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TSETSE AND TRYPANOSOMIASIS INFORMATION

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TSETSE AND TRYPANOSOMIASIS INFORMATION

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

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

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

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

Distribution dates and copy deadlines

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

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

ABBREVIATIONS USED IN *TTI*

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

Organizations

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

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

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

CONTENTS

SECTION A – NEWS

PROGRAMME AGAINST AFRICAN TRYPANOSOMIASIS (PAAT): REPORT OF THE 11TH MEETING OF THE PROGRAMME COMMITTEE (PC)

Foreword

The 11th meeting of the PAAT-PC was convened at WHO headquarters, Geneva, Switzerland, 24-25 April 2007. The meeting focused on (i) achievements of PAAT mandated organizations (i.e. FAO, IAEA, WHO, AU-IBAR) and AU-PATTEC, (ii) implementation of AfDB-PATTEC supported T&T intervention in six sub-Saharan countries (Burkina Faso, Ghana, Mali in West Africa and Ethiopia, Kenya, Uganda in East Africa), (iii) new PAAT partnerships (IFAH, UNIDO, FAO/IGAD-LPI).

Mr Raffaele Mattioli, convenor of the meeting, introduced Mr Lorenzo Savioli, Director of the WHO Neglected Tropical Diseases (NTD) Unit, who warmly welcomed the participants to Geneva on behalf of WHO and officially opened the meeting.

Mr A.A. Ilemobade, PAAT Chairman, welcomed the participants. He reminded them of the constitution of PAAT in 1997 as an international alliance of mandated UN agencies, supported by donors, research institutes and tsetse-affected countries. Mr Ilemobade recalled the role of PAAT in formulating a plan of action whose ultimate goal is to alleviate human suffering, reduce poverty, improve food security and facilitate sustainable agricultural production in tsetse and trypanosomiasis (T&T) infested areas. Although the ultimate goal has not changed, the strategy must, and indeed, needs to be constantly reviewed. Among the major achievements made by PAAT are the establishment of structures to coordinate resources at national and international levels, the creation of an open access Information System and the production of a variety of guidelines concerning different aspects of T&T intervention and related sustainable agriculture and rural development. As regards the implementation of intervention projects, the PAAT Chairman welcomed the release by the African Development Bank (AfDB) of the first instalments of loans and grants to the six countries involved in the first phase of the PATTEC initiative: Burkina-Faso, Mali, Ghana, Kenya, Ethiopia and Uganda. The present achievements and planned activities of these projects are central to this meeting.

Apologies were received from Mr Peter Holmes and Mr Pere Simarro who could not attend the meeting.

The meeting was chaired by Prof. A. A. Ilemobade. FAO provided secretarial assistance.

Minutes of the previous meeting

The report and recommendations of the 10th PAAT-PC meeting were taken as read and, after further deliberation, adopted.

Outcomes of the 11th. PAAT-PC Meeting

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

FAO/PAAT – R.C. Mattioli

FAO/PAAT activities and progress on the implementation of recommendations since the 10th PAAT-PC meeting were presented.

As regards training and capacity building, several actions taken by PAAT were described. In particular, IAEA developed two e-learning modules relevant to the implementation of the SIT i.e. irradiation dosimetry and procedures for strain compatibility testing in tsetse flies. FAO, with the collaboration of IAEA, organized an Interactive Training Workshop (2-week workshop, November–December 2006) on harmonization of GIS-based decision support systems for T&T spatial targeting and on the harmonization of national information systems. Further information on this workshop, as well as on the recommendation to further expand the PAAT-IS resources, can be found below.

The use of GIS to facilitate decision-making was promoted through the publication by FAO, in partnership with DFID, of the book "Mapping the benefits: a new decision tool for T&T interventions" which links economic variables to a GIS spatial framework in order to provide new insights and reinforce the decision making process for intervention. Other activities concerned finalization of the paper "Standardizing land cover mapping for tsetse and trypanosomiasis decision making" and the initiation of the paper entitled "Global datasets for the management of the trypanosomiasis problem: an environmental approach", which reviews state-of-the-art global GIS datasets that can assist T&T decision-making.

The inclusion of policy issues related to trypanosomiasis control in the Greater Horn of Africa within the Inter-Governmental Authority on Development-Livestock Policy Initiative (IGAD-LPI) project activities was pursued at the first meeting of the National Technical Focal Points for IGAD LPI held in Djibouti, 24-29 March 2007. Also, FAO/PAAT and IGAD have agreed to cooperate in defining policy and actions against T&T in the IGAD LPI project area. In this regard a collaborative study will soon be initiated.

Inter-Agency (PAAT mandated organizations, IFAD and UNIDO) collaboration was been further strengthened through an FAO/UNIDO/IFAD/IAEA/IFAH project document that has been elaborated on Quality Control/Quality Assurance of trypanocides. FAO/PAAT has also developed a formal agreement of collaboration with the Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs) and African Livestock (ALIVE, a World Bank initiative). GF-TADs and ALIVE consider PAAT as the reference platform for providing assistance in the definition of appropriate policies and scientific and technical strategies for matters related to T&T.

Progress report from AU-IBAR – M. Traoré

The main recent activity at AU/IBAR was preparation of the 29th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), which will be held in Angola from 1-5 October 2007. A scientific committee was established to discuss the abstracts for presentation. Serious concerns for funding have arisen, mainly related to the high costs of air tickets and daily subsistence allowance (DSA) costs for Angola. Mr Traoré appealed to meeting participants to try and find ways of overcoming these difficulties.

Issues related to the need for training were brought to the attention of the participants. Compared with the 1970s and 1980s, when a critical mass of young technicians dealing with T&T was active, today there is lack of new, young personnel adequately trained and

knowledgeable to guarantee a turnover between generations. A suggestion was made to develop "introductory training modules" to attract technicians towards the problem of T&T.

With a view to improving the effectiveness of efforts to control T&T, a need for a substantial reorganization of the links between institutions at the heart of T&T work and a wish for more "synergistic" interactions were also expressed.

Progress report from PATTEC: further African countries benefiting from AfDB financial support – J. Kabayo

The Head of PATTEC Coordination Office, Mr Kabayo, reported on plans and progress in the implementation of the PATTEC initiative. A brief reminder was given regarding the main features of the PATTEC initiative (decision of the African Heads of State, principles of the "Plan of action", activities of the "PATTEC Coordination Office", nature of PATTEC projects, the reasons behind the creation of PATTEC and its objectives). Mr Kabayo emphasized that PATTEC aims at controlling tsetse using existing means, rather than developing new drugs or new technologies.

The current status and the roadmap for the activities of the PATTEC initiative were presented. With the support of the AfDB, the first phase of multi-national tsetse eradication projects has been started in Burkina Faso, Ghana and Mali in West Africa and in Ethiopia, Kenya and Uganda in East Africa. Activities in four countries in southern Africa (Angola, Botswana, Namibia and Zambia) have also started; Botswana and Namibia are now apparently fly-free, hence focus has shifted to Angola and Zambia, where SAT operations are planned to start in May/June 2007. Operations in these two countries will need to involve also the Democratic Republic of Congo (DRC), which is currently setting up a PATTEC office. South Africa, Mozambique and Zimbabwe are planning operations with the help of IAEA. A proposal to make the Southern Africa Development Community (SADC) a T&T – free zone is on the agenda of the next Summit of SADC countries. Between October 2007 and May 2008 implementation of projects should start in several other countries (these projects should tackle transboundary areas in Rwanda, Tanzania and Burundi, in Benin, Togo, Niger and Nigeria, in Chad, Central African Republic, Cameroon and Nigeria, in Sudan and Ethiopia and in Senegal, Mali and Guinea).

The outcomes of the Special Donors' Conference on PATTEC (Addis Ababa, 2 February 2007) were briefly reported. Significantly, pledges of more than US$ 320 million) came from affected countries rather than donors. The PATTEC Coordinator highlighted the possible support to PATTEC from partners, supervision and backstopping of project implementation, field research, experts consultation and resources mobilization.

Report from WHO – J. Jannin

WHO reported on sleeping sickness surveillance and control programme.

WHO provides support to affected countries in relation to capacity building (hands-on training for staff involved in day to day control activities), surveillance and control (supply of "Card Agglutination Test for Trypanosomiasis" (CATT) reagents, accessories and equipment for screening; free drug distribution for treatments: eflornithine, melarsoprol, pentamidine, suramin; logistics to reach people at risk). Emphasis was placed on collaborations between WHO and Sleeping Sickness National Control Programmes (SSNCP) in Uganda and Burkina

Faso. Other successful WHO collaborations were developed with the Centre International de Recherche-Développement sur l'Elevage en Zone Subhumide (CIRDES) and the Institut de Recherche pour le Développement (IRD-Research Institute for Development) in West Africa, with the University of Edinburgh for its activities in Uganda and with the Institute of Tropical Medicine (ITM), Antwerp on development of new diagnostic tools. The good relationship between WHO and PATTEC was acknowledged, but the need for a closer collaboration with national PATTEC representatives for the control of HAT was stressed.

Progress in dealing with the disease was reviewed. Only three countries report more than 1,000 cases a year (DRC, Angola and Sudan) and evidence suggests that the epidemic in DRC is finally under control. At continental level, three percent of cases are caused by *Trypanosoma brucei rhodesiense*, therefore the main effort must target *T. b. gambiense*.

Mr Jannin also reported on the activities of the Neglected Tropical Diseases (NTDs) Control group aimed at raising the awareness on NTDs. A new agreement signed in November 2006 with Sanofi-Aventis has allowed US$ 25 million to be mobilized for the control of trypanosomiasis and other diseases.

Report from IAEA – A. Robinson

The Agency contributes to international efforts against the T&T problem through three major mechanisms, (i) assistance to "normative" activities; (ii) research and methods development; and (iii) technical cooperation.

The Agency's standpoint and activities for tsetse area-wide integrated pest management (AW-IPM), under regular review as part of Tsetse: the Way Forward (TTWF), were reported. The guiding principle of the Agency's participation is the phased-conditional approach. One of the recommendations of a recent meeting under the Agency's TTWF process was the development of a paper outlining the principles for assessing the feasibility of creating tsetse-free zones. CDs on sampling locations for tsetse in relation to population genetics were distributed together with a GIS tutorial. A draft of the letter to the AU Commissioner is under debate highlighting the need for realistic objectives, implementation plans and budgets for AW-IPM tsetse programmes.

With regards to research and methods development, at the FAO/IAEA Laboratory at Seibersdorf and through coordinated research projects (CRPs), the increasing difficulty in obtaining isotopic radiation sources was mentioned. However new developments in X-ray radiation were mentioned together with the fact that the Agency will purchase an X-ray machine for evaluation at Seibersdorf. Recent data at Seibersdorf on UV irradiation have indicated that it may be an alternative for blood irradiation. The following summary on recently completed, ongoing and new CRPs was also presented:

- The CRP on tsetse genetics was completed and the results published in peer reviewed journals. The data on *G. pallidipes* revealed substantial substructuring in populations;
- The CRP on quality assurance related to tsetse rearing will hold its third meeting in Nairobi, Kenya, 7-11 May 2007;
- A CRP on tsetse symbionts and pathogens was initiated in 2007; and

- A new CRP on the integration of population genetics and GIS for livestock pests will probably be started in 2008.

With regards to technical cooperation, the Agency continued to contribute directly to addressing the objectives of the Pan-African Tsetse and Trypanosomiasis Eradication Campaign (AU-PATTEC) through the implementation of one regional, and nine national Technical Cooperation projects in Botswana, Burkina Faso, Ethiopia, Kenya, Mali, Senegal, South Africa, Uganda and the United Republic of Tanzania. The support was largely through provision of training to Member State personnel; expert services and equipment. Under the regional project, two regional training courses are in preparation in Uganda (tentative dates: 30 August-8 September 2007) on the principles of collecting and processing of tsetse flies for population genetic and morphometric analyses and in Senegal (tentative dates: 5-30 November 2007) on principles of entomological baseline data collection. It is also planned to hold a 3 day satellite workshop (13-15 September 2007) prior to the upcoming ISCTRC meeting in Luanda, Angola, on pre-SIT area-wide tsetse suppression.

PAAT Information System: progress and training activities – G. Cecchi

Mr Cecchi reported on the activities carried out since the last PAAT-PC meeting in the framework of the IFAD-funded project "Strengthening the Information System of the Programme Against African Trypanosomiasis".

The full revision of the PAAT web site has been completed and the gaps in information dissemination have been filled (GIS resources and metadata, manuals and papers). An off-line version of the web site was produced on CD-ROM. The integration of PAAT-IS with other web-based tools and resources (e.g. FAO GeoNetwork-FAO's Spatial Data and Information Portal) has been consolidated.

The draft paper "Standardizing land cover mapping for T&T decision making", which will be published in the PAAT Technical and Scientific Series, was presented in its finalized version. The paper provides methodologies and tools to assist T&T affected countries through the process of customization of readily available, high resolution land cover datasets (FAO-Africover project) for improved tsetse habitat mapping and trypanosomiasis decision-making. The paper also explores the relationship between multi-purpose land cover maps and tsetse habitat on different spatial scales.

Information was provided concerning a 2-week Interactive Training Workshop convened to support decision-making and information management in T&T intervention projects held at FAO Headquarters in Rome from 27 November-8 December 2006. This was attended by 20 participants, including representatives from affected countries, FAO staff and international experts. The Workshop dealt with the availability and utilization of global, national and local GIS datasets, data standardization and dissemination. The outcomes of the Workshop were highly valued by participants, who recommended continued support from PAAT in the fields of GIS, Information Systems management and decision making for T&T intervention.

Following the recommendations made at the Workshop, a draft paper was developed entitled "Spatial datasets for the management of the trypanosomiasis problem: an environmental approach". This provides a review of state-of-the-art geospatial datasets available in the public domain that can assist sounder T&T decision-making. A few examples of data utilization were also included.

Lastly, the aim, scope and preliminary results of an FAO-WHO collaboration for improved HAT data management and mapping were presented. It is believed that GIS and Data Base Management Systems (DBMS) can help to improve spatial targeting of interventions against HAT.

Veterinary trypanocides: the quest for essential similarity - J. Tettey

Mr Tettey, of the University of Strathclyde, UK remarked that the key principles for good drugs are: quality, safety and efficacy. He pointed out that 15 percent of all drugs on the market are fake, exceeding 50 percent in some areas of Africa and Asia. Major problems related to trypanocidal drugs concern low quantity of the active compound, inefficient molecules or commercialization of chemicals from unlicensed producers. The value of this market, in sub-Saharan Africa, exceeds US$ 35 billion/year with veterinary drugs far ahead of human drugs.

For trypanocides, no harmonized, updated monographs are available, the last one dates back to 1965, although 35 million doses are administered per annum (corresponding to US$ 35-40 million), with a significant increase in number of manufacturers since 1995. In some formulations, additional products are included, for example vitamins are added to diminazene aceturate. The results are that there is an inconsistency of chemicals on the market. It should be remembered that production of drugs involves combining components in exact proportions.

A pilot study concerning diminazene involved 11 countries, 102 samples (17 brands) from government, private veterinary clinics, pharmacies and markets. 65 percent of samples showed results that were outside the 5 percent tolerance limits. A similar study for another drug (isometamidium) gave equally negative results.

The most urgent actions to overcome these problems were identified as follows: definition of standard requirements for quality, safety and efficacy, definition of specifications for trypanocides, development of robust and reproducible methods of analysis, transfer of techniques to user laboratories in Africa, and quality control of user laboratory activity.

Update on FAO-IAEA-UNIDO-IFAH collaboration on quality control/quality assurance of trypanocides - R. Mattioli, D. Tezera, F. Van Gool

Although promising signs have recently been received from FAO, negotiations to officially formalize the collaboration (i.e. Memorandum of Understanding, MoU) between FAO and IFAH on Quality Control/Quality Assurance (QC/QA) of trypanocides are still ongoing within FAO. Despite the pending nature of the MoU, however, some activities supported by IFAH, on QC/QA of trypanocides have been carried out by Strathclyde University (see previous section) and the Joint FAO/IAEA Division.

A project proposal to assist African countries in obtaining standardized laboratory equipment for executing tests on QC/QA of trypanocidal drugs was jointly developed by FAO and UNIDO, with inputs from IFAD, IAEA and IFAH. The proposal was subsequently approved by UNIDO.

Mapping the benefits of T&T options in East Africa: a regional proposal – A. Shaw

As part of its contribution to the seven countries of the Inter-Governmental Authority on Development (IGAD)'s Livestock Policy Initiative, FAO's Pro-poor Livestock Policy Initiative (PPLPI) is committed to helping strengthen decision-making capacities and informing policy in a number of fields, including T&T control. In this context it is proposing to adapt the "Mapping the Benefits" approach from West Africa to the countries in the IGAD region most affected by trypanosomiasis.

It has estimated that some 16.5 million cattle, nearly 20 percent of the region's population, live in tsetse infested areas. The methodology involves defining and mapping cattle production systems, modelling their output and population growth in the absence and presence of trypanosomiasis and thus calculating the potential benefits from its removal. These are then applied to the cattle population in each system to allow the production of financial maps. Work has started on defining the production systems in the IGAD region.

Harmonizing methods for assessing socio-economic and environmental impacts of T&T control in context of PATTEC activities - J. Maitima

The major drawbacks in past environmental and socio-economic impact assessments in tsetse control projects were identified as follows: lack of consistency in approaches and methods among projects, great emphasis on impact analysis and little attention given to impact mitigation, lack of a framework for stakeholder involvement.

The peculiarities of PATTEC initiative (area-wide approach, regional projects, emphasis on rural development) call for harmonization of methodologies, which will result in the adoption of a common general framework and similar indicators. This should guarantee the same level of standards across regions and provide comparable results.

