **Goh, J. Y., Lai, C. Y., Tan, L. C., Yang, D., He, C. Y. & Liou, Y. C., 2010.** Functional characterization of two novel parvulins in *Trypanosoma brucei*. *FEBS Letters,* **584** (13): 2901-2908.

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Parvulins belong to a family of peptidyl-prolyl cis/trans isomerases (PPIases) that catalyze the cis/trans conformations of prolyl-peptidyl bonds. Herein, we characterized two novel parvulins, TbPIN1 and TbPAR42, in *Trypanosoma brucei*. TbPIN1, a 115 amino-acid protein, contains a single PPIase domain but lacks the N-terminal WW domain. Using NMR spectroscopy, TbPIN1 was found to exhibit PPIase activity toward a phosphorylated substrate. Overexpression of TbPIN1 can rescue the impaired temperature-sensitive phenotype in a mutant yeast strain. TbPAR42, containing 383 amino acids, comprises a novel FHA domain at its N terminus and a C-terminal PPIase domain but is a non-Pin1-type PPIase. Functionally, a knockdown of TbPAR42 in its procyclic form results in reduced proliferation rates suggesting an important role in cell growth.

15354. **Gunzl, A., 2010.** The pre-mRNA splicing machinery of trypanosomes: complex or simplified? *Eukaryot Cell*. **Published online ahead of print June 25.**

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Trypanosomatids are early-diverged, protistan parasites of which *Trypanosoma brucei, Trypanosoma cruzi*, and several species of *Leishmania* cause severe, often lethal diseases in humans. To better combat these parasites, their molecular biology has been a research focus for more than three decades and the discovery of spliced leader (SL) trans splicing in *T. brucei* established a key difference between parasites and hosts. In SL trans splicing, the capped 5' terminal region of the small nuclear SL RNA is fused onto the 5' end of each mRNA. This process, in conjunction with polyadenylation, generates individual mRNAs from polycistronic precursors and creates functional mRNA by providing the cap structure. The reaction is a two step transesterification process analogous to intron removal by cis splicing which, in trypanosomatids, is confined to very few pre-mRNAs. Both types of pre-mRNA splicing are carried out by the spliceosome consisting of five U-rich small nuclear (sn)RNAs and, in humans, of up to approximately 170 different proteins. While trypanosomatids possess a full set of spliceosomal U snRNAs, only few splicing factors were identified by standard genome annotation because trypanosomatid amino acid sequences are among the most divergent in the eukaryotic kingdom. This review focuses on recent progress made in the characterization of the splicing factor repertoire in *T. brucei* which was achieved by tandem affinity purification of splicing complexes, by systematic analysis of proteins containing RNA recognition motifs, and by mining the genome database. In addition, recent findings about functional differences between trypanosome and human pre-mRNA splicing factors are discussed.

15355. **Gupta, S. K., Hury, A., Ziporen, Y., Shi, H., Ullu, E. & Michaeli, S., 2010**. Small nucleolar RNA interference in *Trypanosoma brucei*: mechanism and utilization for elucidating the function of snoRNAs. *Nucleic Acids Research*. **Published online July 3**.

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15356. **Hill, K. L., 2010.** Parasites in motion: flagellum-driven cell motility in African trypanosomes. *Current Opinion in Microbiology*, **13** (4): 459-465

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Motility of the sleeping sickness parasite, *Trypanosoma brucei*, impacts disease transmission and pathogenesis. Trypanosome motility is driven by a flagellum that harbours a canonical 9+2 axoneme, together with trypanosome-specific elaborations. Trypanosome flagellum biology and motility have been the object of intense research over the last two years. These studies have led to the discovery of a novel form of motility, termed social motility, and provided revision of long-standing models for cell propulsion. Recent work has also uncovered novel structural features and motor proteins associated with the flagellar apparatus and has identified candidate signalling molecules that are predicted to regulate flagellar motility. Together with earlier inventories of flagellar proteins from proteomic and genomic studies, the stage is now set to move forward with functional studies to elucidate molecular mechanisms and investigate parasite motility in the context of host-parasite interactions.

15357. **Holzmuller, P., Herder, S., Cuny, G. & De Meeus, T., 2010.** From clonal to sexual: a step in *T. congolense* evolution? *Trends in Parasitology,* **26** (2): 56-60.

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Although clearly demonstrated in *Trypanosoma brucei*, genetic exchange remains controversial in other trypanosome species. Recently, Morrison and co-workers applied a population-genetics analysis, and established the existence of mating in *Trypanosoma congolense*. Starting from this original discovery, we focus here on the important question of how mating is induced during the trypanosome life cycle and discuss the use of statistics to evidence this type of non-obligatory biological process.

15358. **Jackson, A. P., Sanders, M., Berry, A., McQuillan, J., Aslett, M. A., Quail, M. A., Chukualim, B., Capewell, P., MacLeod, A., Melville, S. E., Gibson, W., Barry, J. D., Berriman, M. & Hertz-Fowler, C., 2010.** The genome sequence of *Trypanosoma brucei gambiense*, causative agent of chronic human African trypanosomiasis. *PLoS Neglected Tropical Diseases,* **4** (4): e658.

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Trypanosoma brucei gambiense is the causative agent of chronic human African trypanosomiasis or sleeping sickness, a disease endemic across often poor and rural areas of Western and Central Africa. We have previously published the genome sequence of a *T. b. brucei* isolate, and have now employed a comparative genomics approach to understand the scale of genomic variation between *T. b. gambiense* and the reference genome. We sought to identify features that were uniquely associated with *T. b. gambiense* and its ability to infect humans. An improved high-quality draft genome sequence for the group 1 *T. b. gambiense* DAL 972 isolate was produced using a whole-genome shotgun strategy. Comparison with *T. b. brucei* showed that sequence identity averages 99.2 percent in coding regions, and gene order is largely collinear. However, variation associated with segmental duplications and tandem gene arrays suggests some reduction of functional repertoire in *T. b. gambiense* DAL 972. A comparison of the variant surface glycoproteins (VSG) in *T. b. brucei* with all *T. b.*

gambiense sequence reads showed that the essential structural repertoire of VSG domains is conserved across *T. brucei*. This study provides the first estimate of intraspecific genomic variation within *T. brucei*, and so has important consequences for future population genomics studies. We have shown that the *T. b. gambiense* genome corresponds closely with the reference, which should therefore be an effective scaffold for any *T. brucei* genome sequence data. As VSG repertoire is also well conserved, it may be feasible to describe the total diversity of variant antigens. While we describe several as yet uncharacterized gene families with predicted cell surface roles that were expanded in number in *T. b. brucei*, no *T. b. gambiense*-specific gene was identified outside of the subtelomeres that could explain the ability to infect humans.

15359. **Kabani, S., Waterfall, M. & Matthews, K. R., 2010.** Cell-cycle synchronisation of bloodstream forms of *Trypanosoma brucei* using Vybrant DyeCycle Violet-based sorting. *Molecular and Biochemical Parasitology,* **169** (1): 59-62.

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Studies on the cell-cycle of *Trypanosoma brucei* have revealed several unusual characteristics that differ from the model eukaryotic organisms. However, the inability to isolate homogenous populations of parasites in distinct cell-cycle stages has limited the analysis of trypanosome cell division and complicated the understanding of mutant phenotypes with possible impact on cell-cycle related events. Although hydroxyurea-induced cell-cycle arrest in procyclic and bloodstream forms has been applied recently with success, such block-release protocols can complicate the analysis of cell-cycle regulated events and have the potential to disrupt important cell-cycle checkpoints. An alternative approach based on flow cytometry of parasites stained with Vybrant DyeCycle Orange circumvents this problem, but is restricted to procyclic form parasites. Here, we apply Vybrant Dyecycle Violet staining coupled with flow cytometry to effectively select different cell-cycle stages of bloodstream form trypanosomes. Moreover, the sorted parasites remain viable, although synchrony is rapidly lost. This method enables cell-cycle enrichment of populations of trypanosomes in their mammal infective stage, particularly at the G1 phase.