The draft document entitled "A methodological guide for assessing environmental and socio-economic impacts of tsetse and trypanosomiasis intervention" was presented. It contains tools for environmental, economic and social impact assessments, as well as methods for scenario analysis. For the environmental impact assessment, the levels of analysis concern individuals, populations, communities, ecosystems and landscapes. For the economic impact assessments the levels concern mainly the direct and indirect effects of the disease at the herd level, farm/household level, sector, national, and international levels, including the economic evaluation of environmental impacts of trypanosomiasis interventions. For the social impact assessments, beneficiaries and structure of societies are considered. With regard to the technical option analysis for T&T interventions, the following methods are reviewed: participatory approach, integrated quantitative modelling and computer simulation. The guidelines also describe how to select tools according to the available technical skills, financial resources and time to carry out the analysis.

Country policy workshops are planned in all the six countries involved in the first phase of the PATTEC initiative. Participants will include as many government officials as possible, other decision makers and the PATTEC Steering Committee. During the workshops policy briefs, framework paper, guidelines, interactive CDs and other relevant background documents will be presented and distributed.

Update on the project "Stamp out Sleeping Sickness in Uganda" – I. Maudlin

In central Uganda, HAT of the *rhodesiense* type has recently moved northwards round the Lake Kyoga, to Soroti district, mainly due to movement of cattle carrying human infective trypanosomes.

The project "Stamp out Sleeping Sickness" is a public-private partnership for control of zoonotic HAT in Uganda, with the contribution of Industri Kapital (VC), CEVA Santé Animale, COCTU (Coordinating Office for the Control of Trypanosomiasis in Uganda), WHO, Ministry of Health, Ministry of Animal Industries and Fisheries, University of Makerere (Veterinary School) and the University of Edinburgh–Centre for Tropical veterinary Medicine (CTVM). The project aims to eliminate the disease by breaking the transmission cycle. 220 000 cattle in a high risk zone were treated with trypanocides. Other measures taken were stopping market introduction of cattle, reinforcement of the government policy for point of sale and treatment, selected application of insecticide on cattle to avoid re-infection and, sensitization of the population.

The results of project activities indicate that the proportion of cattle with human infective trypanosomes has fallen from 20-25 percent to 3 percent. Insecticide spraying activities are still ongoing.

Progress of national strategies and strategic integrated approaches for tsetse and trypanosomiasis (T&T) intervention and related sustainable agriculture and rural development (SARD) in priority areas: country reports

Reports on countries benefiting from AfDB support for T&T intervention were presented by representatives of Burkina Faso, Ethiopia, Ghana, Kenya, Mali and Uganda.

Ethiopia – T. Alemu

After a brief introduction to show the geographical location of the project area, Mr Alemu updated the audience on the latest development concerning tsetse mass rearing facilities. The colony of *Glossina pallidipes* has been successfully established and its size has reached 110 000 units, with a growth in pupae production of 22 000/week. Adult fly mortality is below one percent. The colony is ready for the transition to mass rearing. An embryonic colony of *G. fuscipes fuscipes* has been established through shipment of flies from the Zoological Institute of the Slovak Academy of Sciences in Bratislava, Slovakia. The construction work of a new insectary is 98 percent complete.

The field operations of the STEP project, using a phased approach and existing conventional methods of tsetse suppression, is progressing around villages and livestock areas by means of targets (density 4/km^2) and cattle treatment (20 percent of livestock) with pour-on formulations. With regard to monitoring and reporting, standardized procedures were adopted; entomological monitoring is being conducted using 2 traps/km² around villages and livestock areas, while disease monitoring is conducted on 1 300 km^2. Active community involvement is being pursued through awareness-raising activities among village leaders, livestock owners, trained professionals, technicians and the general public.

The loan provided by AfDB (US$14.6M for a period of six years) and inserted in the on going STEP project will soon disburse an initial advance. The joint Ethiopian Government/FAO/IAEA project, funded by the Government of Japan (US$ 1.7M), through the United Nations Trust Fund for Human Security (UNTFHS), in support of STEP activities started. Initial supplies were received through the IAEA procurement procedure, while the

purchase of substantial field materials including vehicles was finalized through the FAO procurement procedures and consignments are expected in the next few weeks.

Kenya – P. Olet

Kenya targeted tsetse eradication from about 92 000 km^2. In the first phase an area of 24 000 km^2 will be covered, comprising the Lake Victoria basin, the Lake Bogoria region (North Rift) and the Meru/Mwea region (Central Kenya). After slight delays due to the disbursement of funds from AfDB, implementation started in the area of Lake Victoria.

Initial results in the area of the Ruma National Park are promising. Approximately 1,000 targets were deployed within the Park and the number of flies captured per trap per day (FTD) was reduced from 78.4 to 0.5 within 4 months using targets treated with 0.6 percent deltamethrin. Fly suppression activity is ongoing. To ensure that tsetse flies do not reinvade the park, livestock are being sprayed by communities outside the park. Material will also be provided to communities to make traps/targets for monitoring and control. Radio programmes to raise communities awareness will be on air from May 2007. Another achievement has been the commissioning of PATTEC offices; computers were purchased and networked, internet and wireless telephone services were made available and the Project Coordination and Management Unit (PCMU) is now fully functional. Drawing from the experience of previous projects carried out in the same area, concern was expressed with regard to the risk of reinvasion.

For the future, a scale-up in the installation of targets is planned and actions aimed at promoting improved livestock and crop agriculture (e.g. restocking in certain areas) are foreseen. To avoid reinfestation, plans for adjacent areas should be in place in phase two of the PATTEC initiative. A concept note for targeting the tsetse belt in the costal regions of the country is also ready.

Uganda – L. Semakula

The AfDB funded "Creation of sustainable T&T free areas in East and West Africa: the Uganda component" is foreseen to be executed in three phases. The area that will be targeted in the first phase is a vast crescent around the Lake Victoria. The project is implemented by the Ministry of Agriculture, Animal Industry and Fisheries and coordinated by COCTU with the support of the PCMU.

Major advancements were made in the procurement of equipment, with evaluation of tender documents completed by March 2007. Terms of reference for recruitment of consultancies for entomological, parasitological, socio-economic, environment and land use management baseline data collection were developed and submitted to AfDB for approval. Because of the nature of the HAT problem in Uganda, a medical expert was included in the project management team.

As regards the tsetse mass rearing, under financial year 2006/07 the Ugandan Government committed US$ 340,000 for expansion of the tsetse mass rearing seed colony facility at Tororo. Completion of the works is expected in May 2007. The facility will have a holding capacity of approximately 350 000 breeding females (actual colony size estimated at 12,000 units). The capacity of the mass rearing facility in Kaliti (Ethiopia) to supply *G. f. fuscipes* for the project in Uganda has not yet been clarified. Therefore, the Government of Uganda is making a request to the AfDB to use part of US$ 4.2 M available in the project for sourcing flies from Ethiopia to construct a medium tsetse mass rearing facility to complement

Kaliti's efforts. To this aim, job descriptions for critical technical staff (entomologist and laboratory technicians) required for the tsetse mass rearing facility have been made and recruitment will be done in July 2007.

In relation to the project implementation, the baseline data collection will be undertaken during the second half of 2007. Tsetse suppression using the live-bait technique in areas where cattle are available, as well as limited tsetse trapping are ongoing.

In order to address the problem of the possible merger between the two forms of HAT (*gambiense* and *rhodesiense*), Government developed a public-private partnership with CEVA Santé Animale Internationale Kapitale, Cooper Uganda Ltd. Makerere University and Centre for Tropical Veterinary Medicine, Edinburgh. More than 190 000 head of cattle, representing 86.4 percent of the total population in the interface districts of Kaberamaido, Dokolo, Amulata, Apac and Lira, were treated with isometamidium. In Dokolo and Kaberamaido treatment was given to 60 000 head, corresponding to 100 percent of the local cattle population. Diminazine treatment was used in the other three districts. As a result, the average prevalence of Animal African Trypanosomiasis (AAT) was reduced from 34 percent to 0,4 percent (0 percent in some areas) after the first treatment.

The major tsetse suppression method to be implemented in 2008 will be the Sequential Aerosol Technique (SAT) with deltamethrin. During the PATTEC Special Donors Conference in February, 2007, in Addis Ababa, the Government of Uganda made a commitment of US$ 3.0 M to be used for aerial spraying operations.

Burkina Faso - Issa Sidibe

The AfDB-funded project in Burkina Faso will target an area of approximately 96 000 km^2 across the Mouhoun and Bani river basins, which were subdivided into five intervention blocks. The foreseen duration of the project is seven years. The sequence of actions will be as follows: surveys, suppression and eradication, each taking one year.

Activities for the construction of a tsetse mass rearing facility are ongoing with the technical support of the IAEA.

The requirements for the baseline data collection were identified during a workshop held in October 2006. It is foreseen that the collection of parasitological, environmental, land use and socio-economic data would start in May-June 2007. In September it is scheduled to begin the entomological surveys. The project is also active in raising awareness among the project beneficiaries and the planning and management of reclaimed T&T free areas.

With respect to sleeping sickness, Burkina Faso is not considered to be a high risk area. However, the return of approximately 360 000 citizens of Burkina Faso who worked in the HAT endemic zones in Côte d'Ivoire caused worries about a possible surge in HAT cases in southern Burkina. Active surveillance carried out by IRD, CIRDES and PNLTHA (Programme National De Lutte contre la Trypanosomiase Humaine Africaine) did not confirm such worries, even though the situation needs further investigation.

Ghana - Charles Mahama

The Ghanaian component of the PATTEC initiative is financially supported by the AfDB and the Government of Ghana. Following the AfDB loan approval in December 2004, the first disbursement took place in April 2006 and the last one is foreseen for December 2011.

This first phase of the project will cover an area of approximately 20 000 km^2 in the Upper West Region of Ghana, which borders Burkina Faso. Consultants for the parasitological, entomological, socio-economic and integrated land cover/environmental baseline survey were recruited. Human resources and equipment needs for the insectary of the Ghana Atomic Energy Commission (GAEC) were assessed. A report concerning GIS and Spatial Epidemiology was produced for the establishment of a DBMS. Pesticides, drugs, traps and field equipment were supplied.

Capacity building was pursued through information campaigns and raising awareness in 75 communities and extensive training of senior and junior personnel. During a workshop on monitoring and evaluation tasks, responsibilities and a system for information flow among stakeholders were defined.

The major constraint identified in this initial phase of the project was the slowness of the procurement process. The following steps in the implementation of the project will be the execution of the baseline data collection and analysis (second half of 2007) and the initiation of suppression activities. Closer collaboration with Burkina Faso and Mali will be sought.

Fields in which support will be necessary were identified as follows: delineation of a realistic target area using the information generated from baseline studies; harmonization of land cover/land use classification, synchronization among bordering countries of suppression and eradication activities to prevent re-invasion, exploration of the desirability and feasibility of the application of SAT.

Mali - Aligui Djiteye

Mr Djiteye provided ample information regarding past T&T intervention projects in the country. The ongoing AfDB-funded project concerns an area of approximately 37 000 km^2, of which 15 500 km^2 are in the Niger river basin (peri-urban zone of Bamako) and 20 000 km^2 in the Bani river basin at the border with Burkina Faso. The project also receives financial support from the Government of Mali.

The baseline data collection and analysis should clarify the distribution and population dynamics of tsetse flies. Furthermore, studies on animal and human trypanosomiasis prevalence, socio-economic context, and environmental impact will be carried out. Utilization of remote sensing imagery to map land cover/land use are foreseen.

Farming communities' involvement has been actively pursued through the creation of tsetse and trypanosomiasis control groups at village level, regional information and sensitization meetings and community workshops. 455 people, approximately 5 per village, were trained in traps impregnation, installation and surveillance.

The option of using an SIT component in the implementation of the project is being explored. In this regard, collaboration with IAEA and CIRDES is already taking place.

General discussion

One of the major issues which emerged from the general discussion was the need for the six countries involved in the first phase of the PATTEC initiative to develop realistic and detailed workplans and budgets, which should address the delicate question of the timing of operations. In a broader perspective, the need to strengthen the managerial component of the projects was also stressed from different sides. The scientific background of most of the

project managerial staff calls for targeted capacity building actions aimed at endowing project coordinators with the tools necessary for managing such complex interventions.

The usefulness of stronger coordination and interaction among the six national projects was also widely acknowledged; in particular, overlaps and duplications should be avoided and technical cooperation reinforced. These actions will contribute to increased harmonization, cost savings and ultimately to more effective interventions.

Some concerns were expressed about the possibility of re-invasion after completion of the tsetse elimination interventions. Even though intervention areas were selected in an attempt to minimize the risk of re-invasion, actions aimed at monitoring and controlling reinvasion will have to be carefully planned and put in place.

Closing

Mr Ilemobade heartily thanked all participants for their contributions. Thereafter, he declared the meeting closed. Mr Mattioli reminded members that the next PAAT-PAG meeting will be held in Luanda, while the next PAAT-PC meeting will be in Vienna. He thanked Raquel Mercado, Maria Grazia Solari and Giuliano Cecchi for their role in making the meeting a success.

Recommendations

The following recommendations were discussed and agreed to:

Due to the large size of the AfDB funded projects currently implemented in six countries, there is an urgent need for better coordination of technical/operational aspects at national and regional levels. The meeting **recommends**:

- The strengthening of structures at regional and sub-regional levels both to facilitate coordination, especially in the timing of cross-border operations and to avoid duplication of efforts, for example, in commissioning baseline studies many of which could be undertaken using common terms of reference at regional levels.

Action: PATTEC, involved countries.

2. PAAT recognizes the importance of project managers having appropriate managerial skills to ensure the timely realization of project objectives. This would involve a judicious mix of topping up the management competencies of technical staff through appropriate training and of bringing in specialized management skills. The meeting **recommends**:

- That PATTEC give high priority to pursuing this urgent need for expert management inputs as the implementation phase of the projects takes off. PATTEC may seek the support of PAAT and other mandated agencies.

Action: PATTEC.

3. Considering that the PAAT Information System is currently not entirely exploited by PATTEC member countries and endorsing the recommendations made at the Interactive Training Workshop on GIS and Information system management (FAO-Headquarters, Rome, 26 November-8 December 2006), the meeting **recommends** that PATTEC and PATTEC countries:

- Make good use of PAAT-IS as tool to share and harmonize the information generated during project activities;
- Build a community of GIS and IS specialists dealing with T&T intervention. Appropriate skills should be identified and capacity building should be pursued; and
- Give existing, national GIS service centres additional training to handle T&T issues.

Actions: PATTEC, PAAT, member countries.

4. PAAT recognizes the need to address manpower requirements of T&T affected countries, especially at the operational level, for intervention. The meeting **recommends**:

- That PATTEC member countries identify and train a younger generation of personnel for T&T interventions.

Actions: PATTEC, AU-IBAR, PAAT and mandated organizations.

5. PAAT appreciates the contribution of IFAH and recognizes the need to curb the growing occurrence of fake and sub-standard trypanocidal drugs in the African market. It therefore, **recommends**:

- To develop standardized specifications provided by authorized bodies;
- To strengthen capacity of regulatory bodies to enforce adherence to specifications by suppliers; and
- To train and equip existing laboratories, on a regional basis, to conduct quality assurance tests according to specifications provided by authorized bodies and agreed upon by countries.

Actions: AU-IBAR, FAO, IFAH, UNIDO, IAEA.

6. PAAT takes note of the discussion and debate on options for the more appropriate techniques and best combinations of techniques for tsetse suppression and tsetse elimination in different agro-ecological settings and the lack of consensus on their use, despite the extensive scientific literature available. The meeting **recommends**:

- That the various entomological experts work towards achieving a consensus on which technique or combination of techniques is best adapted for which situation, defining their limitations and establishing clear entomological guidelines in a single document, ideally in the form of a paper in the PAAT Technical and Scientific Series.

Action: PAAT.

7. PAAT notes that the issue of re-invasion is still a major concern to all the PATTEC countries. The meeting **recommends**:

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

Action: PATTEC, AfDB-funding benefiting countries.

8. PAAT recognizes the efforts being made by all the AfDB-funded projects in the context of socio-economic data collection and impact analysis. It is **recommended**:

- That criteria and methods be harmonized across countries to facilitate comparisons and avoid duplication of efforts. It is suggested to discuss this matter again at the next PAAT-PAG meeting.

Action: ILRI, FAO.

ACTIONS TAKEN BY FAO/PAAT ON THE RECOMMENDATIONS OF THE 11th PAAT-PC MEETING

RECOMMENDATIONS	**ACTIONS TAKEN**
1 Due to the large size of the AfDB funded projects currently implemented in six countries, there is an urgent need for better coordination at national and regional level of technical/operational aspects. The meeting **recommends**: • To strengthen the structures at regional and sub-regional levels both to facilitate coordination, especially in the timing of cross-border operations and to avoid duplication of efforts, for example in commissioning baseline studies many of which could be undertaken using common terms of reference at regional levels.	**Action**: PATTEC, beneficiary countries involved in the implementation of AfDB projects. • FAO/PAAT participated in the "Regional meeting of National Coordinators", convened by IAEA in July 2007. The meeting acknowledged and recognized the role of PAAT and its mandated organizations in guiding and setting principles for T&T interventions and coordination at international level. • The meeting requested the assistance of PAAT in harmonizing database systems and training in data management and analysis. In this regard, the PAAT-IS is a major tool where information (including datasets) is created, harmonized and shared among partners, stakeholders and PAAT-IS users. Examples are the standardization of land cover mapping for T&T decision making (paper in press), the Global Datasets for African trypanosomiasis management where global geospatial GIS datasets are reviewed, criteria for selection of datasets described, including data access and applications within T&T intervention.