15360. **Kramer, S., Kimblin, N. C. & Carrington, M., 2010.** Genome-wide *in silico* screen for CCCH-type zinc finger proteins of *Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania major*. *BMC Genomics,* **11**: 283.

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CCCH type zinc finger proteins are RNA binding proteins with regulatory functions at all stages of mRNA metabolism. The best-characterized member, tritetraproline (TTP), binds to AU rich elements in 3' UTRs of unstable mRNAs, mediating their degradation. In kinetoplastids, CCCH type zinc finger proteins have been identified as being involved in the regulation of the life cycle and possibly the cell cycle. To date, no systematic listing of CCCH proteins in kinetoplastids is available. We have identified the complete set of CCCH type zinc finger proteins in the available genomes of the kinetoplastid protozoa *Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania major*. One fifth (20 percent) of all CCCH motifs

fall into non-conventional classes and many had not been previously identified. One third of all CCCH proteins have more than one CCCH motif, suggesting multivalent RNA binding. One third have additional recognizable domains. The vast majority are unique to Kinetoplastida or to a subgroup within. Two exceptions are of interest: the putative orthologue of the mRNA nuclear export factor Mex67 and a 3'-5' exoribonuclease restricted to *Leishmania* species. CCCH motifs are absent from these proteins in other organisms and might be unique, novel features of the Kinetoplastida homologues. Of the others, several have a predicted, and in one case experimentally confirmed, connection to the ubiquitination pathways, for instance a HECT-type E3 ubiquitin ligase. The total number of kinetoplastid CCCH proteins is similar to the number in higher eukaryotes but lower than in yeast. A comparison of the genomic loci between the Trypanosomatidae homologues provides insight into both the evolution of the CCCH proteins as well as the CCCH motifs. This study provides the first systematic listing of the Kinetoplastida CCCH proteins. The number of CCCH proteins with more then one CCCH motif is larger than previously estimated, due to the identification of non-conventional CCCH motifs. Experimental approaches are now necessary to examine the functions of the many unique CCCH proteins as well as the function of the putative Mex67 and the *Leishmania* 3'-5' exoribonuclease.

15361. **Li, Z., Umeyama, T., Li, Z. & Wang, C. C., 2010.** Polo-like kinase guides cytokinesis in *Trypanosoma brucei* through an indirect means. *Eukaryotic Cell,* **9** (5): 705-716.

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Polo-like kinase in *Trypanosoma brucei* (TbPLK) is confined to the flagellum attachment zone (FAZ) and regulates only cytokinetic initiation. However, it apparently diffuses into the cytoplasm before the trans-localization of chromosomal passenger complex (CPC) from the midzone of central spindle to FAZ, which is known to be required for initiating cytokinesis. Synchronized *T. brucei* procyclic cells treated with a TbPLK inhibitor, GW843682X (GW), in late S phase were found to go through a full cell cycle at a normal pace before being arrested at cytokinetic initiation in the second cycle. However, synchronized cells treated with GW in G(1) phase were arrested at cytokinetic initiation within the first cell cycle, suggesting that inhibition of TbPLK at its emergence blocks cytokinesis within the same cell cycle. To rule out potential off-target effects from GW, TbPLK RNA interference (RNAi) was induced to deplete TbPLK, and the progression of synchronized cells from late S phase was also found to be arrested at cytokinetic initiation within the first cell cycle. Apparently, TbPLK has accomplished its role in guiding cytokinesis before the late S phase, presumably by phosphorylating a certain substrate(s) during S phase, which may play a critical role in initiating the subsequent cytokinesis.

15362. **Louw, C. A., Ludewig, M. H. & Blatch, G. L., 2010.** Overproduction, purification and characterisation of Tbj1, a novel Type III Hsp40 from *Trypanosoma brucei*, the African sleeping sickness parasite. *Protein Expression and Purification,* **69** (2): 168-177.

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15363. **Lun, Z. R., Lai, D. H., Li, F. J., Lukes, J. & Ayala, F. J., 2010.** *Trypanosoma brucei*: two steps to spread out from Africa. *Trends in Parasitology*. **In press, corrected proof.**

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Trypanosoma brucei equiperdum and *Trypanosoma brucei evansi* are typically considered separate species, although a recent study suggested that these organisms can be classified as subspecies of *Trypanosoma brucei*, which we also favour. Here we present a scenario that attempts to explain the continuing evolution of the dyskinetoplastic and akinetoplastic strains, as a consequence of loss of selective pressure(s) leading to the loss of kinetoplast DNA.

15364. **Ma, J., Benz, C., Grimaldi, R., Stockdale, C., Wyatt, P., Frearson, J. & Hammarton, T. C., 2010.** Nuclear DBF-2-related kinases are essential regulators of cytokinesis in bloodstream stage *Trypanosoma brucei*. *Journal of Biological Chemistry,* **285** (20): 15356-15368.

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Nuclear DBF-2-related (NDR) kinases are essential regulators of cell cycle progression, growth, and development in many organisms and are activated by the binding of an Mps One Binder (MOB) protein partner, autophosphorylation, and phosphorylation by an upstream STE20 family kinase. In the protozoan parasite, *Trypanosoma brucei*, the causative agent of human African trypanosomiasis, the NDR kinase, PK50, is expressed in proliferative life cycle stages and was shown to complement a yeast NDR kinase mutant cell line. However, the function of PK50 and a second NDR kinase, PK53, in *T. brucei* has not been determined to date, although trypanosome MOB1 is known to be essential for cytokinesis, suggesting the NDR kinases may also be involved in this process. Here, we show that specific depletion of PK50 or PK53 from bloodstream stage trypanosomes resulted in the rapid accumulation of cells with two nuclei and two kinetoplasts, indicating that cytokinesis was specifically inhibited. This led to a deregulation of the cell cycle and cell death and provides genetic validation of these kinases as potential novel drug targets for human African trypanosomiasis. Recombinant active PK50 and PK53 were produced and biochemically characterized. Both enzymes autophosphorylated, were able to trans-phosphorylate generic kinase substrates *in vitro*, and were active in the absence of phosphorylation by an upstream kinase. Additionally, both enzymes were active in the absence of MOB1 binding, which was also demonstrated to likely be a feature of the kinases *in vivo*. Biochemical characterization of recombinant PK50 and PK53 has revealed key kinetic differences between them, and the identification of *in*

vitro peptide substrates in this study paves the way for high throughput inhibitor screening of these kinases.

15365. **Macgregor, P. & Matthews, K. R., 2010.** New discoveries in the transmission biology of sleeping sickness parasites: applying the basics. *Journal of Molecular Medicine.* **Published online June 5.**

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The sleeping sickness parasite, *Trypanosoma brucei*, must differentiate in response to the changing environments that it encounters during its complex life cycle. One developmental form, the bloodstream stumpy stage, plays an important role in infection dynamics and transmission of the parasite. Recent advances have shed light on the molecular mechanisms by which these stumpy forms differentiate as they are transmitted from the mammalian host to the insect vector of sleeping sickness, tsetse flies. These molecular advances now provide improved experimental tools for the study of stumpy formation and function within the mammalian bloodstream. They also offer new routes to therapy via high-throughput screens for agents that accelerate parasite development. Here, we shall discuss the recent advances that have been made and the prospects for future research now available.

15366. **Marcoux, V., Wei, G., Tabel, H. & Bull, H. J., 2010.** Characterization of major surface protease homologues of *Trypanosoma congolense*. *Journal of Biomedicine & Biotechnology,* **2010**: 418157.

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Trypanosomes encode a family of proteins known as major surface metalloproteases (MSPs). We have identified six putative MSPs encoded within the partially sequenced *T. congolense* genome. Phylogenic analysis indicates that *T. congolense* MSPs belong to five subfamilies that are conserved among African trypanosome species. Molecular modelling, based on the known structure of *Leishmania major* GP63, reveals subfamily-specific structural variations around the putative active site despite conservation of overall structure, suggesting that each MSP subfamily has evolved to recognize distinct substrates. We have cloned and purified a protein encoding the amino-terminal domain of the *T. congolense* homologue TcoMSP-D (most closely related to *Leishmania* GP63). We detect TcoMSP-D in the serum of *T. congolense*-infected mice. Mice immunized with the amino-terminal domain of TcoMSP-D generate a persisting IgG_1 antibody response. Surprisingly, a low-dose challenge of immunized mice with *T. congolense* significantly increases susceptibility to infection, indicating that immunity to TcoMSP-D is a factor affecting virulence.

15367**. Martinez-Calvillo, S., Vizuet-de-Rueda, J. C., Florencio-Martinez, L. E., Manning-Cela, R. G. & Figueroa-Angulo, E. E., 2010.** Gene expression in trypanosomatid parasites. *Journal of Biomedicine and Biotechnology,* **2010**: 525241.

Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonoma de Mexico, Av. De los Barrios 1, Col. Los Reyes Iztacala, Tlalnepantla, Edo. de Mexico, CP 54090, Mexico. [scalv@campus.iztacala.unam.mx].

The parasites *Leishmania* spp., *Trypanosoma brucei*, and *Trypanosoma cruzi* are the trypanosomatid protozoa that cause the deadly human diseases leishmaniasis, African sleeping sickness, and Chagas disease, respectively. These organisms possess unique mechanisms for gene expression such as constitutive polycistronic transcription of protein-coding genes and trans-splicing. Little is known about either the DNA sequences or the proteins that are involved in the initiation and termination of transcription in trypanosomatids. *In silico* analyses of the genome databases of these parasites led to the identification of a small number of proteins involved in gene expression. However, functional studies have revealed that trypanosomatids have more general transcription factors than originally estimated. Many posttranslational histone modifications, histone variants, and chromatin modifying enzymes have been identified in trypanosomatids, and recent genome-wide studies showed that epigenetic regulation might play a very important role in gene expression in this group of parasites. Here, we review and comment on the most recent findings related to transcription initiation and termination in trypanosomatid protozoa.

15368. **Mehlert, A., Sullivan, L. & Ferguson, M. A., 2010.** Glycotyping of *Trypanosoma brucei* variant surface glycoprotein MITat1.8. *Molecular & Biochemical Parasitology*. **In press, corrected proof.**

Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK. [m.a.j.ferguson@dundee.ac.uk].

Following a switch from variant surface glycoprotein MITat1.4 to variant surface glycoprotein MITat1.8 expression by Lister strain 427 *Trypanosoma brucei brucei* parasites, the latter uncharacterized variant surface glycoprotein was analyzed. Variant surface glycoprotein MITat1.8 was found to be a disulphide-linked homodimer, containing a complex N-linked glycan at Asn58 and a glycosylphosphatidylinositol membrane anchor attached to Asp419. Mass spectrometric analyses demonstrated that the N-glycan is exclusively Galbeta1-4GlcNAcbeta1-2Manalpha1-3(Galbeta1-4GlcNAcbeta1-2Manalpha1-6)Manbeta1-4G lcNAcbeta1-4GlcNAc and that the conserved Man(3)GlcN-myo-inositol glycosylphosphatidylinositol anchor glycan core is substituted with an average of 4 hexose, most likely galactose, residues. The presence of a complex N-glycan at Asn58 is consistent with the relatively acidic environment of the Asn58 N-glycosylation sequon, that predicts N-glycosylation by *T. brucei* oligosaccharyltransferase TbSTT3A with a Man(5)GlcNAc(2) structure destined for processing to a paucimannose and/or complex N-glycan.

15369. **Mohd Ismail, N. I., Yuasa, T., Yuasa, K., Nambu, Y., Nisimoto, M., Goto, M., Matsuki, H., Inoue, M., Nagahama, M. & Tsuji, A., 2010.** A critical role for highly conserved Glu(610) residue of oligopeptidase B from *Trypanosoma brucei* in thermal stability. *Journal of Biochemistry*, **147** (2): 201-211.

Department of Biological Science and Technology, University of Tokushima Graduate School, 2-1 Minamijosanjima, Tokushima 770-8506, Japan. [tsuji@bio.tokushima-u.ac.jp].

15370. **Mosimann, M., Goshima, S., Wenzler, T., Luscher, A., Uozumi, N. & Maser, P., 2010.** A Trk/HKT-type K+ transporter from *Trypanosoma brucei*. *Eukaryotic Cell,* **9** (4): 539-546.

Institute of Cell Biology, University of Bern, Bern, Switzerland. [pascal.maeser@unibas.ch].

15371. **Nganga, J. K., Soller, M. & Iraqi, F. A., 2010.** High resolution mapping of trypanosomosis resistance loci Tir2 and Tir3 using F12 advanced intercross lines with major locus Tir1 fixed for the susceptible allele. *BMC Genomics,* **11**: 394.

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Trypanosomosis is the most economically important disease constraint to livestock productivity in Africa. A number of trypanotolerant cattle breeds are found in West Africa, and identification of the genes conferring trypanotolerance could lead to effective means of genetic selection for trypanotolerance. In this context, high resolution mapping in mouse models are a promising approach to identifying the genes associated with trypanotolerance. In previous studies, using F2 C57BL/6J x A/J and C57BL/6J x BALB/cJ mouse resource populations, trypanotolerance QTL were mapped within a large genomic intervals of 20-40 cM to chromosomes MMU17, 5 and 1, and denoted Tir1, Tir2 and Tir3 respectively. Subsequently, using F6 C57BL/6J x A/J and C57BL/6J x BALB/cJ F6 advanced intercross lines (AIL), Tir1 was fine mapped to a confidence interval (CI) of less than 1 cM, while Tir2 and Tir3, were mapped within 5-12 cM. Tir1 represents the major trypanotolerance QTL. In order to improve map resolutions of Tir2 and Tir3, an F12 C57BL/6J x A/J AIL population fixed for the susceptible alleles at Tir1 QTL was generated. An F12 C57BL/6J x A/J AIL population, fixed for the resistant alleles at Tir1 QTL was also generated to provide an additional estimate of the gene effect of Tir1. The AIL populations homozygous for the resistant and susceptible Tir1 alleles and the parental controls were challenged with *T. congolense* and followed for survival times over 180 days. Mice from the two survival extremes of the F12 AIL population fixed for the susceptible alleles at Tir1 were genotyped with a dense panel of microsatellite markers spanning the Tir2 and Tir3 genomic regions and QTL mapping was performed. Tir2 was fine mapped to less than 1 cM CI while Tir3 was mapped to three intervals named Tir3a, Tir3b and Tir3c with 95 percent confidence intervals (CI) of 6, 7.2 and 2.2 cM, respectively. The mapped QTL regions encompass genes that are vital to innate immune response and can be potential candidate genes for the underlying QTL.

15372. **Paris, Z., Changmai, P., Rubio, M. A., Zikova, A., Stuart, K. D., Alfonzo, J. D. & Lukes, J.,** 2010. The Fe/S cluster assembly protein Isd11 is essential for tRNA thiolation in *Trypanosoma brucei*. *Journal of Biological Chemistry,* **285** (29): July 16.

Institute of Parasitology, Czech Republic. [jula@paru.cas.cz].

15373. **Pillay, D., Boulange, A. F. & Coetzer, T. H., 2010.** Expression, purification and characterisation of two variant cysteine peptidases from *Trypanosoma congolense* with active site substitutions. *Protein Expression & Purification*. **In press, corrected proof.**

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Congopain, the major cysteine peptidase of *Trypanosoma congolense* is an attractive candidate for an anti-disease vaccine and target for the design of specific inhibitors. A complicating factor for the inclusion of congopain in a vaccine is that multiple variants of congopain are present in the genome of the parasite. In order to determine whether the variant congopain-like genes code for peptidases with enzymatic activities different to those of congopain, two variants were cloned and expressed. Two truncated catalytic domain variants were recombinantly expressed in *Pichia pastoris*. The two expressed catalytic domain variants differed slightly from one another in substrate preferences and also from that of C2 (the recombinant truncated form of congopain). Surprisingly, a variant with the catalytic triad Ser(25), His(159) and Asn(175) was shown to be active against classical cysteine peptidase substrates and inhibited by E-64, a class specific cysteine protease inhibitor. Both catalytic domain clones and C2 had pH optima of either 6.0 or 6.5 implying that these congopain-like proteases are likely to be expressed and active in the bloodstream of the host animal.