RECOMMENDATIONS	ACTIONS TAKEN
	FAO/PAAT initiated the FAO GeoNetwork for the PAAT community. The GeoNetwork allows creation of groups of users (networks) to facilitate GIS data sharing within the community.
2 Considering that the PAAT Information System is currently not entirely exploited by PATTEC member countries and endorsing the recommendations made at the Interactive Training Workshop on GIS and information system management (held at FAO Headquarters from 26 November-8 December 2006) the meeting **recommends** that PATTEC and PATTEC countries: • Make good use of PAAT-IS as tool to share and harmonize the information generated during project activities; • Build a community of GIS and IS specialists dealing with T&T intervention. Appropriate skills should be identified and capacity building should be pursued; • Provide existing national GIS service centres with additional training to handle T&T issues.	**Action**: PATTEC, beneficiary countries, PAAT. • FAO/PAAT created the group "PAAT Information System" which allows metadata and GIS datasets to be generated, edited and posted, and through FAO GeoNetwork to create groups of users to facilitate data sharing within the community (In this regard, a paper was published with the title "The role of FAO GeoNetwork in a multinational development programme: the case of PAAT", OSGeo Journal, English and French versions). • In March 2008, experts met at the FAO/IAEA Joint Division to elaborate a detailed programme for a GIS training course for tsetse control personnel. The foreseen period to hold the course is the last quarter of 2008. • A paper soon to be published in the PAAT Technical and Scientific Series deals with "GIS datasets and methods for an environmental approach to African trypanosomiasis".
3 The meeting recognizes the need to address manpower requirements of T&T affected countries, especially at the operational level, for intervention. The meeting **recommends**: • That PATTEC member countries identify and train a younger generation of personnel for T&T interventions.	**Action**: PATTEC, AU-IBAR, PAAT and mandated organizations. • The issue of training has been partially dealt with in Point 3 (i.e. GIS training course for tsetse control personnel). • Training has been provided to staff of the STEP within the framework of the Ethiopian Government IAEA/FAO joint project GCP/ETH/072/UNJ (funded by the UNTFHS/Japanese Government). • Staff involved in T&T planning and field operations are encouraged to make good

RECOMMENDATIONS	**ACTIONS TAKEN**
	use of training manuals produced by PAAT and its mandated organizations. These manuals (hard copies) have been made available to African colleagues. They are also available in electronic format and can be downloaded from the PAAT website. • FAO/IAEA Joint Division continues to provide regular training in tsetse mass rearing, SIT and related matters.
4 The meeting appreciates the contribution of IFAH and recognizes the need to curb the growing occurrence of fake and sub-standard trypanocidal drugs in the African market. The meeting **recommends**: • To develop standardized specifications provided by authorized bodies; • To strengthen capacity of regulatory bodies to enforce adherence to specifications by suppliers; • To train and equip existing laboratories, on a regional basis, to conduct quality assurance tests according to specifications provided by authorized bodies and agreed upon by countries.	**Action**: AU-IBAR, FAO, IFAH, UNIDO, IAEA. • Actions on this recommendation have been slowed pending the official signature of the MoU between FAO and IFAH on QC/QA of trypanocides. In April this year IFAH communicated to FAO the imminent signature of the MoU. • FAO committed itself to enlarge the MoU to anthelmintics, antibiotics, insecticides and acaricides. • FAO approached UEMOA to seek its involvement in the FAO-IFAH initiative and partnership. • Also, FAO and IFAH approached OIE to stimulate its interest in this initiative.
5 The meeting takes note of the discussion and debate on options for the most appropriate techniques and best combinations of techniques for tsetse suppression and tsetse elimination in different agro-ecological settings and the lack of consensus on their use, despite the extensive scientific literature available. The meeting **recommends**: • That the various entomological experts work towards achieving a consensus on which technique or combination of techniques is best adapted for which situation, defining their limitations and establishing clear entomological guidelines in a single document, ideally in the form of	**Action**: PAAT. • Some action has been undertaken in the past (e.g. a paper has been published in the PAAT Technical and Scientific Series "Integrating the sterile insect technique as a key component of area-wide tsetse and trypanosomiasis intervention"). • In June 2007, IAEA published a book entitled "Area-Wide Control of Insect Pests: from Research to Field Implementation", where various case studies related to tsetse control/elimination campaigns in different agro-ecological scenarios are reported. Lessons can be learned from the reported case studies and adapted to specific agro-ecological conditions.

RECOMMENDATIONS	ACTIONS TAKEN
paper in PAAT Technical and Scientific Series.	• The need remains to have a comprehensive (all inclusive), updated publication on entomological guidelines.
6 The meeting notes that the issue of re-invasion is still a major concern to all the PATTEC countries. The meeting **recommends**: • That the risk of re-invasion be comprehensively assessed (e.g. at the time of baseline entomological surveys) and that measures be put in place aimed at minimizing this risk in a sustainable manner.	**Action**: PATTEC, AfDB-funded benefiting countries. • The developed and agreed PAAT-PATTEC criteria and guidelines for the selection of priority areas for T&T intervention are robust tools to assist the countries in the selection of area(s) where the chances of success are highest and risk of tsetse re-invasion minimal. • The flow chart inherent to the phased conditional approach, developed by FAO/IAEA for T&T intervention is a further tool supporting the feasibility (and planning) of field T&T intervention with the aim of increasing the chance of success and minimizing the risk of failure, hence considering the risk of re-invasion.
7 The meeting recognizes the efforts being made by all the AfDB-funded projects in the context of socio-economic data collection and impact analysis. It is **recommended**: • That criteria and methods be harmonized across the countries to facilitate comparisons and avoid duplication of efforts. • It is suggested to discuss this matter again at the next PAAT-PAG meeting	**Action**: ILRI, FAO. • In-house FAO/PAAT-IGAD LPI collaboration on analysis and compilation of livelihood zones and production systems in the Horn of Africa. This collaboration involves socio-economic analysis of farming systems. The methodological approach could be adapted and adopted by other T&T affected countries. • In collaboration with colleagues of Botswana, Burkina Faso, Ghana, Uganda, CIRAD/CIRDES, FAO/PAAT is preparing and harmonizing global GIS datasets and methodologies for planning, managing and evaluating T&T interventions. Also this collaboration considers the socio-economic aspects of T&T interventions.

Other actions related to and/or supporting the PAAT-PC recommendations included:

Technical and scientific support to advance the planning process for intervention and support to planners, policy makers, researchers, and the technical and development community

Publications

- Two issues annually of the Tsetse and Trypanosomiasis Information (TTI) bulletin.
- PAAT-T&S papers in the pipeline:

  i. Linking sustainable agriculture and rural development strategies with sleeping sickness control (Cattand et. al., final stage);
  ii. PAAT brochure entitled "On Target Against Poverty – The Programme against African Trypanosomiasis 1997–2007 and the Millennium Development Goals of the United Nations (final stage);
  iii. Standardizing land cover mapping for T&T decision making (Cecchi et al., in press);
  iv. GIS datasets and methods for an environmental approach to African trypanosomiasis (Cecchi, Mattioli, in preparation);
  v. Global geospatial datasets for African trypanosomiasis management: a review (Cecchi, Mattioli with contributions of Mahama, Mugeny, Kgori, Motsu, Koudougou and Guerrini);
  vi. "Global datasets for the management of the T&T problem: an environmental approach" (Cecchi et al., final stage);
  vii. Creating, harmonizing and sharing the information: the role of PAAT and its Information Systems (Cecchi, Mattioli), presented at ISCTRC. Luanda, Angola, October 20O7;
  viii. Matching land cover and tsetse habitat (Cecchi et al.), presented at the GisVet Conference, Denmark, August. 2007;
  ix. The role of FAO GeoNetwork in a multinational development programme: the case of PAAT. *OSGeo Journal*, **2**: 20-24 (in English and French); and
  x. The PAAT Network: empowering African partners in the fight against African trypanosomiasis (Mattioli); Keynote address at ISCTRC, Luanda, Angola, Oct. 2007.

Projects

- IFAD funded project supporting the PAAT Information System, ended in July 07 and was officially closed in December 2007. A new project proposal entitled "Pro-poor integrated packages to enhance policy and decision making against the African animal disease burden in sub-Saharan Africa" has been submitted. The PRODOC passed all technical and financial screening and is awaiting the signature of IFAD's president for final approval;
- "Development of innovative site-specific integrated animal health packages for the rural poor" project proposal submitted in February 2008 to IFAD. The PRODOC was well received. We have been requested by IFAD to revise and finalize the PRODOC; and

- UNTFHS – Japanese Government funded project in the Southern Rift Valley to support and complement STEP activities on going.

Meetings and partnership initiatives

- FAO/PAAT participated in the annual NTTAT meeting, OIE Headquarters, Paris, May 2007. PAAT activities were presented;
- FAO/PAAT participated in the "Regional meeting of National Coordinators, IAEA Headquarters, Vienna, July 2007;
- FAO, IAEA and WHO convened the 13th PAG meeting at WHO Headquarters, Luanda, September 2007;
- FAO attended ISCTRC Executive Committee meeting and the ISCTRC Conference, Luanda, October 2007. Keynote address on networking and a paper on PAAT IS were presented;
- FAO participated in the Steering Committee and Technical Advisory Committee meetings of STEP–Ethiopia;
- FAO participated in the 3rd Meeting of the Regional Steering Committee of the FAO/OIE Global Framework for Transboundary Animal Diseases (GF-TAD) for Africa (FAO Rome, April 2008) and the 9th ALive Executive Committee meeting (FAO Rome, April 2008). FAO/PAAT activities were presented;
- On going FAO/WHO collaboration on mapping human African trypanosomiasis in sub-Saharan Africa;
- On going in-house PAAT/IGAD LPI collaboration on "Mapping the benefits of T&T removal in the IGAD region" and on analysis and mapping of livelihood production systems;
- FAO-IFAH partnership on QC/QA of trypanocides formally agreed;
- Collaboration with ITM (and possibly with CIRAD) on T&T epidemiological modelling and generating continental maps of disease risk levels (under discussion); and
- Collaboration with ITM for an assessment of the cost-effectiveness of community-based tsetse control operations and their impact on poverty alleviation and food security for small scale farmers.

NEWS FROM THE PATTEC FRONT

Each issue of TTI will in future contain a column (entitled "News from the PATTEC Front") describing progress in the process of implementing the objectives of the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). To set the scene, this first account, submitted by the PATTEC Coordination Office, describes the background and summarizes the events and activities that have punctuated the story of Africa's war against trypanosomiasis since the start of the PATTEC initiative.

Background

During the Summit held in Lomé, Togo in July 2000, African Heads of State and Government adopted a decision urging Member States to act collectively and embark on a Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) aimed at eradicating trypanosomiasis from Africa, in the shortest time possible. The decision by the African leaders to embark on the PATTEC initiative not only underscores the seriousness and significance which African Governments attach to the tsetse and trypanosomiasis problem, but also defines their readiness to assume primary responsibility in implementing the objectives of the initiative. The decision marked a significant departure from past practices, where the direct involvement in, and ownership of, tsetse and trypanosomiasis control activities by governments in the affected African countries was negligible. Within the framework of this historic decision, the Commission of the African Union was charged with the task of initiating and coordinating the activities of the campaign and is required to report to the Summit of the African leaders every year on the progress made.

The PATTEC initiative seeks to generate commitment, mobilize support and sustain the action necessary to effectively address the tsetse and trypanosomiasis problem. This will be accomplished through emphasizing the strategic importance of the ownership, leadership and direct involvement of African governments in mobilizing the human, organizational and financial resources required to initiate and sustain the necessary intervention programmes. The trans-boundary nature of tsetse infestation and trypanosomiasis prevalence imparts a multi-national character on the PATTEC initiative, which in turn calls for maximum inter-state co-operation and co-ordination for effective intervention action.

The PATTEC concept and Plan of Action

A Plan of Action, which outlines the approaches and methods by which the process of implementing the decision to eradicate trypanosomiasis is organized and executed, has been developed. It proposes tackling individual zones of tsetse fly infestation, applying principles of the area-wide approach and utilizing appropriate tsetse suppression methods, integrated to maximize their combined effect to achieve tsetse eradication. By successively and systematically tackling individual areas of tsetse infestation, while preventing re-invasion into treated areas, it will be possible to sequentially create an ever-expanding tsetse-free zone, and thus ultimately eliminate the disease. The PATTEC Plan of Action was endorsed and referred for implementation to relevant offices in the affected countries. Various development partners and stakeholders active in tsetse and trypanosomiasis control also adopted the Plan within the framework of their declared support for PATTEC. Many countries have now developed their national strategies and prepared proposals for the implementation of the

PATTEC initiative. Based on information on the level of mobilization to implement the objectives of PATTEC compiled by the PATTEC Coordination Office from the affected countries, the 37 countries affected by tsetse and trypanosomiasis can be categorized in four groups, viz:

- Two countries (Botswana and Namibia) that recently succeeded in achieving tsetse and trypanosomiasis-free status
- Ten countries, comprising Angola, Burkina Faso, Ghana, Guinea, Kenya, Ethiopia, Mali, Senegal, Uganda and Zambia where the execution of activities to eradicate tsetse has actually started.
- Fifteen countries, which have prepared plans or declared their intention to embark on tsetse eradication activities, namely: Benin, Burundi, Cameroon, Central African Republic, Chad, Gambia, Ghana, Malawi, Mozambique, Nigeria, Rwanda, South Africa, Sudan, Tanzania and Togo
- Ten countries where there are still no reported arrangements to initiate tsetse eradication activities, comprising Congo, Cote d'Ivoire, Democratic Republic of Congo, Equatorial Guinea, Gabon, Guinea Bissau, Liberia, Sierra Leone, Niger and Somalia. However, in some of these countries activities aimed at treating human African trypanosomiasis are being undertaken with the support of WHO.

The PATTEC Coordination Office

Within the framework of the decision by the African Heads of State and Government to embark on the PATTEC initiative, the Commission of the African Union was assigned the task of initiating and coordinating the activities of the campaign. In the context of this assignment, the Commission of the African Union established the PATTEC Coordination Office at the Commission Headquarters in Addis Ababa, to help in the initiation and coordination of activities to implement the PATTEC initiative.

The activities of the PATTEC Coordination Office include the translation of the PATTEC Plan of Action into definitive programmes of work, aiming to achieve tangible demonstrable results in a given time frame. While the individual affected African countries execute the actual intervention activities, the PATTEC Co-ordination Office serves to initiate, coordinate, harmonize, guide, support and sustain the activities of the PATTEC initiative. Through contact with affected countries, national and international organizations and other partners, the Office helps to bring together the political, financial and technical components of the tsetse and trypanosomiasis eradication campaign. The Office also takes a leadership role in the development of work programmes, preparation of project proposals, training of personnel involved in intervention activities, preparation and dissemination of publicity and public information on the PATTEC and designing proposals for exploiting tsetse-free land. The PATTEC Coordination Office maintains contact with country Focal Points in each of the affected countries and generally serves to remind affected countries and partners about their individual and collective obligations in the PATTEC initiative.

Consultations with stakeholders and partners

In the past 5 years, visits were made by the African Union Commission (PATTEC Coordination Office) to 32 of the 37 tsetse-affected countries for consultations with relevant government officials, national experts and other stakeholders aimed at generating commitment for the implementation of PATTEC and drumming up action. These visits routinely included meetings with the relevant key Ministers and other senior officials, notably those concerned with Livestock, Agriculture, Health, Finance, Foreign Affairs and Rural Development. In several cases the delegation from the Commission was granted audience with the President of the respective countries to discuss issues of PATTEC and sometimes also with the First Lady and other dignitaries in the country. Meetings with such high profile personalities were instrumental in creating awareness and generating commitment among decision makers and thus attracting attention to PATTEC, followed by support and facilitation for action.

Visits were also made to Africa's development partners, including the African Development Bank, BADEA, IFAD, USDA, USAID, World Bank and DFID; as well as the technical partners, including IAEA, WHO, FIND, FAO, ILRI, ICIPE, CIRDES and ITC for discussions on the plans and mechanisms for supporting African countries in the implementation of PATTEC.

Technical meetings, workshops and project development

The PATTEC Coordination Office has been involved in project identification, preparation and appraisal. A number of technical planning workshops were organized for groups of national experts and senior policy officials to discuss proposals for implementing PATTEC, develop modalities of cooperation in executing tsetse eradication projects in tsetse belts across national boundaries, discuss plans and strategies for project development and execution, analyse implementation protocols, appraise project proposals and review progress. Resulting from such work, a multi-national project proposal to create sustainable tsetse and trypanosomiasis-free areas in six countries located in East and West Africa was prepared and submitted to the African Development Fund with a request for funding. Other workshops and project proposals were prepared for a number of country groups, including: Ethiopia and Sudan; South Africa and Mozambique; Angola, Botswana, Namibia and Zambia; Burundi, Rwanda and Tanzania; Benin, Burkina Faso, Niger, Nigeria and Togo; Cameroon, Central African Republic, Chad and Nigeria; Malawi, Mozambique, Zambia and Zimbabwe. These project proposals are being used to mobilize resources and serve to define and evaluate the work and inputs required to eradicate trypanosomiasis in the identified project areas.

Resources mobilization

The PATTEC Coordination Office played a key role in mobilizing support from the African Development Bank, which provided support to six countries (Burkina Faso, Ethiopia, Ghana, Kenya, Mali and Uganda) worth US$ 70 million in soft loans and grants, in the first phase of the AfDB-supported PATTEC Programme. More support has also been secured from other partners, including US$ 90 000 from the WHO to support the preparation and dissemination of publicity and public information materials; US$ 250 000 from the USA State Department in the form of a contract to ILRI to support research in the development of procedures for identifying, avoiding and mitigating any negative impacts created by the process or

consequence of trypanosomiasis eradication; US$ 450 000 from the Leverhulme Trust in the form of a grant to a network of research scientists conducting morphometric analysis for assessment of the extent of isolation of tsetse populations and investigating optimal methods for tsetse suppression in different tsetse habitats; the International Atomic Energy Agency, which has recently pledged over US$ 2 million per year to support African countries in their efforts to implement PATTEC; the WHO which has successfully mobilized over US$ 25 million from the private sector to provide free trypanocidal drugs and diagnostic tools for sleeping sickness. In addition, the PATTEC Coordination Office signed a MoU with the Latin American Network for Research and Control of Triatominae (ECLAT) concerning the establishment and management of a joint Trypanosomiasis Vector Control and Research (TVRC) Foundation, a charity registered in the USA as a 501(c) (3) charitable foundation designed to seek funds in support of trypanosomiasis research and control activities. Also, a donors' conference jointly organized by the AU Commission and the African Development Bank, which was held on 2 February 2007 and attended by over 250 people from 39 countries and realized over US$ 350 million in pledges and expressed commitment, and within the framework of the MoU signed between the AU Commission and the Foundation for Innovative New Diagnostics (FIND), the PATTEC Coordination Office will receive support worth US$ 850,000 over the next three years to advance advocacy work on trypanosomiasis eradication.