15374. **Price, H. P., Guther, M. L., Ferguson, M. A. & Smith, D. F., 2010.** Myristoyl-CoA:protein N-myristoyltransferase depletion in trypanosomes causes avirulence and endocytic defects. *Molecular & Biochemical Parasitology,* **169** (1): 55-58.

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The enzyme myristoyl-CoA:protein N-myristoyltransferase (NMT) catalyses the co-translational covalent attachment of the fatty acid myristate to the N-terminus of target proteins. NMT is known to be essential for viability in *Trypanosoma brucei* and *Leishmania major*. Here we describe phenotypic analysis of *T. brucei* bloodstream form cells following knockdown of NMT expression by tetracycline-inducible RNA interference. Cell death occurs from 72h post-induction, with approximately 50 percent of cells displaying a defect in endocytic uptake by this time. The majority of these induced cells do not have an enlarged flagellar pocket typical of a block in endocytosis but vesicle accumulation around the flagellar pocket indicates a defect in vesicular progression following endocytic fusion. Induced parasites have a wild-type or slightly enlarged Golgi apparatus, unlike the phenotype of cells with reduced expression of a major N-myristoylated protein, ARL1. Critically we show that following NMT knockdown, *T. brucei* bloodstream form cells are unable to establish an infection in a mouse model, therefore providing further validation of this enzyme as a target for drug development.

15375. **Richmond, G. S., Gibellini, F., Young, S. A., Major, L., Denton, H., Lilley, A. & Smith, T. K., 2010**. Lipidomic analysis of bloodstream and procyclic form *Trypanosoma brucei*. *Parasitology,* **137** (9): 1357-1392.

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The biological membranes of *Trypanosoma brucei* contain a complex array of phospholipids that are synthesized *de novo* from precursors obtained either directly from the host, or as catabolized endocytosed lipids. This paper describes the use of nanoflow electrospray tandem mass spectrometry and high resolution mass spectrometry in both positive and negative ion modes, allowing the identification of approximately 500 individual molecular phospholipids species from total lipid extracts of cultured bloodstream and procyclic form *T. brucei*. Various molecular species of all of the major subclasses of glycerophospholipids were identified including phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol as well as phosphatidic acid, phosphatidylglycerol and cardolipin, and the sphingolipids sphingomyelin, inositol phosphoceramide and ethanolamine phosphoceramide. The lipidomic data obtained in this study will aid future biochemical phenotyping of either genetically or chemically manipulated commonly used bloodstream and procyclic strains of *Trypanosoma brucei.* Hopefully this will allow a greater understanding of the bizarre world of lipids in this important human pathogen.

15376. **Richterova, L., Vavrova, Z. & Lukes, J., 2010.** DEAD-box RNA helicase is dispensable for mitochondrial translation in *Trypanosoma brucei*. *Experimental Parasitology*. **In press, corrected proof.**

Biology Centre, Institute of Parasitology, Czech Academy of Sciences, and Faculty of Sciences, University of South Bohemia, Ceske Budejovice (Budweis), Czech Republic. [jula@paru.cas.cz].

15377. **Roy, N., Nageshan, R. K., Pallavi, R., Chakravarthy, H., Chandran, S., Kumar, R., Gupta, A. K., Singh, R. K., Yadav, S. C. & Tatu, U., 2010.** Proteomics of *Trypanosoma evansi* infection in rodents. *PLoS One,* **5** (3): e9796.

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Trypanosoma evansi infections, commonly called “surra”, cause significant economic losses to the livestock industry. While this infection is mainly restricted to large animals such as camels, donkeys and equines, recent reports indicate their ability to infect humans. There are no World Animal Health Organization (WAHO) prescribed diagnostic tests or vaccines available against this disease and the available drugs show significant toxicity. There is an urgent need to develop improved methods of diagnosis and control measures for this disease. Unlike its related human parasites *T. brucei* and *T. cruzi* whose genomes have been fully sequenced, the *T. evansi* genome sequence remains unavailable and very little effort is being made to develop improved methods of prevention, diagnosis and treatment. With a view to identifying potential diagnostic markers and drug targets we have studied the clinical proteome of *T. evansi* infection using mass spectrometry (MS). Using shot-gun proteomic approach involving nano-lc Quadrupole Time Of Flight (QTOF) mass spectrometry we have identified over 160 proteins expressed by *T. evansi* in mice infected with a camel isolate.

Homology driven searches for protein identification from MS/MS data led to most of the matches arising from related *Trypanosoma* species. Proteins identified belonged to various functional categories including metabolic enzymes; DNA metabolism; transcription; translation as well as cell-cell communication and signal transduction. TCA cycle enzymes were strikingly missing, possibly suggesting their low abundances. The clinical proteome revealed the presence of known and potential drug targets such as oligopeptidases, kinases, cysteine proteases and more. Previous proteomic studies on trypanosomal infections, including human parasites *T. brucei* and *T. cruzi*, have been carried out from lab-grown cultures. For *T. evansi* infection this is indeed the first ever proteomic study reported thus far. In addition to providing a glimpse into the biology of this neglected disease, our study is the first step towards identification of diagnostic biomarkers, novel drug targets as well as potential vaccine candidates to fight against *T. evansi* infections.

15378. **Sevova, E. S., Goren, M. A., Schwartz, K. J., Hsu, F. F., Turk, J., Fox, B. G. & Bangs, J. D., 2010.** Cell-free synthesis and functional characterization of sphingolipid synthases from parasitic trypanosomatid protozoa. *Journal of Biological Chemistry*, **285** (27): 20580-20587.

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The *Trypanosoma brucei* genome has four highly similar genes encoding sphingolipid synthases (TbSLS1-4). TbSLSs are polytopic membrane proteins that are essential for viability of the pathogenic bloodstream stage of this human protozoan parasite and, consequently, can be considered as potential drug targets. TbSLS4 was shown previously to be a bifunctional sphingomyelin/ethanolamine phosphorylceramide synthase, whereas functions of the others were not characterized. Using a recently described liposome-supplemented cell-free synthesis system which eliminates complications from background cellular activities, we now unambiguously define the enzymatic specificity of the entire gene family. TbSLS1 produces inositol phosphorylceramide, TbSLS2 produces ethanolamine phosphorylceramide, and TbSLS3 is bifunctional, like TbSLS4. These findings indicate that TbSLS1 is uniquely responsible for synthesis of inositol phosphorylceramide in insect stage parasites, in agreement with published expression array data. This approach also revealed that the *Trypanosoma cruzi* orthologue (TcSLS1) is a dedicated inositol phosphorylceramide synthase. The cell-free synthesis system allowed rapid optimization of the reaction conditions for these enzymes and site-specific mutagenesis to alter end product specificity. A single residue at position 252 (TbSLS1, Ser(252); TbSLS3, Phe(252)) strongly influences enzymatic specificity. We also have used this system to demonstrate that aureobasidin A, a potent inhibitor of fungal inositol phosphorylceramide synthases, does not significantly affect any of the TbSLS activities, consistent with the phylogenetic distance of these two clades of sphingolipid synthases. These results represent the first application of cell-free synthesis for the rapid preparation and functional annotation of integral membrane proteins and thus illustrate its utility in studying otherwise intractable enzyme systems.

15379**. Siegel, T. N., Hekstra, D. R., Wang, X., Dewell, S. & Cross, G. A., 2010.** Genome-wide analysis of mRNA abundance in two life-cycle stages of *Trypanosoma brucei*

and identification of splicing and polyadenylation sites. *Nucleic Acids Research*. **Published online April 12**.

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Transcription of protein-coding genes in trypanosomes is polycistronic and gene expression is primarily regulated by post-transcriptional mechanisms. Sequence motifs in the untranslated regions regulate mRNA trans-splicing and RNA stability, yet where UTRs begin and end is known for very few genes. We used high-throughput RNA-sequencing to determine the genome-wide steady-state mRNA levels ("transcriptomes") for approximately 90 percent of the genome in two stages of the *Trypanosoma brucei* life cycle cultured *in vitro*. Almost 6 percent of genes were differentially expressed between the two life-cycle stages. We identified 5' splice-acceptor sites (SAS) and polyadenylation sites (PAS) for 6959 and 5948 genes, respectively. Most genes have between one and three alternative SAS, but PAS are more dispersed. For 488 genes, SAS were identified downstream of the originally assigned initiator ATG, so a subsequent in-frame ATG presumably designates the start of the true coding sequence. In some cases, alternative SAS would give rise to mRNAs encoding proteins with different N-terminal sequences. We could identify the introns in two genes known to contain them, but found no additional genes with introns. Our study demonstrates the usefulness of the RNA-sequencing technology to study the transcriptional landscape of an organism whose genome has not been fully annotated.

15380. **Sienkiewicz, N., Ong, H. B. & Fairlamb, A. H., 2010**. *Trypanosoma brucei* pteridine reductase 1 is essential for survival *in vitro* and for virulence in mice. *Molecular Microbiology,* **77** (3): 658-671.

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Gene knockout and knockdown methods were used to examine essentiality of pteridine reductase (PTR1) in pterin metabolism in the African trypanosome. Attempts to generate PTR1 null mutants in bloodstream form *T. brucei* proved unsuccessful; despite integration of drug selectable markers at the target locus, the gene for PTR1 was either retained at the same locus or elsewhere in the genome. However, RNA interference (RNAi) resulted in complete knockdown of endogenous protein after 48 h, followed by cell death after 4 days. This lethal phenotype was reversed by expression of enzymatically active *Leishmania major* PTR1 in RNAi lines or by addition of tetrahydrobiopterin to cultures. Loss of PTR1 was associated with gross morphological changes due to a defect in cytokinesis, resulting in cells with multiple nuclei and kinetoplasts, as well as multiple detached flagella. Electron microscopy also revealed increased numbers of glycosomes, while immunofluorescence microscopy showed increased and more diffuse staining for glycosomal matrix enzymes, indicative of mis-localisation to the cytosol. Mis-localization was confirmed by digitonin fractionation experiments. RNAi cell lines were markedly less virulent than wild-type (WT) parasites in mice and virulence was restored in the (oe)RNAi line. Thus, PTR1 may be a drug target for human African trypanosomiasis.

15381. **Simo, G., Herder, S., Cuny, G. & Hoheisel, J., 2010.** Identification of subspecies specific genes differentially expressed in procyclic forms of *Trypanosoma brucei* subspecies. *Infection, Genetics & Evolution,* **10** (2): 229-237.

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Trypanosoma brucei subspecies undergo establishment and maturation in tsetse flies mid-gut and salivary glands, respectively. Successful establishment of trypanosomes in tsetse mid-gut as well as their migration to saliva gland depends on the ability of these parasites to adapt rapidly to new environmental conditions and to negotiate the physical barriers. To identify subspecies specific genes which are differentially regulated during the establishment of *T. brucei* subspecies in tsetse flies mid-gut, a comparative genomic analysis between different *T. brucei* subspecies was performed using microarrays containing about 23 040 *T. brucei* shotgun fragments. The whole genome analyses of RNA expression profiles revealed about 274 significantly differentially expressed genes between *T. brucei* subspecies. About 7 percent of the differentially regulated clones did not match to any *T. brucei* predicted genes. Most of the differentially regulated transcripts are involved in transport across cell membrane and also in the purines metabolism. The genes selectively up regulated in *T. brucei gambiense* and *T. brucei rhodesiense* (human infective *T. brucei*) like snoRNA and HSP70 are expressed in response to stress. The high failure rate in the process of establishment and maturation of *T. brucei gambiense* during cyclical transmission in tsetse flies may result from the incapacity of this parasite to regulate its growth due to the expression of a variety of chaperones or heat shock proteins. Genes selectively up regulated in *T. brucei brucei* like NT8.1 nucleoside/nucleobase transporters and S-adenosylmethionine synthetase may favour the establishment of this subspecies in tsetse mid-gut. These genes appear as potential targets for investigations on the development of vaccine blocking the transmission of trypanosomes in tsetse flies.

15382. **Simo, G., Njiokou, F., Tume, C., Lueong, S., De Meeus, T., Cuny, G. & Asonganyi, T., 2010**. Population genetic structure of Central African *Trypanosoma brucei gambiense* isolates using microsatellite DNA markers. *Infection, Genetics & Evolution,* **10** (1): 68-76.

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Genetic variation of microsatellite loci is a widely used method for the analysis of population genetic structure of microorganisms. Seven microsatellite markers were used here to characterize *Trypanosoma brucei gambiense* isolates from Central Africa sub-region in order to improve knowledge on the population genetic structure of this subspecies. These markers confirmed the low genetic polymorphism within Central African *T. b. gambiense* isolates from the same focus and strong differentiation between different foci. The presence of many multilocus genotypes of *T. b. gambiense* and the excess of heterozygotes found in this study play in favour of a clonal reproduction of this parasite. But some data may be indicative of a unique recombination event in one subsample. The high F_{ST} value indicates

low migration rates between *T. b. gambiense* subpopulations (foci). Very negative F_{IS} suggests fairly small clonal population sizes of this pathogen in the different human trypanosomosis foci of Central Africa.

15383. **Smith, T. K. & Butikofer, P., 2010.** Lipid metabolism in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **172** (2): 66-79.

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Trypanosoma brucei membranes consist of all major eukaryotic glycerophospholipid and sphingolipid classes. These are *de novo* synthesized from precursors obtained either from the host or from catabolised endocytosed lipids. In recent years, substantial progress has been made in the molecular and biochemical characterization of several of these lipid biosynthetic pathways, using gene knockout or RNA interference strategies or by enzymatic characterization of individual reactions. Together with the completed genome, these studies have highlighted several possible differences between mammalian and trypanosome lipid biosynthesis that could be exploited for the development of drugs against the diseases caused by these parasites.

15384. **Sprehe, M., Fisk, J. C., McEvoy, S. M., Read, L. K. & Schumacher, M. A., 2010.** Structure of the *Trypanosoma brucei* p22 protein, a cytochrome oxidase subunit II-specific RNA-editing accessory factor. *Journal of Biological Chemistry,* **285** (24): 18899-18908.

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15385. **Stagno, J., Aphasizheva, I., Bruystens, J., Luecke, H. & Aphasizhev, R., 2010.** Structure of the mitochondrial editosome-like complex associated TUTase 1 reveals divergent mechanisms of UTP selection and domain organization. *Journal of Molecular Biology,* **399** (3): 464-475.

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RNA uridylylation reactions catalyzed by terminal uridylyl transferases (TUTases) play critical roles in the formation of the mitochondrial transcriptome in trypanosomes. Two mitochondrial RNA editing TUTases have been described: RNA editing TUTase 1 catalyzes guide RNA, ribosomal RNA, and mRNA 3'-uridylylation, and RNA editing TUTase 2 acts as a subunit of the RNA editing core complex (also referred to as the 20S editosome) to perform guided U-insertion mRNA editing. Although RNA editing TUTase 1 and RNA editing TUTase 2 carry out distinct functions and possess dissimilar enzymatic properties, their catalytic N-terminal domain and base recognition C-terminal domain display a high degree of similarity, while their middle domains are less conserved. MEAT1 (mitochondrial editosome-like complex associated TUTase 1), which interacts with an editosome-like assembly and is

exclusively U-specific, nonetheless shows limited similarity with editing TUTases and lacks the middle domain. The crystal structures of apo MEAT1 and UTP-bound MEAT1 refined to 1.56 A and 1.95 A, respectively, reveal an unusual mechanism of UTP selection and domain organization previously unseen in TUTases. In addition to established invariant UTP-binding determinants, we have identified and verified critical contributions of MEAT1-specific residues using mutagenesis. Furthermore, MEAT1 possesses a novel bridging domain, which extends from the C-terminal domain and makes hydrophobic contacts with the N-terminal domain, thereby creating a cavity adjacent to the UTP-binding site. Unlike the minimal TUT4 TUTase, MEAT1 shows no appreciable conformational change upon UTP binding and apparently does not require RNA substrate to select a cognate nucleoside triphosphate. Because MEAT1 is essential for the viability of the bloodstream and insect forms of *Trypanosoma brucei*, the unique organization of its active site renders this protein an attractive target for trypanocide development.