Publicity and public information materials

The PATTEC Coordination Office has prepared publicity and public information leaflets and newsletters, brochures, T-shirts, caps, posters and pamphlets, whose value in explaining and advancing the causes and purposes of the PATTEC initiative is well appreciated. The Office has also produced a uniform, which was endorsed by the ISTRC in September 2005 for use by field personnel in PATTEC projects all over Africa to signify the unity and mentality of fighting the same war in all countries.

Conclusion

The process of implementing PATTEC has started in several countries and is gathering speed. A previously neglected disease is slowly becoming one that is currently attracting attention and receiving support. Resources mobilized from Africa's development partners or provided by affected countries have enabled a number of countries to initiate action, and two countries, Botswana and Namibia have recently been rendered tsetse and trypanosomiasis-free. The Presidents of the two countries were each presented with the newly introduced "African Union Trophy of the Last Tsetse Fly" during the African Union Summit held in Addis Ababa in January 2007. PATTEC now provides a good example of the functional cooperation between African countries to solve a common problem within the framework of the African Union. The war against trypanosomiasis has begun; and every six months, throughout the years to come, "News from the PATTEC Front" will provide information about the progress in this war. So, watch this space!!!

REPORTS AND RECOMMENDATIONS FROM THE 29th MEETING OF THE INTERNATIONAL SCIENTIFIC COUNCIL FOR TRYPANOSOMIASIS RESEARCH AND CONTROL (ISCTRC), LUANDA, ANGOLA 1-5 OCTOBER 2007

Reports from International and Regional Organizations

Food and Agriculture Organization of the United Nations (FAO)

This report was published in Volume 30 Part 2 of TTI, pp. 16-18.

African Development Bank (AfDB)

The AfDB recognizes trypanosomiasis as a major debilitating disease affecting both humans and animals. Trypanosomiasis also impairs socio-economic development and livelihood of entire rural communities, limits land use and, hence having a negative impact on agricultural production and frustrating efforts to attain food security and reducing rural poverty. The social and economic consequences of the disease are enormous. FAO reports indicate that if trypanosomiasis were to be eradicated, 40 percent of the population in sub-Saharan Africa would benefit and 55,000 deaths per year from sleeping sickness would be avoided.

The AfDB decided to provide assistance to the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). In this initial phase, the Bank supports six sub-Saharan countries, i.e. Burkina Faso, Ghana, Mali in West Africa and Ethiopia, Kenya, Uganda in East Africa. Funds provided amount to approximately US$ 70 million in soft loans and grants and are intended to create tsetse and trypanosomiasis free areas in well demarcated parts of these six countries. The success of this initial phase will greatly enhance the plans and efforts of African countries aimed at improving human and livestock health, eradicating poverty and increasing food security through improved agricultural and livestock production.

PATTEC programme would require the sum of US$ 3 billion within the next 15 years. In line with PATTEC's road map, to accomplish the task of T&T eradication on the African continent, countries are grouped to ensure that the attack will be efficient and sustainable. Based on this principle, the next set of countries proposed includes Angola, Zambia, Tanzania, Rwanda, Burundi, Benin, Chad, Democratic Republic of Congo, Niger, Nigeria and Togo. The estimated cost is projected to be about US$ 467 million. The Bank started the preparation of multinational project to assist the above countries. At the jointly organized AfDB/AU donors' conference, held in Addis Ababa, February 2007, AfDB showed the intention to further support PATTEC by pledging to commit US$ 75 million under the upcoming ADF XI replenishment. The sum of US$ 392 million constitutes the financial gap in the next five years to which PATTEC will require the support of African member countries and the international community.

The Bank renews its commitment to combat the disease to enable the people to improve their livelihood. However, this noble objective will require the concerted efforts and commitment of member countries, international organizations, donors, research institutions, all stakeholders and the benefiting rural communities.

International Atomic Energy Agency (IAEA)

The IAEA has continued to provide assistance to African Member States in the peaceful use of nuclear and related techniques for the reduction of poverty and the enhancement of sustainable rural and agricultural development. Of particular importance has been the further development and transfer of the sterile insect technique (SIT) as an additional control tactic for the creation of tsetse-free zones as part of an area-wide integrated pest management (AW-IPM) approach.

The IAEA concurs with other partners that the T&T problem is one of the major root causes of rural poverty in sub-Saharan Africa. In this regard, the Agency provides support to the implementation of the PATTEC Plan of Action through one regional and ten national technical cooperation projects in Botswana, Burkina Faso, Ethiopia, Kenya, Mali, Senegal, south Africa, Uganda, the United Republic of Tanzania and Zimbabwe. Under these projects, the IAEA implemented technology transfer to the Member States in the areas of feasibility assessment, capacity building and pre-operational support on the SIT for tsetse, through the provision of training, expert services and equipment. In collaboration with FAO and WHO, IAEA has continued to offer coordinated assistance to the six countries, Burkina Faso, Ethiopia, Ghana, Kenya, Mali and Uganda.

The Agency believes that AW-IPM approach is the best strategy that can lead to sustainable tsetse-free areas, but it is by no means a guarantee for success. IAEA, in collaboration with other partners, has developed a set of pre-requisite conditions for successful AW-IPM T&T interventions. In addition, IAEA is not promoting the use of SIT in all tsetse projects and in all circumstances. SIT is just one of several techniques that should be used only in areas where it has comparative advantages and where other techniques alone can not lead to complete elimination of targeted tsetse population(s).

The IAEA recognizes that the creation of tsetse-free zones will require a concerted action by many partners over many years, including appropriate policy, institutional and technological interventions and substantial human resources and financial commitments. A phased conditional approach, whereby support to a next phase will be subject to achieving pre-agreed milestones in the previous years is essential. The Agency also puts high importance on the Member States' ownership and that the development of an overall road map for the creation of tsetse-free zones is formally a matter of the Member States. Respecting this, the IAEA is placing more and more emphasis on the principle of national and regional ownership and international harmonization, solidarity and prioritization. In doing this, the IAEA will continue to provide support that lies within its mandate and therefore limited to aspects that are relevant to the "SIT- packages" i.e. SIT has to be applied in the context of multidisciplinary efforts aiming at sustainable agriculture and rural development. Therefore, enhanced partnerships with other UN agencies and other organizations, institutions and stakeholders will be essential to attain the overall objective.

The IAEA will continue to provide support to the PATTEC initiative for the concerned Member States. In this respect, several activities have been implemented and range from the production of normative and technical guidelines, provision of training and human resource development, funding Coordinated Research Projects (CRPs) and Technical Research Contracts, development and production of manuals, models, databases and textbooks to assist and facilitate the programming, the implementation and the day-to-day decision making in tsetse intervention programmes.

The Agency will continue to assist its Member States in enhancing the effectiveness of their activities by strengthening not only their human resources and infrastructures, but also leveraging financial resources required to intervene against T&T with the aim at developing an environment conducive for improved human well-being through sustainable agriculture and rural development.

World Health Organization (WHO)

During the period 1997-2006, the *gambiense* form of the disease, which represents 97 percent of the total sleeping sickness cases reported at continental level, declined due to intensified control activities on the human reservoir. The role of an animal reservoir is minor in the transmission process of this disease form. A decrease was also observed in the number of new cases. Conversely, intervention activities, supported by WHO, of the *rhodesiense* form (accounting for 3 percent of total cases) and focusing mainly on the human reservoir were insufficient to control the disease. Therefore, in countries endemic for *T. b. rhodesiense* infections, an integrated human and animal disease, and vector control approach should be adopted and should rely on close collaboration between human and veterinary health services and include an entomological component to ensure successful control of the *rhodesiense* form.

The worrisome scenario described during the World Health Assembly in 1997 has been dramatically improved. In addition to the political will at the highest level, capacities for surveillance and control in the endemic countries have been strengthened through capacity building based on training by "learning by doing" of staff involved and provision of equipment for screening, diagnosis and treatment, facilitated by WHO financial and technical support. WHO also secured production and distribution of drugs through public-private sector partnerships. These actions have resulted in 69 percent reduction of cases in the last ten years. Unfortunately, with low number of cases detected, there is a shift in country priorities leading to reduced attention to sleeping sickness. A similar situation occurred 50 years ago with a subsequent resurgence of sleeping sickness cases. To avoid this experience, a cost-effective sustainable sleeping sickness surveillance and control is the challenge of the immediate future. This sustainability can only be achieved through an integration of surveillance and control activities within reinforced health systems. The approach should foresee specialized teams and health systems working together. Health staff should be sensitized and trained to be able to integrate sleeping sickness surveillance and control in the day-to-day activities and supported by teams of specialists/experts in monitoring and evaluation to ensure performance quality.

Technical bottlenecks for the implementation of the above strategy are the availability of a sensitive and specific diagnostic test, cheap and easy to perform under field conditions and to be used at any level of the health system, and a new oral drug, cheap, safe and easy to administer, able to cure both stages of the disease.

During an informal consultation (May 2007), endemic countries concluded that (i) the elimination of the disease is possible, (ii) the participation of the national health system is necessary to sustain surveillance and control, (iii) the development of new diagnostic tools together with simple and adapted drugs is crucial to guarantee the effective participation of health structures, (iv) the maintenance of a specialized national central structure is required for coordination and overall necessary technical assistance, (v) the support of WHO to

endemic countries must be maintained for the implementation of the different measures required to achieve the objective of the sustainable elimination of the sleeping sickness public health problem.

In order to pursue the decline of the number of sleeping sickness cases, effective surveillance and control will continue to require appropriate human resources, adequate control activities, effective reporting, awareness, advocacy for priority ranking and for fund raising. Research and Development must be maintained and priorities must be driven to provide adequate tools for a sustainable elimination of the disease. In this regard, WHO is ready to take up the challenge and continue to lead country support and coordinate the work of all the involved actors.

Centre International de Recherche-Développement Sur L'Élevage en Zone Sub-Humide (CIRDES)

Several projects, funded by various donors (e.g. UEMOA, CORAF/AfDB, BMZ, the Wellcome Trust) have been implemented since the last ISCTRC. The Centre is in the process to strengthen its capacities in biotechnology techniques (molecular genetics, diagnostic tools), human resource development on trypanosomiases and their vectors, technology transfer and information dissemination.

Research activities focus on tsetse and trypanosomiasis, ticks and cowdriosis, disease epidemiology, evaluation of disease risk factors, disease/vector ecology, integrated disease control (also in peri-urban areas), genetic markers for trypanosomiasis resistance/susceptibility in animals. In addition to animal trypanosomiasis, sleeping sickness (the human form of the disease) is also among the research themes of CIRDES. Most of the research actions are carried out in partnership in the West African region. Major CIRDES partners are IRD/CIRAD France, WHO and LTTRN. The expansion of partnerships has allowed the Centre to increase its critical mass in terms of scientific personnel and to diversify, reinforce and complement research activities. It is worth mentioning that the research teams of CIRDES provide also a constant support to disease control field-based actions in the West African sub-region and to the AfDB-PATTEC funded project in Burkina Faso.

Human resource development and training focused mainly on trypanosomiasis and their vectors. Most of the trainees were from the West African region. The training activities benefited from the support of IAEA. The foreseen collaborations of CIRDES with IRD France, ITM Belgium, Bill and Melinda Gates Foundation, EU and Belgian Development Cooperation will provide further support to develop sustainable regional capacity.

International Centre of Insect Physiology and Ecology (ICIPE)

ICIPE's mission is to improve the well being of the peoples of the tropics through research and capacity building in insect science and its application. This is done by addressing the interlinked problems of poverty, low agricultural productivity, poor health and degradation of the environment. The Centre tackles these issues through its operative Research and Development paradigm, addressing human, animal, plant and environmental health, by integrated pest and vector management (IPVM). Another important constraint is capacity,

both human and institutional, to solve developmental problems. Therefore, each of ICIPE's projects includes an important capacity building component.

The Animal Health Division's goal is to improve livestock health and productivity. A full research continuum, from strategic to adaptive research and finally to technology development and transfer through strategic partnerships is pursued. Research and experience in tsetse and ticks have generated technologies, which enables farmers to undertake better ecological management of major disease vectors, help in intensifying and diversifying small holders farming systems to generate more cash income and enhance food security. Emphasis has been on developing environmentally safe methods. In tsetse, the Centre has considerable experience in community mobilization, empowerment and organization for undertaking T&T control in different agro-ecosystems and animal husbandry practices. Capacity building at all levels of society is an integral part of all Division activities. Several post-graduate student, technicians and farmers have been trained in research and control from different African countries in order to ensure sustainability of vector control activities. A key element in ICIPE's strategy will continue to be to develop, introduce and adapt new tools and strategies for arthropod management that are environmentally safe, affordable, appropriate, socially acceptable and applicable by the targeted end users with full community participation.

The Centre's accomplishments in capacity building are well known. ICIPE has trained 200 PhD scientists and 150 MSc students through the African Regional post-graduate Programme (ARPIS) in insect science in collaboration with 32 African universities. Over 15,000 farmers and some 2,000 extension workers have been trained in IPVM.

In order to ensure sustainability of vector control, the Animal Health Division continues to undertake capacity and capability building at all levels including service providers, practitioners, technicians, community health workers and managers of control operations. The Division helps communities in the establishment of organizational, management and financial community structures.

As the only international institute working primarily on arthropods, ICIPE has the comparative advantage in addressing the complex cross-cutting challenges of tsetse and trypanosomiasis. The Centre will continue to develop new and strategic partnerships for managing the enormous vector and disease burden that Africa bears.

UNICEF–UNDP–WORLD BANK–WHO: Special Programme for Research and Training in Tropical Diseases (TDR)

The TDR mission is "an effective global research effort on infectious diseases of poverty in which disease endemic countries play a pivotal role". Human African Trypanosomiasis (HAT) is among the diseases considered by TDR as major cause of poverty in sub-Saharan Africa.

Major research work on African trypanosomiasis should focus on developing new and improved tools for treatment and diagnosis of Human African Trypanosomiasis (HAT), providing new knowledge, tools and strategies for vector control, and strengthening capacity of African scientists and institutions to address country research priorities.

Research on new and improved drugs relates mainly in optimising use of available drugs (i.e. eflornithine-niflurtimox for late stage and pentamidine for early stage of

gambiense infections). Concerning diagnosis, novel and improved diagnostic tools should be developed and bio-banks of well characterized specimens should be established to facilitate assay development efforts. TDR is about to prepare reference standards (set of rules) for the evaluation of HAT diagnostics. Also, it is foreseen to commission a systematic review of HAT diagnostic research.

In vector research, TDR promotes the development and testing of novel methods for improving HAT vector mass-trapping system, and to support the generation and exploitation of *Glossina* genome sequence data. Expected outputs that relate to this research theme range from improved odour baits and odour release system, improved large scale tsetse mass trapping methods and publication of tsetse genome sequence data.

Training objective focuses on empowering scientists from disease endemic countries and provide them opportunities for Masters, PhDs, grants for new graduates, organizations of short courses and seminars through strengthening HAT consortium. The training activities should lead to increased leadership skills to, inter alia, plan and manage research, apply research best practices, negotiate role in partnerships, apply research results into policy.

TDR will continue to work along its three main objectives: stewardship, empowerment and research on neglected disease priority needs, with the ultimate goal of empowering scientists from disease endemic countries through capacity strengthening.

International Livestock Research Institute (ILRI)

ILRI's mandate is to reduce poverty and make sustainable development possible trough livestock-related research. Four main thematic areas are addressed: (i) targeting research and development activities; (ii) enhancing access to market opportunities; (iii) securing assets through biotechnology; and (iv) livestock systems (people, livestock and the environment). Main partners in the research programme implementation are national, regional and international institutions. Collaborations with civil society, NGOs and private sector have also been established.

Research on trypanosomiasis concerns mainly molecular genetics and breeding for trypanotolerance, managing trypanocide resistance, socio-economics and environmental monitoring of tsetse and trypanosomiasis control programmes, and sustainable land management in tsetse-freed areas. The trypanosomiasis research package is carried out mainly in the East and West African regions. Research on environmental and socio-economic impact assessment has generated a framework and guidelines for assessing the impact of T &T interventions. This work was done in collaboration with AfDB-PATTEC funded projects. The framework and guidelines are now being field-tested in Kenya with the support of Kenya Government. Additional financial contributions are expected from the National Institute of Health (NIH), Michigan State University to identify linkages between climate, land use, land cover, socio-demographic factors and tsetse distribution. The UNEP/GEF financial assistance will support research to identify and apply best practices to sustain land and forest productivity in tsetse-freed areas.

Twenty years of work in the formerly tsetse-infested Ghibe valley in the Southwest of Ethiopia have recently been transformed into community-driven livestock disease control via the successful formation of animal health "cooperatives". The farmer-to-farmer knowledge transfer is now paving the way for community-based schemes for livestock disease control.

Eastern Africa Network for Trypanosomiasis (EANETT)

EANETT is a network comprising of institutions in five countries (Sudan, Uganda, Kenya, Tanzania, Malawi) undertaking research and control of tsetse and HAT. The network was inaugurated in 2000 and is supported by the Swiss Development Cooperation. Three main research priority areas have been identified: (i) determination of prevalence, extent of spread and risk of overlap of *T. b. rhodesiense* and *T. b. gambiense* in selected areas of Eastern Africa; (ii) isolation and characterization of melarsoprol refractory *T. b. gambiense* and investigation of host and parasite factors involved in relapses; (iii) transmission studies to assess the risk of spread of *T. b. rhodesiense* and *T. b. gambiense* sleeping sickness foci in Eastern Africa by *Glossina* spp.

Capacity building, through training, information and provision of technical equipment is also among the activities of EANETT. Particular attention is devoted to improve laboratory facilities for research and diagnosis. Human resource development focuses on transfer of technology and promotion of MSc and PhD programmes. The Network has also organized technical workshops and scientific conferences in East Africa. Links and collaborations have been established with WHO, PAAT, MSF/DNDi, ITM in Belgium, Yale University in USA and PATTEC.

Organisation de Coordination pour la Lutte Contre les Endémies en Afrique (OCEAC)

In line with the WHO principle of the elimination of sleeping sickness, the OCEAC has established the sub-regional programme against HAT in Central Africa. This Programme, under the OCEAC coordination, comprises all relevant actors and stakeholders involved in fighting HAT in the sub-region. The strategy for the elimination of sleeping sickness based on four pillars: (i) strengthening of surveillance activity in 26 known sub-regional foci, with particular attention to the transboundary foci; (ii) the establishment of a network of passive surveillance, with the support of 26 health agents trained in disease diagnosis and treatment; (iii) anti-vectorial intervention in suspected residual hot-spots; and (iv) the development of operational research aiming at make available to the operators simple diagnostic methods and treatment schemes adapted to field conditions. These four pillars of activity are spread over eight years. The final objective of sleeping sickness elimination in Central Africa is foreseen in 2014-2015.

Foundation for Innovative New Diagnostics (FIND)

Control of HAT relies on diagnosis and treatment of infected individuals. At present, none of the diagnostic tests in use for sleeping sickness has been produced commercially. Available tests suffer from inadequate sensitivity and specificity. Therefore, there is a need to develop diagnostic tools that are accurate and applicable in endemic regions.

FIND is an independent Swiss Foundation dedicated to the development of diagnostic tests for poverty related infectious diseases of public health importance. The FIND tuberculosis programme has been used as a model to develop a rigorous and systematic approach to the needs-driven development, evaluation and demonstration of diagnostic technologies. This is done in partnership with academic and research institutes, and with biotechnology companies in the private sector to ensure eventual access to affordable tools in the public sector of developing countries.

HAT diagnostic is a vertical programme within FIND, alongside other disease-specific diagnostic development projects. The HAT programme benefits from active scouting of technologies and the development of technology platforms that can serve multiple diseases. Since its launch in early 2006, with a grant of US$ 9.8 million from the Bill and Melinda Gates Foundation, the HAT programme, executed jointly with WHO, has established links with industry, academic and research institutes in developed and endemic countries.

FIND is currently screening a large number of antigens obtained from laboratories around the world to develop a sufficiently sensitive and specific serological test which should guide treatment without the need for a confirmatory test. For molecular diagnosis, a highly sensitive, specific, simple and potentially cheap molecular test, based on isothermal amplification for DNA, is expected to complete its feasibility very soon. In addition, FIND has invested in the development of a more accurate method for staging HAT and follow up treatment. A number of projects have been initiated, which range from improvement of existing tests, marker validation, to discovery research.

With the concerted efforts in T & T control that are evident today, elimination of sleeping sickness is a distinct possibility.

Recommendations

The meeting:

1. **Welcomes** the ongoing efforts, activities and achievements of the international organizations and institutions in support of national projects/actions and PATTEC initiative in tackling the tsetse and trypanosomiasis problem (T&T) in sub-Saharan Africa;
2. **Notes** that the problem posed by T & T is vast and complex and that the complexity derives from its medical, veterinary, agricultural and rural development dimensions;
3. **Acknowledges** the existence of criteria and guiding principles for the selection of priority areas for T & T interventions in the context of Sustainable Agriculture and Rural Development (SARD) and human health;
4. **Acknowledges** the research efforts made to support T & T programmes in disease endemic countries and to strengthen capacity to undertake appropriate research;
5. **Notes** that to eliminate the disease, there is critical need for country commitment in order to mobilize all available resources and to establish effective transboundary integration;
6. **Notes** the need for a phased-conditional approach in the implementation of area-wide integrated pest management (AW-IPM) (i.e. moving to the next phase will only happen when achievement have been made in the previous phase);
7. **Notes** that various prerequisites have been identified for the successful planning and implementation of tsetse AW-IPM (i.e. availability of accurate, recent baseline data; quality assurance of the sterile males, in case SIT is incorporated; autonomous and independent management structures; adequate funding, resources and expertise;

continuity in the implementation of all project components; independent programme review; and commitment of all stakeholders.

Recommends that:

1. The policy and the strategy implemented be comprehensive and beyond the entomological and parasitological aspects. It needs to be oriented towards:
    - Food security and poverty alleviation;
    - The conservation and protection of the environment; and
    - Capacity building and institutional strengthening for enhanced decision making capacity.
2. Efforts and activities of international organizations should be directed to the harmonization of research strategies, for production of tools for T & T field programmes for policy makers and advisors, planners, scientific and technical staff.
3. Efforts should be made by countries with endemic Human African Trypanosomiasis (HAT) to involve as much as possible the health systems (in particular primary health care system) in the surveillance, control and appropriate research for HAT. Sleeping sickness national programmes or specialized bodies at national level should be maintained to monitor and provide appropriate support to the integration process.
4. An integrated vector, human and animal disease control approach should be adopted relying not only on human health but also setting a multisectoral approach including veterinary and entomological services. Coordination between these different services is needed to ensure successful control of both forms (*gambiense* and *rhodesiense*) of the disease.
5. Efforts should be made to strengthen research capacity in T & T through training and empowering the disease endemic countries (DECs) to lead and direct research needs in countries.
6. Projects use the document "Assessing the feasibility of implementing AW-IPM" to clearly assess the status of project implementation and to assess which next steps to take.
7. Projects ensure that the prerequisites identified for the successful planning and implementation of tsetse AW-IPM are in place before embarking with the operational phase of the project.

Recommendation on PAAT

The ISCTRC notes with appreciation the continued contribution of PAAT and its organs in moving forward the fight against T & T through the provision of guidelines for assessing the feasibility of creating T & T free zones, vital information and decision-making tools, publications, access to PAAT information system and harmonization of GIS-based decision support.

Council urges PAAT to continue to support, in increasing measures, T & T interventions in the spirit of increased agricultural production, poverty reduction and sustainable rural development.

PATTEC and Country Reports

Moderator: T.K Phillemon-Motsu

Rapporteur: C.I. Mahama

The session on country reports was preceded by a keynote presentation on the "Pan African Tsetse and Trypanosomiasis Eradication Campaign" (PATTEC). The presenter, Dr John Kabayo, who is also the Coordinator of the campaign, expressed satisfaction at the level of commitment and dedication tsetse-infested countries have demonstrated in the fight against tsetse and trypanosomiasis. There was, in his view, widespread support for PATTEC and that this was evident in the plans that are currently being put in place by affected countries for tsetse eradication. Dr Kabayo reminded participants that the declaration made by African Heads of State and Government in Lomé in 2000, to eradicate tsetse and trypanosomiasis from the continent, was based on the realization that the economic and social losses caused by the disease are colossal and that there can no longer be any justification for prolonging the suffering of the majority of Africans. He reminded participants that past control efforts failed due to their uncoordinated nature and also to the fact that they were not sustained. The new approach supported by PATTEC therefore, is the systematic removal of tsetse on an Area-wide basis and in a sustained manner. Where a group of countries have identified a common zone of intervention, tsetse and trypanosomiasis eradication projects can be jointly executed. Countries in East Africa (Ethiopia, Kenya and Uganda) and West Africa (Mali, Burkina Faso and Ghana) received funding from the African Development Fund to support the creation of tsetse-free zones in those countries.

Dr Kabayo informed the meeting that other countries in West, Central and Southern Africa are in the process of developing projects for the creation of tsetse-free zones. The main responsibility of the PATTEC Coordination Office, based at the AU Commission in Addis Ababa, is to drum-up action and assist to mobilize resources for the sustenance of the activities of the campaign and to continually remind African countries of their individual and collective obligations to the objectives of the campaign. He informed the meeting that PATTEC has high profile recognition, to the extent that a report on the status of the campaign is submitted to African Heads of State and Government at their annual summits. The presenter urged all affected countries to include T & T in their national development priorities and called on scientists to give support to the campaign through the provision of technical and scientific advice that would facilitate the removal of tsetse and trypanosomiasis from the Continent in the shortest possible time.

Seventeen countries presented their reports. The first set of country reports was given by the respective representatives of Burkina Faso, Ghana, Mali, Kenya and Uganda. A presentation from Ethiopia could not be made because the representative could not attend the conference. Under the guidance of the PATTEC Coordination Office, existing baseline biological and socio-economic and environmental information was packaged by the six countries to develop projects that enabled them obtain loans from the African Development Bank to create tsetse and trypanosomiasis-free zones. The projects started in 2006 and will end in 2011. The countries planned baseline studies between 2007 and 2008. These studies

are aimed at improving on existing information and providing decision support for tsetse and trypanosomiasis eradication. The presenters informed the meeting that the choice of techniques for tsetse eradication would be determined following baseline studies. PATTEC was exploring the introduction of the Sequential Aerosol Technique for tsetse suppression and plans were advanced in the recruitment of consultants for assessing its feasibility.

Other country reports were from Somalia, Democratic Republic of Congo, Botswana, Mozambique, Zimbabwe, Angola, Guinea, Benin, Togo, Tanzania, Sudan and Zambia. The countries reported the occurrence of both Animal trypanosomiasis and Human African Trypanosomiasis. Countries in Central and Southern Africa focused mainly on sleeping sickness situation. It was generally observed that effective and sustained treatments often lead to significant decline in disease prevalence and that resurgence occurs when surveillance and control are relaxed. Certain countries notably Benin, Togo, Angola, Tanzania, Sudan and Zambia were gathering baseline data that would enable them prepare project documents for funding by development partners. These efforts were being coordinated by PATTEC. The presentation by Botswana demonstrated the successful application of the Sequential Aerosol Technique (SAT) initiated and funded by the Government.

During the discussion, participants urged the countries to integrate activities carried out by Veterinary Services and the Ministry of Health, with regard to the control and or eradication of tsetse and trypanosomiasis. There was a general consensus that all methods for the elimination of tsetse and trypanosomiasis be explored and used as and when necessary. There was also a call on International Organisations and the Private Sector to continue providing support to the current effort being made by tsetse affected countries.

Recommendations

Following the general concern that the situation of tsetse and trypanosomiasis in many countries has worsened in the last decade and appreciation on the progress made by AU-PATTEC in the creation of awareness and the mobilization of human and financial resources towards the eradication of tsetse and trypanosomiasis, the meeting commended concerted and sustained action for the realization of the objective of the campaign and recommends to AU-PATTEC to:

1. Support countries implementing tsetse and trypanosomiasis eradication programmes to develop bankable projects for submission to development partners.
2. Ensure that projects developed are based on sound information and are Regional in character.

and recommends countries to:

1. Continue to accord high priority to the removal of tsetse and trypanosomiasis in their effort to promote Sustainable Agricultural and Rural Development.

2. Harmonize, coordinate and integrate, as much as possible, plans and efforts aimed at eradicating tsetse and both Human and Animal African trypanosomiasis.

Glossina Biology and Control

Moderator: William Shereni

Rapporteur: Mweemba Hamukombwe

Five papers were presented during this session covering population genetics, use of insecticide-treated mosquito nets in zero-grazing units, restricted application of pesticides to cattle and on the prediction models for tsetse densities. Results on tsetse genetics were presented and their usefulness in the understanding of tsetse ecology was demonstrated. Genetic differences between tsetse fly populations separated by physical barriers were presented as an important factor in the establishment of area-wide projects.

Two innovative tsetse control techniques on the use of insecticide-treated nets were presented under varying ecological settings in West and East Africa. Enormous reductions of tsetse flies resulting in the corresponding lowering of trypanosomiasis incidences were recorded in trials involving the use of insecticide-treated mosquito fences to protect livestock in Ghana and the confinement of goats in zero-grazing units using polyethylene nets. Studies in Ghana demonstrated the effective reduction of *G. p. palpalis* and the successful protection of pig-pens in tsetse infested areas. The technique was also successfully used in Guinea in an integrated tsetse control campaign programme. In Kenya, goats were protected against trypanosome infections through the use of 1 percent deltamethrin sc treated polyethylene nets in zero-grazing units.

The fourth paper was on the restricted application of pesticides in foot baths. The treatment of cattle lower legs in foot baths offered a valuable technique for the simultaneous control of tsetse and ticks.

The final paper was on the need to adapt PAAT-IS tsetse distribution models through the use of the high resolution landsat (30 m x 30 m pixels) and spot (10 m x 10 m pixels) satellite imagery. The high resolution maps were useful particularly in making population density predictions in riverine areas.

Recommendations

1. Taking into consideration the limited number of research papers on tsetse biology and control during the 29th ISCTRC conference, the conference calls upon research and academic institutions, scientists, governments and donors to put more resources to strengthen and enhance research. Research should however be focused on the constraints to effective implementation of PATTEC initiative programmes to improve the field operations.
2. Studies on tsetse genetics should be promoted to develop a tool for the development of PATTEC projects based on a better understanding of differences between tsetse fly populations geographically separated by physical barriers and therefore important in designing area wide operations.
3. Further research is required to assess the efficacy of insecticide treated nets in extensive livestock production systems. The technique needs to be further optimized to determine under which conditions animals can be maximally protected. Specifications of the nets and insecticides dosages need to be standardised to ensure proper usage. Similarly, the restricted use of insecticide on animals needs to be further

optimised taking into consideration the behaviours of different tsetse species and the possibility of tick resistance against the pesticides applied.

4. Low technology and low cost techniques that can easily be integrated at the farmer level such as the use of insecticide-treated nets and the restricted application of pesticides to cattle are recommended for use in the peri-urban and other areas with high human population to improve health and productivity of improved stock especially during the implementation of PATTEC projects.

5. There is need to adapt PAAT-IS tsetse distribution models to local situations using high resolution satellite imagery (Landsat or spot images) in the collection of baseline data especially in riverine areas to predict tsetse densities and distributions in areas that are proposed for PATTEC Projects.

Human African Trypanosomiasis (HAT)

Moderator: Perr Simarro

Rapporteur: Consantin Miaka Mia Bilenge

Nine presentations were made in the two sessions that were allocated to the theme. One presentation dealt with bio-informatics, three on epidemiology, two of which were related to the implementation of control programmes, their challenges and perspectives. Four presentations dealt with treatment while the last one was about genetics. Five papers were presented as posters.

The presentation on bio-informatics demonstrated how this area can contribute to, among other things, the search for new molecules requiring extensive *in vitro* and *in vivo* clinical tests for their use.

Among the three presentations on epidemiology, one dealt with achievements of the programme in three phases, with successes reported from Yei, in Southern Sudan for more than five years, by the international NGO, Malteser. However, the withdrawal of this NGO after the emergency phase could pose a serious problem for the continuity of the control activities, with the risk of a possible resurgence if the Government and local partners do not get involved on time. The second one is about Mali whose real epidemiological situation is unknown due to the lack of effective interventions. The third one was based on the need to identify risk factors for the transmission of HAT that have to do with human-fly contact in the urban settings, such as the city of Kinshasa, in order to better conduct an integrated control programme and inform strategies.

Out of the four presentations on the subject of treatment, the first one dealt with Phase III of confirmation of the effectiveness and tolerance of pafuramidine (DB 289), which is a new oral drug used in the treatment of sleeping sickness in its primary phase involving 250 patients among whom there were pregnant and breastfeeding women from six different centres, one of which was in Angola, four in DRC and one in Southern Sudan. The second presentation demonstrated the problem of the 14 percent failure rate in the treatment of *T. b. gambiense* type Trypanosomiasis using DFMO (difluoromethylornithine) as a monotherapy in patients monitored in North-western Uganda. The third one demonstrated the success of preliminary results of clinical tests using a combination of nifurtimox at 15mg per kg per day every eight hours for ten days and eflornithine 400mg per kg per day every 12 hours for

seven days for the advanced stage. The fourth presentation was based on the retrospective consideration of the treatment of intermediate phase infection in Angola, (6 to 20 white blood cells in the CSF), using pentamidine, a 19 percent reinfection rate was observed.

The presentation on genetics demonstrated the existence of a genetic diversity in *T. brucei* in two countries (Côte d'Ivoire and Guinea). Studies on the genetic variability of trypanosomes and their vectors should be encouraged. The IGGI initiative (International *Glossina* Genomics Initiative) will result in significant progress because of sequencing of the *Glossina* genome.

In addition to the papers that were presented, a keynote address highlighted successes and constraints in pharmaceutical industry with emphasis on identification and development of new drugs.

Recommendations

1. The session recommends that bio-informatics be made part of the same network as other partners who are involved in *in vivo* experimentation in order to speed up the process of the development of new drugs against trypanosomiasis.
2. The Government of Southern Sudan should put in place a facility for the coordination and organisation of HAT control.
3. Countries that use DFMO in monotherapy should put in place drug efficacy programmes for this product.
4. The presence of PATTEC in Mali should facilitate the updating of the epidemiological and trypanosomiasis situation to facilitate the planning of joint activities.

General Recommendations

1. ISCTRC recommends that the establishment of PATTEC in all countries serve as an entry point for the organization of integrated control programmes (AAT, HAT, LAV).
2. ISCTRC recommends that the DRC be accorded the appropriate support by the international community, all international and financial organisations, given this country's epidemiological situation (scope of the problem, high HAT prevalence, massive presence of *Glossina* and of AAT) and its geographical situation, given that it has common borders with nine countries.