15386. **Stewart, M., Haile, S., Jha, B. A., Cristodero, M., Li, C. H. & Clayton, C., 2010.** Processing of a phosphoglycerate kinase reporter mRNA in *Trypanosoma brucei* is not coupled to transcription by RNA polymerase II. *Molecular & Biochemical Parasitology,* **172** (2): 99-106.

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15387. **Subramanya, S., Armah, D. A. & Mensa-Wilmot, K., 2010**. *Trypanosoma brucei:* reduction of GPI-phospholipase C protein during differentiation is dependent on replication of newly transformed cells. *Experimental Parasitology,* **125** (3): 222-229.

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The protozoan parasite *Trypanosoma brucei* lives in the bloodstream of vertebrates or in a tsetse fly. Expression of a GPI-phospholipase C polypeptide (GPI-PLCp) in the parasite is restricted to the bloodstream form. Events controlling the amount of GPI-PLCp expressed during differentiation are not completely understood. Our metabolic "pulse-chase" analysis reveals that GPI-PLCp is stable in bloodstream form. However, during differentiation of bloodstream to insect stage (procyclic) *T. brucei*, translation GPI-PLC mRNA ceases within 8h of initiating transformation. GPI-PLCp is not lost precipitously from newly transformed procyclic trypanosomes. Nascent procyclics contain 400-fold more GPI-PLCp than established insect stage *T. brucei*. Reduction of GPI-PLCp in early-stage procyclics is linked to parasite replication. Sixteen cell divisions are required to reduce the amount of GPI-PLCp in newly differentiated procyclics to levels present in the established procyclic. GPI-PLCp is retained in strains of *T. brucei* that fail to replicate after differentiation of the bloodstream to the procyclic form. Thus, at least two factors control levels of GPI-PLCp during differentiation of bloodstream *T. brucei*; (i) repression of GPI-PLC mRNA translation, and (ii) sustained replication of newly transformed procyclic *T. brucei*. These studies illustrate the importance of repeated cell divisions in controlling the steady-state amount of GPI-PLCp during differentiation of the African trypanosome.

15388**. Swift, R. V. & Amaro, R. E., 2009**. Discovery and design of DNA and RNA ligase inhibitors in infectious microorganisms. *Expert Opinion on Drug Discovery,* **4** (12): 1281-1294.

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Members of the nucleotidyltransferase superfamily known as DNA and RNA ligases carry out the enzymatic process of polynucleotide ligation. These guardians of genomic integrity share a three-step ligation mechanism, as well as common core structural elements. Both DNA and RNA ligases have experienced a surge of recent interest as chemotherapeutic targets for the treatment of a range of diseases, including bacterial infection, cancer, and the diseases caused by the protozoan parasites known as trypanosomes. In this review, we will focus on efforts targeting pathogenic microorganisms; specifically, bacterial NAD^+-dependent DNA ligases, which are promising broad-spectrum antibiotic targets, and ATP-dependent RNA editing ligases from *Trypanosoma brucei*, the species responsible for the devastating neurodegenerative disease, African sleeping sickness. High quality crystal structures of both NAD^+-dependent DNA ligase and the *Trypanosoma brucei* RNA editing ligase have facilitated the development of a number of promising leads. For both targets, further progress will require surmounting permeability issues and improving selectivity and affinity.

15389. **Szoor, B., 2010.** Trypanosomatid protein phosphatases. *Molecular & Biochemical Parasitology,* **173** (2): 53-63.

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Protein phosphorylation is one of the most important post-translational modifications regulating various signalling processes in all known living organisms. In the cell, protein phosphatases and protein kinases play a dynamic antagonistic role, controlling the phosphorylation state of tyrosine (Tyr), serine (Ser) and threonine (Thr) side chains of proteins. The reversible phosphorylation modulates protein function, through initiating conformational changes, which influences protein complex formation, alteration of enzyme activity and changes in protein stability and subcellular localization. These molecular changes affect signalling cascades regulating the cell cycle, differentiation, cell-cell and cell-substrate interactions, cell motility, the immune response, ion-channel and transporter activities, gene transcription, mRNA translation, and basic metabolism. In addition to these processes, in unicellular parasites, like *Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania* spp., additional signalling pathways have evolved to enable the survival of parasites in the changing environment of the vector and host organism. In recent years the genome of five trypanosomatid genomes have been sequenced and annotated allowing complete definition of the composition of the trypanosomatid phosphatomes. The very diverse environments involved in the different stages of the kinetoplastids' life cycle might have played a role to develop a set of trypanosomatid-specific phosphatases in addition to orthologues of many

higher eukaryote protein phosphatases present in the kinetoplastid phosphatomes. In spite of their well-described phosphatomes, few trypanosomatid protein phosphatases have been characterized and studied *in vivo*. The aim of this review is to give an up to date scope of the research, which has been carried out on trypanosomatid protein phosphatases.

15390. **Szoor, B., Ruberto, I., Burchmore, R. & Matthews, K. R., 2010.** A novel phosphatase cascade regulates differentiation in *Trypanosoma brucei* via a glycosomal signalling pathway. *Genes and Development,* **24** (12): 1306-1316.

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In the mammalian bloodstream, the sleeping sickness parasite *Trypanosoma brucei* is held poised for transmission by the activity of a tyrosine phosphatase, TbPTP1. This prevents differentiation of the transmissible "stumpy forms" until entry into the tsetse fly, whereupon TbPTP1 is inactivated and major changes in parasite physiology are initiated to allow colonization of the arthropod vector. Using a substrate-trapping approach, we identified the downstream step in this developmental signalling pathway as a DxDxT phosphatase, TbPIP39, which is activated upon tyrosine phosphorylation, and hence is negatively regulated by TbPTP1. *In vitro*, TbPIP39 promotes the activity of TbPTP1, thereby reinforcing its own repression, this being alleviated by the trypanosome differentiation triggers citrate and cis-aconitate, generating a potentially bistable regulatory switch. Supporting a role in signal transduction, TbPIP39 becomes rapidly tyrosine-phosphorylated during differentiation, and RNAi-mediated transcript ablation in stumpy forms inhibits parasite development. Interestingly, TbPIP39 localizes in glycosomes, peroxisome-like organelles that compartmentalize the trypanosome glycolytic reactions among other enzymatic activities. Our results invoke a phosphatase signalling cascade in which the developmental signal is trafficked to a unique metabolic organelle in the parasite: the glycosome. This is the first characterized environmental signalling pathway targeted directly to a peroxisome-like organelle in any eukaryotic cell.