African Animal Trypanosomiasis

Moderator: Solomon Haile Mariam

Rapporteur: Mamadou Lamine Dia

The presentations that were made during this session can be categorized as follows:

- A keynote presentation on animal trypanosomiasis
- Two presentations on diagnosis of the disease
- Four presentations on the epidemiological status of animal trypanosomiasis, two of which focused on camel *Trypanosoma evansi*
- Four presentations on drug resistance

- One presentation on trypanotolerance markers
- One presentation on vectors

The keynote address provided an overview of past, current and future control and research strategies, a synthesis of the current situation regarding animal trypanosomiasis control and the analysis of perspectives of various control options. In conclusion, the long-term vision must focus on the eradication of tsetse flies to eliminate human and animal trypanosomiasis

With regard to diagnosis, the first presentation dealt with standardised analysis in search of new molecular targets based on purified proteomes and secretomes for two sub-genus, i.e. *Trypanozoon* and *Nannomonas* obtained from bloodstream trypanosomes. The second presentation in this category was on inhibition ELISA with the assistance of a recombinant antigen whose apparent specificity (97.3 percent) and sensitivity (84.4 percent) are excellent. Both presentations were in the experimental phase and need to be validated.

Four presentations were made on the epidemiological situation of animal trypanosomiasis in concerned countries. Two papers were on bovine trypanosomiasis in the Kachia grazing reserve (Nigeria). From the presentations, it emerged that bovine trypanosomiasis constitutes a real challenge in the grazing reserve where *T. vivax* was dominant. The other report was on the cross-sectional surveys conducted in South Darfur State outside the tsetse belt where *T. vivax* infection prevalence was very high and inside the tsetse belt where *T. congolense* infections (58.5 percent) was higher when compared with *T. vivax* infections (17.9 percent). Two presentations in this category pertained to trypanosomiasis in camels in 4 countries (Algeria, Morocco, Mauritania and Tunisia) and camel calves in Kenya.

Four presentations discussed drug resistance. Two of these were on the development of molecular tools for the rapid detection of *T. congolense* and *T. b. brucei* resistance to isometamidium and diminazene. A presentation was made on the seasonal variation and risk factors in the context of drug resistance in Sikasso (Mali). From a parasitological perspective, the study revealed the existence of heterogeneity in the evolution of the disease. In some villages significant variation in treatment failures (resistance) to diminazene was observed during rainy seasons. The objective of the presentation on the modelling of productivity of trypanocides under the risk of drug resistance in West Africa was to determine if the use of the drugs is characterised by path dependency. The results on isometamidium in all epidemiological conditions and diminazene in high disease prevalence and subsequently high drug resistance reveal a sub-optimal use of the two molecules.

The presentation on trypanotolerance seeks to detect quantitative trait loci controlling trypanotolerance in a backcross of N'Dama and Boran.

The presentation on vectors highlighted the use of screens and traps by the community as a means to control trypanosomiasis.

Recommendations

1. The session noted that during the 28th ISCTRC meeting, thirty presentations were made on human trypanosomiasis and very few on animal trypanosomiasis. During the 29th Conference, was a change with more presentations animal trypanosomiasis with emphasis on drug resistance. In countries like Sudan, it was revealed that drug

resistance was also reported in camels. Drug resistance should therefore be the subject of further investigations in order to address its causes and develop regimes for those that have been identified.

2. It is vital that farmers receive advice on the use of trypanocides which have two aspects: one facilitates an increase in the livestock productivity to encouraging farmers to continue using trypanocides and the other aspect is reduce mortality of livestock from overdoses.
3. Concerning *T. evansi* infections, it is proposed that investigations be conducted on the equine family and livestock that are sensitive to this parasite.
4. The ISCTRC commends the effort of WHO for its special recognition and advocacy to bring HAT to the level of HIV/AIDS, malaria and tuberculosis. Similar advocacy on AAT will assist the PATTEC programme to have access to the Global Fund. The sensitization must be strengthened at national and regional levels. ISCTRC should play a role in increasing advocacy.
5. Following the effort that is being made to eradicate tsetse in many countries in Africa and noting that some countries may have achieved this goal, ISCTRC was called upon urgently to initiate development of guidelines for International protocol to declare reclaimed land tsetse free. This could be achieved in collaboration with other partners.

Socio-Economics, Environment and Land Use/GIS

Moderator: Hippolyte Affognon

Rapporteur: Joseph Maitima

Papers scheduled for oral presentation in this section were five but only two were presented. Twelve others were to be presented as posters and are reported in the posters section. In addition to the two papers presented, a very informative keynote paper was presented that set a stage for a very interactive plenary discussion.

The recommendations generated in this session were according to the conclusions made in each of the papers presented and in response to the discussions during the plenary. There was sufficient time for discussions on the papers presented.

The issues that dominated plenary discussions in this session were:

- The need a political framework to support tsetse and trypanosomiasis interventions;
- The need for T & T interventions to be pro-poor and focused on poverty;
- The need for T & T interventions to be participatory especially with the rural communities and be user and environmentally friendly;
- The need for integrating trypanosomiasis interventions into the social paradigms of the rural poor; and
- Mainstreaming environmental and socio-economics impacts assessment in tsetse and trypanosomiasis eradication campaigns with emphasis on base data, development of appropriate indicators.

Recommendations

1. The conference recognizes the need for developing a political framework to enhance the success and sustainability of T & T interventions to solve the problems of T&T and that such framework should involve all stakeholders including the rural communities;
2. The conference recommends that T & T interventions be focused on improving rural livelihoods, improving access to markets for livestock and livestock products, and market chains;
3. The conference recommends that the methods for assessing economic and environmental impacts of T & T interventions must employ modern techniques including use of modern predictive models and adopt more holistic approaches that link with other economic sectors;
4. The conference recognizes the need for developing pro-poor public policies to create an enabling environment for the participation of rural communities in T & T interventions and private sector involvement;
5. The conference recognizes the importance of trypanotolerance in the search for ways to reduce risks or losses caused by trypanosomiasis and recommends further research on how this can be exploited and applied. Further the meeting recommends efforts to conserve trypanotolerant livestock to prevent them from going extinct; and
6. The conference recommends that T & T interventions practitioners should be more proactive in making vector control more attractive to livestock keepers and by recognizing the role played by trypanocidal drugs as a first line of defence in the fight against trypanosomiasis, the meeting recommends more work to be done on how to improve the use of drugs.

Poster Session

Moderator: Issa Sidibé

Rapporteur: Joyce Daffa

Two sessions were allocated to posters and one hour plenary at the end of the presentation to facilitate discussion. There were 24 posters displayed out of 53 listed or expected for the conference. Format guideline and themes were well adhered by all presenters i.e. introduction followed by objective, materials and methods, results and conclusion.

The majority of them were large with, visible or readable fonts, nice colours and well illustrated. However few posters carried too much detail, making quick reading and comprehension difficult. The different themes and related sessions are given in the following table.

THEMES	TITLE	POSTERS PRESENTED	NOT PRESENTED	TOTAL	PERCENT PRESENTED
I	PATTEC and Country reports				
II	*Glossina* Biology and Control	4	12	16	25
III	Human African Trypanosomiasis (HAT)	5	4	9	56
IV	Animal African Trypanosomiasis (AAT)	12	5	17	71
V	Socio-economics, Land use & Environnent	3	8	11	27
	TOTAL	24	29	53	45

Discussion

The meeting noted that posters were given less attention compared with oral papers. Apparently the general perception is that if the presented abstracts are selected for posters then they may not be interesting or important. It also appears that some sponsors prefer oral presentations to posters.

The meeting appealed to institutions and sponsors to give opportunity to presenters, especially young scientists to prepare posters for them to learn and gain experience for international meetings. Some presenters indicated their intention to present a poster on submission.

The Council Secretariat was requested to consider setting up a competition among posters to attract and motivate young presenters by giving prizes.

Conclusions

- From papers on *Glossina* biology, two posters were on genetic structure or diversity of *Glossina* and its implication on the control. The majority of the papers on this theme were focused on repellents, except one on the ability of tsetse flies to acquire an infection of the second trypanosome species.
- All the posters on sleeping sickness were related to diagnosis: B cells methods, centrifugation technique and Loop Mediated Isothermal Amplification (LAMP). The first two posters provide data on potential early stage detection and, the LAMP of DNA is in validation process.
- Regarding AAT control, farmers' capacity building for AAT Management and Environment was recommended by most presenters. This will allow livestock owners to have improved and quality animal husbandry practices to reduce drug resistance. Other animals have been surveyed in Sudan to assess the importance of *T. evansi* in camels and in donkeys.
- The need for a study on strategic environmental, socio-economic assessment and baseline surveys prior to conducting T & T control/eradication was emphasized.

Recommendations

1. It was observed that two consecutive sessions in day two were not enough for participants to visit all posters so as to be able to comprehend materials for discussion and recommendation. Day four is probably better for poster sessions to give more time for reading.
2. The Council welcomes the continued improvement of Poster sessions and recommends the provision of appropriate poster room to encourage authors including young scientists to explain their presentations to the participants fully. Action: ISCTRC Secretariat and hosting member nation.
3. In order to further encourage increased participation in the Poser sessions, Council recommends that prizes be awarded to the three best posters during the Conference and that the Private Sector be approached to sponsor such prizes. In this case, clear guidelines for poster selection should be worked out and agreed upon. The details should be included in conference announcement. Action: ISCTRC Secretariat.

Networking Session

Chairperson: A. A. Ilemobade

Rapporteur: M. Vreysen

Networks are an interconnected group or system having the same interest and objective and working together towards its achievement. A good example of a network that functions properly is the Programme Against African Trypanosomiasis (PAAT). PAAT is a specialized, comprehensive network that is composed of four mandated organisations i.e. AU-IBAR, FAO, IAEA and WHO who constitute the secretariat. PAAT aims at networking and coordinating international alliances towards harmonized interventions against T & T both in human and animals, to promote partnerships, dialogue and assistance, to encourage and enhance multi-stakeholders' initiatives, and to build consensus on common agricultural policy in T & T affected areas.

Networking for T & T is needed in view of the magnitude of the problem and the fact than none of the specialized international organisations can cope with all aspects of the T & T problem. The root problem that the international organisations are called upon to help solve is poverty, under nourishment and food insecurity. Therefore, strategies are needed that are comprehensive to positively impact the roots of the causative effects on poverty posed by T & T, and hence the need for a good functional network.

PAAT, as a unique international forum, also promotes open access to high-quality information for the 37 T & T affected countries. In that respect, the recently upgraded PAAT Information System provides new tools, methodologies and datasets for the benefit of all stakeholders. The use of GIS techniques, the sharing and exchange of spatial datasets through the creation of meta-documentation and the integration with other web-based resources (e.g. FAO GeoNetwork) has been advanced. Standardized, high-resolution land cover maps of eight T & T-affected countries, based on the FAO Africover dataset, have been produced in support of tsetse habitat mapping. In addition, global spatial datasets have been reviewed, selected and analysed to facilitate their use, capacity building and harmonization have been pursued through workshops and training courses. Finally, the PAAT Information System offers renewed and novel opportunities for knowledge sharing for all stakeholders and

provides an ideal institutional, scientific and technical framework for information dissemination with a still largely unexploited potential.

Another example of a functioning network is the HAT Platform. The platform aims at providing assistance in the attempt to remove several obstacles such as the delays in obtaining authorisation for clinical trials, the need for adapted expertise, and the need for appropriate methodologies for clinical trials.

The discussion topics focused on the following: (i) various networks are in place, but it was suggested that several of these networks are not working properly, and their operations need to be urgently optimised, (ii) the importance of networks was underlined to make each stakeholder understand "who is doing what", (iii) the need for better communication within the network, the importance of a common purpose within each of the networks, the importance that the members of a network have complementary expertise, and the importance of a bottom-up approach were emphasized, and (iv) networks need to make sure that they can be reached and that they can be used by all stakeholders to the maximum.

Recommendations

The ISCTRC recognizes:

- The work of the Programme Against African Trypanosomiasis (PAAT) in networking and coordination within the international community; and,
- The achievements concerning the development of harmonized intervention against T & T both in humans and animals.
- The advisory role of PAAT for policy and technical issues for T & T management and in assisting Member States and PATTEC in planning implementation of field T & T programmes and operations.

The ISCTRC recommends:

1. To further strengthen the coordination, communication and information networking activities of PAAT;

2. That member countries and regional networks increase the dialogue and exchange of information with other networks, in particular PAAT, with a view to promote consensus in common agriculture and human and animal health policy in T & T affected countries; and

3. That the existing networks are strengthened through more active participation and improved dissemination of technical and scientific knowledge and through increased sharing of relevant datasets.

Capacity Building

Moderator: Rajinder Saini

Rapporteur: Victorin Codjia

Dr. Grace Murila in her keynote address highlighted the Research Capacity Strengthening being undertaken by KARI-TRC with funding from WHO-TDR. Dr. Saini presented the training needs assessment undertaken for six PATTEC countries (Burkina Faso, Mali, Ghana, Ethiopia, Uganda and Kenya).

The main points arising out of the discussion were:

- An acute shortage of well-trained and experienced staff at all levels in T&T affected countries for the implementation of large-scale area wide T & T control/eradication programmes;
- Capacity of scientists and managers in vector and disease research in Africa was limited;
- A coherent and targeted programme for building capacity was lacking;
- That African scientists need to be trained in modern cutting edge techniques in molecular sciences and in GIS;
- Further R & D is needed to develop new innovative tools for sustainable surveillance and control of HAT;
- The necessity to train more lab technicians and medical assistants for control of HAT;
- Available training manuals need to be reviewed for appropriateness and adopted;
- Lack of funding and necessity of attracting more funds from national systems and other international donors and institutes;
- Need to implement comprehensive training programmes urgently; and
- To involve all stakeholders for developing and implementing research and training programmes.

Recommendations

Recognizing the shortage of well trained and experienced staff in the region, and the need to expedite the implementation of large-scale area wide T & T control/eradication programmes, the session recommends;

1. That the shortage of scientists and managers in vector and disease research in Africa needs to be urgently addressed;
2. That African scientists need to be trained in cutting edge techniques in molecular sciences and in GIS;
3. That further R & D is needed to develop new innovative tools for sustainable surveillance and control of HAT;
4. The major role played by laboratory technicians and medical assistants in the reduction of HAT cases in the last ten years should be more widely recognized; and
5. The need for specific training modules and manuals.

Noting with appreciation the capacity building efforts of International Organizations like FAO, IAEA, WHO, the International Research Institutes like ICIPE, ILRI, CIRDES, CIRAD, National Institute like KARI-TRC and continental projects like PATTEC among others, the session recommends:

1. A more coordinated, focused and holistic approach is needed for capacity building in African countries, so that trained manpower can be built at all levels based on needs assessments for each country;
2. Steps should be taken to strengthen the institutional capacity of African countries in research and in undertaking large-scale control /eradication programmes;
3. There is an urgent need to continue training of middle and senior staff in implementation of the control programmes especially for PATTEC countries;
4. Senior staff to be trained in projects management;
5. There is an urgent need to produce training modules and manuals and refine existing ones for use by technical staff implementing PATTEC activities;
6. WHO HAT Surveillance and Control Programme should continue enhancing the capacity of health workers in disease diagnosis and management, for people living in remote rural areas where the disease is endemic;
7. WHO/TDR is requested to continue its approach of enhancing the research capacity of African scientists;
8. National systems to ensure that the required capacity to undertake large-scale control programmes exists and is optimally used; and
9. That resource mobilization for capacity building is given priority.

THE INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE (ILRI)

Publication of Manuals and Guidelines

Rodriguez, L. C., Kshatriya, M., Mugatha & S. Maitima, J. M., (2007). A conceptual framework for integrated impact assessment of tsetse and trypanosomiasis interventions. *ILRI Manuals and Guides No. 5,* 37 pp., ISBN 92-9146-213-6. Report to the US State Department and the AU's PATTEC. Available from ILRI through contact with j.maitima@cgiar.org.

At the heart of any effort to foster sustainable development, lie scientific analysis and the application of scientific knowledge. Providing scientifically correct and appropriate information that respond to and anticipate the needs of policy makers and other stakeholders is essential when addressing issues from the plight of widespread poverty, global changes and environmental degradation. Attainment of the Millennium Development Goals (MDG) can not be possible without sufficient client technological progress and improved policies to address the global challenges that face the resource poor regions of the world. Coupled with efforts to increase agricultural productivity, natural resources management (NRM) has become one of the cornerstones of research and development within the national, sub-regional and international agricultural research systems. The Consultative Group on International Agricultural Research (CGIAR) has devoted significant resources into this area of research. Development investors, policy makers and researchers alike are keen to assess and evaluate investments in NRM. In the past, progress has been limited by the lack of scientific calmly valid ways to evaluate the complex environmental outcomes associated with these interventions that need new methods and techniques to enhance their effectiveness. Understanding of how the interventions affect environmental and socio-economic systems

and pathways is crucial in order to sustain the benefits of these developments and safeguard the destiny of our future generations. Impact analysis must become more problem-focused, and apply an interdisciplinary approach to sustainable development issues in order for science to become more policy relevant.

This document focuses on these needs and synthesizes a framework for evaluating the impacts of tsetse and trypanosomiasis interventions. The need to eradicate or eliminate the problem of trypanosomiasis and at the same time promote sustainable utilization of land on which millions of poor families depend, are real concerns for the future of 37 countries of Africa that are faced with this problem. An understanding of how the interventions affect all the other systems that contribute to attainment of the desired goals is now a matter of urgency.

In the past numerous efforts to control trypanosomiasis, have been made largely focusing on reduction of the abundance and distribution of the vector. However, all gains made through these control efforts have always been short lived due to resurgence of fly populations and disease prevalence in the controlled areas. Based on the experience of past control failures to sustain low tsetse populations, the African Union is now promoting the elimination of the tsetse flies in order to eradicate the disease from the continent through the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) with government resources and the support from the African Development Bank and other development agencies

Eradication has costs and benefits that need to be evaluated in order to estimate the viability of the investment and the sustainability of the interventions. All interventions on tsetse control and eradication have expected environmental and socio-economic implications depending on the approach used and the place where the control is undertaken, so the direct and indirect effects of the T&T interventions should be evaluated across the natural, social and economic systems in order to properly estimate the costs and benefits of the eradication campaign.

These natural, economic and social systems are dynamic and complex systems, thus they exhibit non linear behavior and spatial and temporal lags in their response to T&T interventions, making it difficult to estimate their direct impacts on an individual system. In addition environmental, economic and social systems are linked and interdependent. In consequence, interventions on the natural systems can have indirect effects on the economic sector and human societies and in a similar way impact on the economy or society can have indirect effects on the natural environment. Understanding of how the natural, economic and social systems are inter-linked and inter-dependent is necessary in order to identify the direction of the flows and the magnitude of indirect effects of T&T interventions to estimate with higher accuracy the cost and benefit t of the eradication campaign, identify areas where further interventions are needed to avoid negative feedbacks and design strategies to mitigate adverse effects across systems.

Considering the complex nature of the involved systems, their inter-linkages and feedback mechanisms, assessing the impact of T&T interventions could be a difficult task. The challenge is to understand and quantify how the interventions directly affect the environmental, the economic and social systems, and how the indirect effects of an intervention implemented on one system spread through the others affecting their processes and modifying the flow and direction of the linkages between them. Such assessments require

first a conceptual framework to help in mapping out the main cause effect relationships and response patterns.

Integrated impact assessments serve to provide decision makers with better information on the extent to which different trypanosomiasis interventions affect the target population and the magnitude of the effect of the interventions on the welfare of the intended beneficiaries. It is currently recognized that environmental, economic and social systems interchange flows of material and energy and the links and processes among these systems are evident at different temporal and spatial scales. It is expected, therefore, that the direct or indirect effects of the trypanosomiasis interventions on a given system might affect the quality or quantity of the flows of matter and energy to the other systems, finally having an impact on human wellbeing.

These impacts can be evaluated following a series of logical stages described in this framework and applying a set of different methodologies which are summarized in this document. The results of the integrated impact assessment can be used to prioritize actions, justify interventions, evaluate the attainment of the objectives of the programme and provide information of the pathways through which observed impacts have occurred. The results can also be used to develop mitigation and monitoring plans for undesired impacts, provide adequate policies, improve management strategies and spread the results among target groups.

Maitima, J. M., Rodriguez, L. C., Kshatriya & M. Mugatha, S., (2007). Guidelines for assessing environmental and socio-economic impacts of tsetse and trypanosomiasis interventions, 183 pp., ISBN 92-9146-212-8. Report to the US State Department and the AU's PATTEC. Available from ILRI through contact with j.maitima@cgiar.org

Tsetse-transmitted trypanosomiasis in both people and domestic animals is a critical constraint to economic development in Africa. Economic losses have been estimated at over US$ 1.3 million dollars annually. In addition, about US$ 30 million per year is estimated to be spent on prophylaxis and treatment. The Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), has designed an extensive programme in support of the eradication of tsetse and trypanosomiasis in sub-Saharan Africa, through a range of vector and disease management techniques integrating suppression, and eradication technologies. These interventions are likely to have significant environmental and socio-economic impacts that will be felt far beyond the target organisms, populations and regions where they will be implemented. It is therefore important that the design and implementation of PATTEC project activities lead to sustainable development outcomes. Sustainable development interventions need to be based on scientific evidence. To be useful for decision making, such scientific evidence must be relevant and responsive to the needs of policy and other decision-makers who are looking for practical research and development options to reduce poverty through sustainable land management.

Improved technologies, policies, and institutions are central in efforts to control tsetse and trypanosomiasis. Alternative control strategies have direct and indirect impact on the environment as well as on livestock keepers and other poor people who depend on livestock for their livelihoods. Understanding the direct and indirect environmental and socio-economic impact of alternative control strategies is important to sustain livelihood benefits, enhance long term livestock and crop productivity and safeguard the integrity of the natural

resource base for use by future generations. Careful evaluation of project impacts that provides timely information to decision makers is an important strategic input in the design and implementation of PATTEC investments. Given the multi-dimensional impact of project interventions, it is important that impact is assessed from inter-disciplinary perspectives for the results to have practical policy relevance and operationally useful.

This book responds to the expressed needs of decision makers and impact assessment practitioners who are looking for strategic guidance and practical information for evaluating the impacts of tsetse and trypanosomiasis interventions. It synthesizes information on key challenges in assessing impacts of natural resource interventions and provides a conceptual framework for assessing impact as well as guidelines and methods for assessing environmental, economic, and social impacts of project interventions. It also provides information on integrated approaches for assessing impacts. Practical advice is provided on the appropriateness, data needs, and resource requirements for each method that is described. The diversity of the methods and tools described in the book makes it a key reference source for the design, implementation, and evaluation of PATTEC projects.

New Tsetse Project: A Dynamic Ecological Simulation Model of Tsetse transmitted Trypanosomiasis in Kenya.

The overall goal of this new four year project being implemented with funds from the US National Institute of Health (NIH) is to examine, build, and test a predictive model that defines the relationships between climate change, land use and land cover change, social systems, and ecological disturbance on the ecological distribution of tsetse flies and African Trypanosomiasis or sleeping sickness across Kenya. This study will employ an interdisciplinary team to develop and deploy an innovative advanced special simulation system, "ATcast"–African Trypanosomiasis Forecasting system that integrates dynamic multi- scale multi-agent models, geographic and epidemiological methods, and a regional climatic mode. The information produced will directly benefit on going tsetse suppression programmes and make a substantial understanding of broader patterns of human-environmental impacts, ecologically related changes, disease emergence, transmission, prevention and control.

This project is being implemented in collaboration with Michigan State University and ILRI's specific contribution will include:

1. Research on the dynamics of savannah vegetation in relation to climate change:
    a. Analysis of the spatial and temporal trends in climate variables, and the responses of people, livestock, and wildlife to changes in rainfall and temperature;
    b. Development of a typology of savannah vegetation based on climate and soil GIS variables, and literature review; and
    c. Collect land use and cover ground-truth data
2. Model validation of tsetse distributions:
    a. ILRI will coordinate and support in conjunction with the MSU team field work to two sites identified by the model.

3. Analysis of how agro-pastoral livelihoods are affected by altered climate and how households and communities respond by altering their livelihood system and land management decisions.
    a. Contribute to an understanding of how communities and households respond, and how their land management has evolved by developing a timeline of changing land management in relation to the livelihood system, and by discussing how coping strategies to climate change have evolved with key informant interviews.
4. Share the research results and scenarios with policy makers, communities, scientists and others, to contribute towards research and development planning to sustain pastoral and agro-pastoral lands and livelihoods
    a. Write journal articles and produce other informational materials; and
    b. Conduct feedback workshops at the community and at the national level.
5. Contribute to the project's inter-institutional collaboration by hosting project workshops

SECTION B - ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

14341. **Alter, H. J., 2008**. Pathogen reduction: a precautionary principle paradigm. *Transfusion Medicine Reviews,* **22** (2): 97-102.

Department of Transfusion Medicine, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, MD 20892, USA. [halter@dtm.cc.nih.gov].

Although remarkable advances have been made in the prevention of the major transfusion-transmitted diseases, long intervals have transpired between the first recognition of transfusion risk and the implementation of a preventive strategy. For hepatitis B virus, that interval was 30 years; for non-A, non-B/hepatitis C virus, 15 years; and for human immunodeficiency virus, West Nile virus, *Trypanosoma cruzi*, and bacteria, 3, 4, 5, and 18 years, respectively. In our existing reactive approach, there is a fundamental and inevitable delay before we can react; and thus, infections are destined to occur. The continued emergence or re-emergence of transfusion-transmitted infections calls for a new paradigm of pre-emptive pathogen reduction (PR). Two PR systems, psoralen/UV-A and riboflavin/UV-A, have shown efficacy and safety for platelets and plasma; and psoralen/UV-A technology has been successfully implemented for platelets in Europe. Pathogen reduction can eliminate or reduce the risk for any nucleic acid containing agent, including bacteria, and thus will be effective for all but prion diseases. It is possible to introduce PR for platelets and plasma now and to concentrate resources on developing PR for red cells. This will require an intellectual and financial commitment from the National Institutes of Health, the Food and Drug Administration, industry, and the blood bank establishment, just as occurred for nucleic acid testing (NAT) technology. This can be done if there is sufficient will to do it.

14342. **Baton, L. A., Garver, G., Zhiyong, X., & Dimopoulis, G., 2008** Functional genomics studies on the innate immunity of disease vectors. *Insect Science*, **15**: 15-27.

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The increasing availability of genome sequences and the development of high-throughput techniques for gene expression profiling and functional characterization are transforming the study of innate immunity and other areas of insect biology. Already, functional genomic approaches have enabled a quantum advance in the characterization of mosquito immune responses to malaria parasite infection, and similar high-throughput functional genomic studies of other vector-pathogen interactions can be expected in the near future. The application of microarray-based and other expression analyses provide genome-wide transcriptional profiles that can be used to identify insect immune system components that are differentially regulated upon exposure to various classes of pathogens, including many important aetiologic agents of human and animal diseases. The role of infection-

responsive or other candidate immune genes identified through comparative genomic approaches can then be functionally characterized, either *in vivo*, for instance in adult mosquitoes, or in vitro using cell lines. In most insect vectors of human pathogens, germ-line transgenesis is still technically difficult and maintenance of multiple transgenic lines logistically demanding. Consequently, transient RNA interference (RNAi)-mediated gene-silencing has rapidly become the method of choice for functional characterization of candidate innate immune genes. The powerful combination of transcriptional profiling in conjunction with assays using RNAi to determine gene function, and identify regulatory pathways, together with downstream cell biological approaches to determine protein localization and interactions, will continue to provide novel insights into the role of insect innate immunity in a variety of vector-pathogen interactions. Here we review advances in functional genomics studies of innate immunity in the insect disease vectors, over the past decade, with a particular focus on the *Anopheles* mosquito and its responses to malaria infection.

14343. **Bletter, N., 2007.** A quantitative synthesis of the medicinal ethnobotany of the Malinke of Mali and the Ashaninka of Peru, with a new theoretical framework. *Journal of Ethnobiology and Ethnomedicine*, **3**: 36.

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Although ethnomedically and taxonomically guided searches for new medicinal plants can improve the percentage of plants found containing active compounds when compared to random sampling, ethnobotany has fulfilled little of its promise in the last few decades to deliver a bounty of new, laboratory-proven medicinal plants and compounds. It is quite difficult to test, isolate, and elucidate the structure and mechanism of compounds from the plethora of new medicinal plant uses described each year with limited laboratory time and resources and the high cost of clinical trials of new drug candidates. A new quantitative theoretical framework of mathematical formulas called "relational efficacy" is proposed that should narrow down this search for new plant-derived medicines based on the hypothesis that closely related plants used to treat closely related diseases in distantly related cultures have a higher probability of being effective because they are more likely to be independent discoveries of similar plant compounds and disease mechanisms. A prerequisite to this hypothesis, the idea that empirical testing in traditional medicine will lead to choosing similar medicinal plants and therefore the medicinal flora of two distant cultures will prove to be more similar than their general flora, is tested using resampling statistics on cross-cultural field data of the plants used by the Malinke of Mali and the Ashaninka of Peru to treat the following diseases: malaria, African sleeping sickness, Chagas' disease, leishmaniasis, diabetes, eczema, asthma, and uterine fibroids. In this case, the similarity of the medicinal floras is found to be significantly greater than the similarity of the general floras, but only when the diseases in question are grouped into the categories of parasitic and autoimmune diseases. If the central theoretical framework of this hypothesis is shown to be true, it will allow the synthesis of medicinal plant information from around the world to pinpoint the species with the highest potential efficacy to take into the laboratory and analyze further, ultimately saving much field and laboratory time and resources.

14344. **Brown, K., 2008**. From Ubombo to Mkhuzi: disease, colonial science, and the control of nagana (livestock trypanosomosis) in Zululand, South Africa, c. 1894-1953. *Journal of the History of Medicine and Allied Sciences,* **63:** 1-38**.**

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This article looks at the scientific studies and debates that surrounded the control of nagana (trypanosomosis in livestock) in Zululand, South Africa, from the late nineteenth century until the 1950s. By 1953 the disease appeared to be contained following the use of DDT to exterminate the tsetse fly that spread the infection from immune wildlife to susceptible livestock. It argues that South Africa made an important contribution to western knowledge about trypanosomosis in terms of its aetiology and possibilities for its control-a fact that has often been overlooked in the historical literature that has tended to focus on events in colonial central and east Africa. It explores Zulu conceptualizations of nagana, which influenced early researchers, the evolution of veterinary, entomological, and ecological sciences as "tools" for understanding and suppressing disease, as well as the difficulties involved in reconciling game conservation with colonial settlement. The article also shows how an animal disease contributed to the development of colonial science and encouraged the expansion of scientific networks with African colonies and beyond.

14345. **Chaves, L. F., Cohen, J. M., Pascual, M. & Wilson, M. L., 2008**. Social exclusion modifies climate and deforestation impacts on a vector-borne disease. *PLoS Neglected Tropical Diseases,* **2** (1): e176.

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The emergence of American cutaneous leishmaniasis has been associated with changes in the relationship between people and forests, leading to the view that forest ecosystems increase infection risk and subsequent proposal that deforestation could reduce re-emergence of this disease. We analyzed county-level incidence rates of the disease in Costa Rica (1996-2000) as a function of social and environmental variables relevant to transmission ecology with statistical models that incorporate breakpoints. Once social marginality was taken into account, the effect of living close to a forest on infection risk was small, and diminished exponentially above a breakpoint. Forest cover was associated with the modulation of temporal effects of El Nino Southern Oscillation (ENSO) at small spatial scales, revealing an additional complex interplay of environmental forces and disease patterns. In conclusion, social factors which previously have not been evaluated rigorously together with environmental and climatic factors appear to play a critical role that may ultimately determine disease risk.

14346. **Dacks, J. B., Walker, G. & Field, M. C., 2008.** Implications of the new eukaryotic systematics for parasitologists. *Parasitology International*, **57** (2): 97-104.

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An accurate understanding of evolutionary relationships is central in biology. For parasitologists, understanding the relationships among eukaryotic organisms allows the prediction of virulence mechanisms, reconstruction of metabolic pathways, identification of potential drug targets, elucidation of parasite-specific cellular processes and understanding of interactions with the host or vector. Here we consider the impact of major recent revisions of eukaryotic systematics and taxonomy on parasitology. The previous, ladder-like model placed some protists as early diverging, with the remaining eukaryotes "progressing" towards a "crown radiation" of animals, plants, Fungi and some additional protistan lineages. This model has been robustly disproven. The new model is based on vastly increased amounts of molecular sequence data, integration with morphological information and the rigorous application of phylogenetic methods to those data. It now divides eukaryotes into six major supergroups; the relationships between those groups and the order of branching remain unknown. This new eukaryotic phylogeny emphasizes that organisms including *Giardia*, *Trypanosoma* and *Trichomonas* are not primitive, but instead highly evolved and specialised for their specific environments. The wealth of newly available comparative genomic data has also allowed the reconstruction of ancient suites of characteristics and mapping of character evolution in diverse parasites. For example, the last common eukaryotic ancestor was apparently complex, suggesting that lineage-specific adaptations and secondary losses have been important in the evolution of protistan parasites. Referring to the best evidence-based models for eukaryotic evolution will allow parasitologists to make more accurate and reliable inferences about pathogens that cause significant morbidity and mortality.

14347. **Dodd, R. Y., 2007**. Current risk for transfusion transmitted infections. *Current Opinion in Hematology,* **14** (6): 671-676.

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

14348. **Esch, G., 2007.** *Parasites and infectious diseases: discovery by serendipity and otherwise.* Cambridge University Press, 348 pp. Paperback ISBN-13: 9780521675390. Price $ 45

The author of this book retells several of the famous stories of discovery in the field of vector-borne and parasitic diseases through the eyes of some of the most prominent

researchers working in this field today. He goes on to connect these early stories with more recent watershed contributions as recounted through a series of interviews he references throughout the course of the book. For example, he describes the discovery of the African sleeping sickness agent, *Trypanosoma gambiense*, and the contributions in the early 1900s by Dutton, Castellani, and Bruce. He then moves on to more recent discoveries regarding immunity, antigenic variation, and the role of variable surface glycoproteins. He describes the seminal studies that were performed in this area as recounted through interviews with prominent parasitologists. Through this process, he weaves a tapestry of the new and old as it relates to the history of important tropical diseases such as African typanosomiasis, malaria, yellow fever, HIV/AIDS, hookworm, and schistosomiasis, which continue to plague humankind.

14349. **Futse, J. E., Brayton, K. A., Dark, M. J., Knowles, D. P., Jr. & Palmer, G. H., 2008.** Superinfection as a driver of genomic diversification in antigenically variant pathogens. *Proceedings of the National Academy of Sciences USA*, **105** (6): 2123-2127.

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A new pathogen strain can penetrate an immune host population only if it can escape immunity generated against the original strain. This model is best understood with influenza viruses, in which genetic drift creates antigenically distinct strains that can spread through host populations despite the presence of immunity against previous strains. Whether this selection model for new strains applies to complex pathogens responsible for endemic persistent infections, such as anaplasmosis, relapsing fever, and sleeping sickness, remains untested. These complex pathogens undergo rapid within-host antigenic variation by using sets of chromosomally encoded variants. Consequently, immunity is developed against a large repertoire of variants, dramatically changing the scope of genetic change needed for a new strain to evade existing immunity and establish coexisting infection, termed strain superinfection. Here, we show that the diversity in the alleles encoding antigenic variants between strains of a highly antigenically variant pathogen was equal to the diversity within strains, reflecting equivalent selection for variants to overcome immunity at the host population level as within an individual host. This diversity among strains resulted in expression of nonoverlapping variants that allowed a new strain to evade immunity and establish superinfection. Furthermore, we demonstrated that a single distinct allele allows strain superinfection. These results indicate that there is strong selective pressure to increase the diversity of the variant repertoire beyond what is needed for persistence within an individual host and provide an explanation, competition at the host population level, for the large genomic commitment to variant gene families in persistent pathogens.

14350. **Gould, F., 2008** Broadening the application of evolutionarily based genetic pest management. *Evolution*, **62**: 500-510.