15391. **Takcz, I. D., Gupta, S. K., Volkov, V., Romano, M., Haham, T., Tulinski, P., Lebenthal, I. & Michaeli, S., 2010.** Analysis of spliceosomal proteins in trypanosomatids reveals novel functions in mRNA processing. *Journal of Biological Chemistry*. **In press, corrected proof.**

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In trypanosomatids, all mRNAs are processed via trans-splicing, though cis-splicing also occurs. In trans-splicing, a common small exon, the spliced leader (SL), which is derived from a small SL RNA species, is added to all mRNAs. Sm and Lsm proteins are core proteins that bind to U snRNAs and are essential for both these splicing processes. In this study, SmD3 and Lsm3 associated complexes were purified to homogeneity from *Leishmania tarentolae.* The purified complexes were analyzed by mass spectrometry and 54 and 39 proteins were purified from SmD3 and Lsm complexes, respectively. Interestingly, among the proteins purified from Lsm3, no mRNA degradation factors were detected, as in Lsm complexes from other eukaryotes. The U1A complex was purified and mass-spectrometry

analysis identified, in addition to U1 snRNP proteins, additional co-purified proteins including the polyadenylation factor, CPSF73. Defects observed in cells silenced for U1 snRNP proteins suggest that the U1 snRNP functions exclusively in cis-splicing, though U1A also participates in polyadenylation and affects trans-splicing. The study characterized several trypanosome-specific nuclear factors involved in snRNP biogenesis, whose function was elucidated in *Trypanosoma brucei*. Conserved factors, such as PRP19, which functions at the heart of every cis-spliceosome, also affects SL RNA modification; GEMIN2, a protein associated with SMN (survival of motor neurons) and implicated in selective association of U snRNA with core Sm proteins in trypanosomes, is a master regulator of snRNP assembly. This study demonstrates the existence of trypanosomatid-specific splicing factors, but also that conserved snRNP proteins possess trypanosome-specific functions.

15392**. Tyc, J., Faktorova, D., Kriegova, E., Jirku, M., Vavrova, Z., Maslov, D. A. & Lukes, J., 2010.** Probing for primary functions of prohibitin in *Trypanosoma brucei*. *International Journal of Parasitology,* **40** (1): 73-83.

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Prohibitins (PHBs) 1 and 2 are small conserved proteins implicated in a number of functions in the mitochondrion, as well as in the nucleus of eukaryotic cells. The current understanding of PHB functions comes from studies of model organisms such as yeast, worm and mouse, but considerable debate remains with regard to the primary functions of these ubiquitous proteins. We exploit the tractable reverse genetics of *Trypanosoma brucei*, the causative agent of African sleeping sickness, in order to specifically analyse the function of PHB in this highly divergent eukaryote. Using inducible RNA interference (RNAi) we show that PHB1 is essential in *T. brucei*, where it is confined to the cell's single mitochondrion forming a high molecular weight complex. PHB1 and PHB2 appear to be indispensible for mitochondrial translation. Their ablation leads to a decrease in mitochondrial membrane potential, however no effect on the level of reactive oxygen species was observed. Flagellates lacking either PHB1 or both PHB1 and PHB2 exhibit significant morphological changes of their organelle, most notably its inflation. Even long after the loss of the PHB proteins, mtDNA was unaltered and mitochondrial cristae remained present, albeit displaced to the periphery of the mitochondrion, which is in contrast to other eukaryotes.

15393. **Tyc, J., Long, S., Jirku, M. & Lukes, J., 2010**. YCF45 protein, usually associated with plastids, is targeted into the mitochondrion of *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **173** (1): 43-47.

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15394. **Vanhollebeke, B., Uzureau, P., Monteyne, D., Perez-Morga, D. & Pays, E., 2010**. Cellular and molecular remodelling of the endocytic pathway during differentiation of *Trypanosoma brucei* bloodstream forms. *Eukaryotic Cell*, **9** (8) 1272-1282.

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During the course of mammalian infection, African trypanosomes undergo extensive cellular differentiation, as actively dividing long slender forms (SL) progressively transform into intermediate forms (I) and finally quiescent G1/G0-locked short stumpy forms (ST). ST forms maintain adaptations compatible with their survival in the mammalian bloodstream such as high endocytic activity, but they already show pre-adaptations to the insect midgut conditions. The nutritional requirements of ST forms must differ from those of SL forms because they do not multiply any longer. We report that in ST forms the uptake of several ligands was reduced as compared with SL forms. In particular, the haptoglobin-haemoglobin (Hp-Hb) complex was no longer taken up due to dramatic down-regulation of its cognate receptor TbHpHbR. As this receptor also allows uptake of trypanolytic particles from human serum, ST forms were resistant to trypanolysis by human serum lipoproteins. These observations allowed both flow cytometry analysis of SL to ST differentiation and the generation of homogeneous ST populations after positive selection upon exposure to trypanolytic particles. In addition, we observed that in ST forms the lysosome relocates anterior to the nucleus. Altogether, we identified novel morphological and molecular features that characterize SL to ST differentiation.

15395. **Vaughan, S., 2010**. Assembly of the flagellum and its role in cell morphogenesis in *Trypanosoma brucei*. *Current Opinion in Microbiology,* **13** (4): 453-458.

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Eukaryotic flagella are microtubule-based structures required for a variety of functions including cell motility and sensory perception. Most eukaryotic flagella grow out from a cell into the surrounding medium, but when the flagellum of the protozoan parasite *Trypanosoma brucei* exits the cell via the flagellar pocket, it is attached along the length of the cell body by a cytoskeletal structure called the flagellum attachment zone (FAZ). The exact reasons for flagellum attachment have remained elusive, but evidence is emerging that the attached flagellum plays a major role in cell morphogenesis in this organism. In this review we discuss evidence published in the past four years that is unravelling the role of the flagellum in organelle segregation, inheritance of cell shape and cytokinesis.

15396. **Veitch, N. J., Johnson, P. C., Trivedi, U., Terry, S., Wildridge, D. & MacLeod, A., 2010.** Digital gene expression analysis of two life cycle stages of the human-infective parasite, *Trypanosoma brucei gambiense* reveals differentially expressed clusters of co-regulated genes. *BMC Genomics,* **11**: 124.

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The evolutionarily ancient parasite, *Trypanosoma brucei*, is unusual in that the majority of its genes are regulated post-transcriptionally, leading to the suggestion that transcript abundance of most genes does not vary significantly between different life cycle stages despite the fact that the parasite undergoes substantial cellular remodelling and metabolic changes throughout its complex life cycle. To investigate this in the clinically relevant sub-species, *Trypanosoma brucei gambiense*, which is the causative agent of the fatal human disease African sleeping sickness, we have compared the transcriptome of two different life cycle stages, the potentially human-infective bloodstream forms with the non-human-infective procyclic stage using digital gene expression (DGE) analysis. Over eleven million unique tags were generated, producing expression data for 7 360 genes, covering 81 percent of the genes in the genome. Compared to microarray analysis of the related *T. b. brucei* parasite, approximately 10 times more genes with a 2.5-fold change in expression levels were detected. The transcriptome analysis revealed the existence of several differentially expressed gene clusters within the genome, indicating that contiguous genes, presumably from the same polycistronic unit, are co-regulated either at the level of transcription or transcript stability. DGE analysis is extremely sensitive for detecting gene expression differences, revealing firstly that a far greater number of genes are stage-regulated than had previously been identified and secondly and more importantly, this analysis has revealed the existence of several differentially expressed clusters of genes present on what appears to be the same polycistronic units, a phenomenon which had not previously been observed in microarray studies. These differentially regulated clusters of genes are in addition to the previously identified RNA polymerase I polycistronic units of variant surface glycoproteins and procyclin expression sites, which encode the major surface proteins of the parasite. This raises a number of questions regarding the function and regulation of the gene clusters that clearly warrant further study.

15397. **Worthen, C., Jensen, B. C. & Parsons, M., 2010**. Diverse effects on mitochondrial and nuclear functions elicited by drugs and genetic knockdowns in bloodstream stage *Trypanosoma brucei. PLoS Neglected Tropical Diseases,* **4** (5): e678.

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The options for treating the fatal disease human African trypanosomiasis are limited to a few drugs that are toxic or facing increasing resistance. New drugs that kill the causative agents, subspecies of *Trypanosoma brucei*, are therefore urgently needed. Little is known about the cellular mechanisms that lead to death of the pathogenic bloodstream stage. We therefore conducted the first side by side comparison of the cellular effects of multiple death inducers that target different systems in bloodstream form parasites, including six drugs (pentamidine, prostaglandin D_2, quercetin, etoposide, camptothecin, and a tetrahydroquinoline) and six RNAi knockdowns that target distinct cellular functions. All compounds tested were static at low concentrations and killed at high concentrations. Dead parasites were rapidly quantified by forward and side scatter during flow cytometry, as confirmed by ethidium homodimer and esterase staining, making these assays convenient for quantitating parasite death. The various treatments yielded different combinations of defects in mitochondrial potential, reactive oxygen species, cell cycle, and genome segregation. No evidence was seen for phosphatidylserine exposure, a marker of apoptosis. Reduction in ATP levels lagged behind decreases in live cell number. Even when the impact on growth was

similar at 24 hours, drug-treated cells showed dramatic differences in their ability to further proliferate, demonstrating differences in the reversibility of effects induced by the diverse compounds. Parasites showed different phenotypes depending on the treatment, but none of them were clear predictors of whether apparently live cells could go on to proliferate after drugs were removed. We therefore suggest that clonal proliferation assays may be a useful step in selecting anti-trypanosomal compounds for further development. Elucidating the genetic or biochemical events initiated by the compounds with the most profound effects on subsequent proliferation may identify new means to activate death pathways.

15398. **Wright, J. R., Siegel, T. N. & Cross, G. A., 2010.** Histone H3 trimethylated at lysine 4 is enriched at probable transcription start sites in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology,* **172** (2): 141-144.

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Recent studies have identified histone modifications and suggested a role for epigenetic gene regulation in *Trypanosoma brucei*. The histone modification H4K10ac and histone variants H2AZ and H2BV localize to probable sites of transcription initiation. Although all *T. brucei* histones have very evolutionarily divergent N-terminal tails, histone H3 shows conservation with other eukaryotic organisms in 6 of 8 amino acids encompassing lysine 4. Tri-methylation of H3K4 is generally associated with transcription. We therefore generated a specific antibody to *T. brucei* H3K4me3 and performed chromosome immunoprecipitation and high-throughput sequencing. We show that H3K4me3 is enriched at the start of polycistronic transcription units at divergent strand-switch regions and at other sites of RNA polymerase II transcription reinitiation. H3K4me3 largely co-localizes with H4K10ac, but with a skew towards the upstream side of the H4K10ac peak, suggesting that it is a component of specific nucleosomes that play a role in Pol II transcription initiation.

15399. **Xia, Y., Zhang, Y., Jiang, S. & Cheng, H., 2010.** CD4(+) T-cell anergy induced by lin(-)CD117(c-kit$^+$) stem cell-derived immature dendritic cells loaded with nuclear antigen derived from *Trypanosoma equiperdum*. *Autoimmunity*. **e publication ahead of print April 7**

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Dendritic cells (DCs) are professional antigen-presenting cells, which have the extraordinary capacity to initiate naive T-cell-mediated primary immune responses. To investigate the role of DCs in the induction of antigen-specific tolerance, the immature DCs (imDCs) and mature DCs (mDCs) were generated *in vitro* from lin(-)CD117(c-kit$^+$) stem cells isolated from mice bone marrow. Flow cytometry and confocal microscopy were used to characterize the phenotypes of DCs. These cells were loaded with nuclear antigen derived from *Trypanosoma equiperdum* and then co-cultured with naive CD4$^+$ T cells. It was found that imDC-treated T cells had lower proliferation level and cytokine expression of interleukin (IL)-2, IL-4, IL-12, and interferon-gamma compared with mDC-treated T cells. These results demonstrated that the maturation status of DCs is critical for preventing the production of autoantibodies.

15400. **Yao, Y., Gao, G. & Li, D., 2010.** Cloning, expression, purification and activity assay of *Trypanosoma brucei* phenylalanyl-tRNA synthetase in *Escherichia coli*. *Sheng Wu Gong Cheng Xue Bao,* **26** (1): 130-135.

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15401. **Young, S. A. & Smith, T. K., 2010.** The essential neutral sphingomyelinase is involved in the trafficking of the variant surface glycoprotein in the bloodstream form of *Trypanosoma brucei*. *Molecular Microbiology,* **76** (6): 1461-1482.

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Sphingomyelin is the main sphingolipid in *Trypanosoma brucei,* the causative agent of African sleeping sickness. *In vitro* and *in vivo* characterization of the *T. brucei* neutral sphingomyelinase demonstrates that it is directly involved in sphingomyelin catabolism. Gene knockout studies in the bloodstream form of the parasite indicate that the neutral sphingomyelinase is essential for growth and survival, thus highlighting that the *de novo* biosynthesis of ceramide is unable to compensate for the loss of sphingomyelin catabolism. The phenotype of the conditional knockout has given new insights into the highly active endocytic and exocytic pathways in the bloodstream form of *T. brucei*. Hence, the formation of ceramide in the endoplasmic reticulum affects post-Golgi sorting and rate of deposition of newly synthesized GPI-anchored variant surface glycoprotein on the cell surface. This directly influences the corresponding rate of endocytosis, via the recycling endosomes, of pre-existing cell surface variant surface glycoprotein. The trypanosomes use this coupled endocytic and exocytic mechanism to maintain the cell density of its crucial variant surface glycoprotein protective coat. TbnSMase is therefore genetically validated as a drug target against African trypanosomes, and suggests that interfering with the endocytic transport of variant surface glycoprotein is a highly desirable strategy for drug development against African trypanosomasis.

15402. **Zhou, Q., Gheiratmand, L., Chen, Y., Lim, T. K., Zhang, J., Li, S., Xia, N., Liu, B., Lin, Q. & He, C. Y., 2010.** A comparative proteomic analysis reveals a new bi-lobe protein required for bi-lobe duplication and cell division in *Trypanosoma brucei*. *PLoS One,* **5** (3): e9660.

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A Golgi-associated bi-lobed structure was previously found to be important for Golgi duplication and cell division in *Trypanosoma brucei*. To further understand its functions, comparative proteomics was performed on extracted flagellar complexes (including the flagellum and flagellum-associated structures such as the basal bodies and the bi-lobe) and purified flagella to identify new bi-lobe proteins. A leucine-rich repeats containing protein, TbLRRP1, was characterized as a new bi-lobe component. The anterior part of the TbLRRP1-labeled bi-lobe is adjacent to the single Golgi apparatus, and the posterior side is tightly associated with the flagellar pocket collar marked by TbBILBO1. Inducible depletion

of TbLRRP1 by RNA interference inhibited duplication of the bi-lobe as well as the adjacent Golgi apparatus and flagellar pocket collar. Formation of a new flagellum attachment zone and subsequent cell division were also inhibited, suggesting a central role of bi-lobe in Golgi, flagellar pocket collar and flagellum attachment zone biogenesis.

15403. **Zimmermann, R., Eyrisch, S., Ahmad, M. & Helms, V., 2010.** Protein translocation across the ER membrane. *Biochimica et Biophysica Acta*. **In press, corrected proof**.

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Protein translocation into the endoplasmic reticulum (ER) is the first and decisive step in the biogenesis of most extracellular and many soluble organelle proteins in eukaryotic cells. It is mechanistically related to protein export from eubacteria and archaea and to the integration of newly synthesized membrane proteins into the ER membrane and the plasma membranes of eubacteria and archaea (with the exception of tail anchored membrane proteins). Typically, protein translocation into the ER involves cleavable amino terminal signal peptides in precursor proteins and sophisticated transport machinery components in the cytosol, the ER membrane, and the ER lumen. Depending on the hydrophobicity and/or overall amino acid content of the precursor protein, transport can occur co- or posttranslationally. The respective mechanism determines the requirements for certain cytosolic transport components. The two mechanisms merge at the level of the ER membrane, specifically, at the heterotrimeric Sec61 complex present in the membrane. The Sec61 complex provides a signal peptide recognition site and forms a polypeptide conducting channel. Apparently, the Sec61 complex is gated by various ligands, such as signal peptides of the transport substrates, ribosomes (in cotranslational transport), and the ER lumenal molecular chaperone, BiP. Binding of BiP to the incoming polypeptide contributes to efficiency and unidirectionality of transport. Recent insights into the structure of the Sec61 complex and the comparison of the transport mechanisms and machineries in the yeast *Saccharomyces cerevisiae*, the human parasite *Trypanosoma brucei*, and mammals have various important mechanistic as well as potential medical implications.

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