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Insect- and tick-vectored diseases such as malaria, dengue fever, and Lyme disease cause human suffering, and current approaches for prevention are not adequate. Invasive plants and animals such as Scotch broom, zebra mussels, and gypsy moths continue to cause environmental damage and economic losses in agriculture and forestry. Rodents transmit diseases and cause major pre- and post harvest losses, especially in less affluent countries. Each of these problems might benefit from the developing field of Genetic Pest Management that is conceptually based on principles of evolutionary biology. This article briefly describes the history of this field, new molecular tools in this field, and potential applications of those tools. There will be a need for evolutionary biologists to interact with researchers and practitioners in a variety of other fields to determine the most appropriate targets for genetic pest management, the most appropriate methods for specific targets, and the potential of natural selection to diminish the effectiveness of genetic pest management. In addition to producing environmentally sustainable pest management solutions, research efforts in this area could lead to new insights about the evolution of selfish genetic elements in natural systems and will provide students with the opportunity to develop a more sophisticated understanding of the role of evolutionary biology in solving societal problems.

14351. **Greer, A., Ng, V. & Fisman, D., 2008.** Climate change and infectious diseases in North America: the road ahead. *Canadian Medical Association Journal*, **178** (6): 715-722.

Research Institute of the Hospital for Sick Children, Toronto, Canada.

Global climate change is inevitable-the combustion of fossil fuels has resulted in a build-up of greenhouse gases within the atmosphere, causing unprecedented changes to the earth's climate. The Fourth Assessment Report of the Intergovernmental Panel on Climate Change suggests that North America will experience marked changes in weather patterns in coming decades, including warmer temperatures and increased rainfall, summertime droughts and extreme weather events (e.g. tornadoes and hurricanes). Although these events may have direct consequences for health (e.g. injuries and displacement of populations due to thermal stress), they are also likely to cause important changes in the incidence and distribution of infectious diseases, including vector-borne and zoonotic diseases, water-and food-borne diseases and diseases with environmental reservoirs (e.g. endemic fungal diseases). Changes in weather patterns and ecosystems, and health consequences of climate change will probably be most severe in far northern regions (e.g. the Arctic). We provide an overview of the expected nature and direction of such changes, which pose current and future challenges to health care providers and public health agencies.

14352. **Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L. & Daszak, P., 2008.** Global trends in emerging infectious diseases. *Nature*, **451** (7181): 990-993.

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Emerging infectious diseases (EIDs) are a significant burden on global economies and public health. Their emergence is thought to be driven largely by socio-economic,

environmental and ecological factors, but no comparative study has explicitly analysed these linkages to understand global temporal and spatial patterns of EIDs. Here we analyse a database of 335 EID "events" (origins of EIDs) between 1940 and 2004, and demonstrate non-random global patterns. EID events have risen significantly over time after controlling for reporting bias, with their peak incidence (in the 1980s) concomitant with the HIV pandemic. EID events are dominated by zoonoses (60.3 percent of EIDs): the majority of these (71.8 percent) originate in wildlife (for example, severe acute respiratory virus, Ebola virus), and are increasing significantly over time. We find that 54.3 percent of EID events are caused by bacteria or rickettsia, reflecting a large number of drug-resistant microbes in our database. Our results confirm that EID origins are significantly correlated with socio-economic, environmental and ecological factors, and provide a basis for identifying regions where new EIDs are most likely to originate (emerging disease "hotspots"). They also reveal a substantial risk of wildlife zoonotic and vector-borne EIDs originating at lower latitudes where reporting effort is low. We conclude that global resources to counter disease emergence are poorly allocated, with the majority of the scientific and surveillance effort focused on countries from where the next important EID is least likely to originate.

14353. **Kalluri, S., Gilruth, P., Rogers, D. & Szczur, M., 2007**. Surveillance of arthropod vector-borne infectious diseases using remote sensing techniques: a review. *PLoS Pathogens*, **3** (10): 1361-1371.

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Epidemiologists are adopting new remote sensing techniques to study a variety of vector-borne diseases. Associations between satellite-derived environmental variables such as temperature, humidity, and land cover type and vector density are used to identify and characterize vector habitats. The convergence of factors such as the availability of multi-temporal satellite data and georeferenced epidemiological data, collaboration between remote sensing scientists and biologists, and the availability of sophisticated, statistical geographic information system and image processing algorithms in a desktop environment creates a fertile research environment. The use of remote sensing techniques to map vector-borne diseases has evolved significantly over the past 25 years. In this paper, we review the status of remote sensing studies of arthropod vector-borne diseases due to mosquitoes, ticks, blackflies, tsetse flies, and sandflies, which are responsible for the majority of vector-borne diseases in the world. Examples of simple image classification techniques that associate land use and land cover types with vector habitats, as well as complex statistical models that link satellite-derived multi-temporal meteorological observations with vector biology and abundance, are discussed here. Future improvements in remote sensing applications in epidemiology are also discussed.

14354. **Karp, C. L. & Auwaerter, P. G., 2007**. Coinfection with HIV and tropical infectious diseases. I. Protozoal pathogens. *Clinical Infectious Diseases,* **45** (9): 1208-1213.

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The brunt of the human immunodeficiency virus (HIV) pandemic has been borne disproportionately by resource-poor regions of the world, where tropical infectious diseases continue to hold greatest sway. As a result, our understanding of the epidemiological, biological, and clinical interactions between HIV and tropical pathogens has lagged, compared with our understanding of the interactions between HIV and pathogens that are common in the industrialized world. Because of the current rapid expansion of HIV care in the tropics, with increasing resources being made available, an overview of the available data is timely. Tropical protozoa are discussed here; other tropical pathogens are discussed in a related mini-review in this issue of Clinical Infectious Diseases.

14355. **Lebarbenchon, C., Brown, S. P., Poulin, R., Gauthier-Clerc, M., Thomas, F., 2008**. Evolution of pathogens in a man-made world. *Molecular Biology* **17** (1): 475-484.

Génétique et Evolution des Maladies Infectieuses, UMR CNRS/IRD 2724, IRD, 911 Avenue Agropolis, BP 64501, 34394 Montpellier cedex 5, France, Station Biologique de la Tour du Valat, Le Sambuc, 13200 Arles, France, Section of Integrative Biology, University of Texas at Austin, TX 78712, USA, Department of Zoology, University of Otago, PO Box 56, Dunedin 9015, New Zealand. [frederic.thomas@mpl.ird.fr].

Human activities have resulted in substantial, large-scale environmental modifications, especially in the past century. Ecologists and evolutionary biologists are increasingly coming to realize that parasites and pathogens, like free-living organisms, evolve as the consequence of these anthropogenic changes. Although this area now commands the attention of a variety of researchers, a broad predictive framework is lacking, mainly because the links between human activities, the environment and parasite evolution are complex. From empirical and theoretical examples chosen in the literature, we give an overview of the ways in which humans can directly or indirectly influence the evolution of different traits in parasites (*e.g.* specificity, virulence, polymorphism). We discuss the role of direct and indirect factors as diverse as habitat fragmentation, pollution, biodiversity loss, climate change, introduction of species, use of vaccines and antibiotics, ageing of the population, etc. We also present challenging questions for further research. Understanding the links between anthropogenic changes and parasite evolution needs to become a cornerstone of public health planning, economic development and conservation biology.

14356. **MacGregor, P. & Matthews, K. R., 2008**. Modelling trypanosome chronicity: VSG dynasties and parasite density. *Trends in Parasitology,* **24** (1): 1-4.

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A new mathematical model developed by Lythgoe *et al.* shows that the semi-predictable order of trypanosome antigenic variation can be generated by two parasite-intrinsic factors. The first is the different probabilities of antigen-gene activation that result from the different molecular mechanisms by which the genes become expressed. The second

is the density-dependent differentiation of slender to stumpy cells. The study has important implications for understanding the dynamics of antigenic variation and for modelling the consequences of therapeutic strategies directed against trypanosomes.

14357. **Myler, P. J., 2008**. Searching the Tritryp genomes for drug targets. *Advances in Experimental Medicine and Biology,* **625**: 133-140.

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The recent publication of the complete genome sequences of *Leishmania major*, *Trypanosoma brucei* and *Trypanosoma cruzi* revealed that each genome contains 8,300-12,000 protein-coding genes, of which approximately 6,500 are common to all three genomes, and ushers in a new, post-genomic, era for trypanosomatid drug discovery. This vast amount of new information makes possible more comprehensive and accurate target identification using several new computational approaches, including identification of metabolic "choke-points", searching the parasite proteomes for orthologues of known drug targets, and identification of parasite proteins likely to interact with known drugs and drug-like small molecules. This chapter describes several databases (such as Genedb, Brenda, Kegg, Metacyc, the Therapeutic Target Database, and Chembank) and algorithms (including Pathologic, Pathway Hunter Tool, and Autodock) which have been developed to facilitate the bioinformatic analyses underlying these approaches. While target identification is only the first step in the drug development pipeline, these new approaches give rise to renewed optimism for the discovery of new drugs to combat the devastating diseases caused by these parasites. Traditionally, drug discovery against the trypanosomatids (and other organisms) has proceeded from two different starting points: screening large numbers of existing compounds for activity against whole parasites or more focused screening of compounds for activity against defined molecular targets. Most existing anti-trypanosomatids drugs were developed using the former approach, although the latter has gained much attention in the last twenty years under the rubric of "rational drug design". Until recently, one of the major bottlenecks in anti-trypanosomatid drug development has been our ability to identify good targets, since only a very small percentage of the total number of trypanosomatid genes were known. That has now changed forever, with the recent (July, 2005) publication of the "Tritryp" (*Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*) genome sequences. This vast amount of information now makes possible several new approaches for target identification and ushers in a post-genomic era for trypanosomatid drug discovery.

14358. **Nesse, R. M., Stearns, S. C., 2008.** The great opportunity: evolutionary applications to medicine and public health. *Evolutionary Applications*, **1** (1): 28-48.

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Evolutionary biology is an essential basic science for medicine, but few doctors and medical researchers are familiar with its most relevant principles. Most medical schools have geneticists who understand evolution, but few have even one evolutionary biologist to suggest other possible applications. The canyon between evolutionary biology and medicine is wide. The question is whether they offer each other enough to make bridge building

worthwhile. What benefits could be expected if evolution were brought fully to bear on the problems of medicine? How would studying medical problems advance evolutionary research? Do doctors need to learn evolution, or is it valuable mainly for researchers? What practical steps will promote the application of evolutionary biology in the areas of medicine where it offers the most? To address these questions, we review current and potential applications of evolutionary biology to medicine and public health. Some evolutionary technologies, such as population genetics, serial transfer production of live vaccines, and phylogenetic analysis, have been widely applied. Other areas, such as infectious disease and aging research, illustrate the dramatic recent progress made possible by evolutionary insights. In still other areas, such as epidemiology, psychiatry, and understanding the regulation of bodily defenses, applying evolutionary principles remains an open opportunity. In addition to the utility of specific applications, an evolutionary perspective fundamentally challenges the prevalent but fundamentally incorrect metaphor of the body as a machine designed by an engineer. Bodies are vulnerable to disease – and remarkably resilient–precisely because they are not machines built from a plan. They are, instead, bundles of compromises shaped by natural selection in small increments to maximize reproduction, not health. Understanding the body as a product of natural selection, not design, offers new research questions and a framework for making medical education more coherent. We conclude with recommendations for actions that would better connect evolutionary biology and medicine in ways that will benefit public health. It is our hope that faculty and students will send this article to their undergraduate and medical school Deans, and that this will initiate discussions about the gap, the great opportunity, and action plans to bring the full power of evolutionary biology to bear on human health problems.

14359. **Simarro, P. P., Jannin, J. & Cattand, P., 2008**. Eliminating human African trypanosomiasis: where do we stand and what comes next? *PLoS Medicine,* **5** (2): e55.

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While the number of new detected cases of HAT is falling, sleeping sickness could suffer the "punishment of success," receiving lower priority by public and private health institutions with the consequent risk of losing the capacity to maintain disease control. While waiting for new tools for sleeping sickness control, the greatest challenge for the coming years will be to increase and sustain the current control efforts using existing tools. Effective surveillance and control followed by good reporting will be crucial. Furthermore, advocacy in endemic countries should continue to be maintained in the face of decreasing cases reported; sleeping sickness should retain its high priority with health policy makers and planners. Research must be encouraged to resolve the technical issues preventing the development of a new approach to surveillance and control that could be sustained by countries themselves. Since elimination of the disease has been considered feasible, WHO will adopt the conclusions of countries where HAT is endemic, who have demonstrated that: (i) the participation of existing health systems is not only desirable but essential for surveillance and control sustainability; (ii) the development of new diagnostic tools and drugs is crucial to guarantee the effective participation of existing health structures; and (iii) the maintenance of

a specialised central structure at national level is necessary to ensure the coordination and overall technical assistance needed. In that context, WHO is ready to take up the challenge and continue to lead countries, supporting and coordinating the work of all the actors involved.

14360. **Steverding, D., 2008**. The history of African trypanosomiasis. *Parasites and Vectors,* **1** (1): 3.

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The prehistory of African trypanosomiasis indicates that the disease may have been an important selective factor in the evolution of hominids. Ancient history and medieval history reveal that African trypanosomiasis affected the lives of people living in sub-Saharan African at all times. Modern history of African trypanosomiasis revolves around the identification of the causative agents and the mode of transmission of the infection, and the development of drugs for treatment and methods for control of the disease. From the recent history of sleeping sickness we can learn that the disease can be controlled but probably not be eradicated. Current history of human African trypanosomiasis has shown that the production of anti-sleeping sickness drugs is not always guaranteed, and therefore, new, better and cheaper drugs are urgently required.

14361. **Stuart, K., Brun, R., Croft, S., Fairlamb, A., Gurtler, R. E., McKerrow, J., Reed, S. & Tarleton, R., 2008**. Kinetoplastids: related protozoan pathogens, different diseases. *Journal of Clinical Investigation,* **118** (4): 1301-1310.

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Kinetoplastids are a group of flagellated protozoans that include the species *Trypanosoma* and *Leishmania*, which are human pathogens with devastating health and economic effects. The sequencing of the genomes of some of these species has highlighted their genetic relatedness and underlined differences in the diseases that they cause. As we discuss in this Review, steady progress using a combination of molecular, genetic, immunologic, and clinical approaches has substantially increased understanding of these pathogens and important aspects of the diseases that they cause. Consequently, the paths for developing additional measures to control these "neglected diseases" are becoming increasingly clear, and we believe that the opportunities for developing the drugs,

diagnostics, vaccines, and other tools necessary to expand the armamentarium to combat these diseases have never been better.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

[See **31**: 14369, 14370, 14374]

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

[See also **31**: 14368, 14371, 14372, 14375, 14376, 14377, 14378, 14379]

14362. **Abd-Alla, A. M., Cousserans, F., Parker, A. G., Jehle, J. A., Parker, N. J., Vlak, J. M., Robinson, A. S. & Bergoin, M., 2008**. Genome analysis of a *Glossina pallidipes* salivary gland hypertrophy virus reveals a novel, large, double-stranded circular DNA virus. *Journal of Virology,* **82** (9): 4595-4611.

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Several species of tsetse flies can be infected by the *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV). Infection causes salivary gland hypertrophy and also significantly reduces the fecundity of the infected flies. To better understand the molecular basis underlying the pathogenesis of this unusual virus, we sequenced and analyzed its genome. The GpSGHV genome is a double-stranded circular DNA molecule of 190,032 bp containing 160 nonoverlapping open reading frames (ORFs), which are distributed equally on both strands with a gene density of one per 1.2 kb. It has a high A+T content of 72 percent. About 3 percent of the GpSGHV genome is composed of 15 sequence repeats, distributed throughout the genome. Although sharing the same morphological features (enveloped rod-shaped nucleocapsid) as baculoviruses, nudiviruses, and nimaviruses, analysis of its genome revealed that GpSGHV differs significantly from these viruses at the level of its genes. Sequence comparisons indicated that only 23 percent of GpSGHV genes displayed moderate homologies to genes from other invertebrate viruses, principally baculoviruses and entomopoxviruses. Most strikingly, the GpSGHV genome encodes homologues to the four baculoviral *per os* infectivity factors (p74 [pif-0], pif-1, pif-2, and pif-3). The DNA polymerase encoded by GpSGHV is of type B and appears to be phylogenetically distant from all DNA polymerases encoded by large double-stranded DNA viruses. The majority of the remaining ORFs could not be assigned by sequence comparison. Furthermore, no homologues to DNA-dependent RNA polymerase subunits were detected. Taken together, these data indicate that GpSGHV is the prototype member of a novel group of insect viruses.

14363. **Parker, N. J. & Parker, A. G., 2008**. Simple tools for assembling and searching high-density picolitre pyrophosphate sequence data. *Source Code for Biology and Medicine,* **3** (1): 5.

Entomology Unit, FAO/IAEA Agriculture & Biotechnology Laboratory, IAEA Laboratories, A-2444 Seibersdorf, Austria.

The advent of pyrophosphate sequencing makes large volumes of sequencing data available at a lower cost than previously possible. However, the short read lengths are difficult to assemble and the large dataset is difficult to handle. During the sequencing of a virus from the tsetse fly, *Glossina pallidipes*, we found the need for tools to search quickly a set of reads for near exact text matches. A set of tools is provided to search a large data set of pyrophosphate sequence reads under a "live" CD version of Linux on a standard PC that can be used by anyone without prior knowledge of Linux and without having to install a Linux setup on the computer. The tools permit short lengths of *de novo* assembly, checking of existing assembled sequences, selection and display of reads from the data set and gathering counts of sequences in the reads. Demonstrations are given of the use of the tools to help with checking an assembly against the fragment data set; investigating homopolymer lengths, repeat regions and polymorphisms; and resolving inserted bases caused by incomplete chain extension. The additional information contained in a pyrophosphate sequencing data set beyond a basic assembly is difficult to access due to a lack of tools. The set of simple tools presented here would allow anyone with basic computer skills and a standard PC to access this information.

14364. **Siozios, S., Sapountzis, P., Ionnidis, P., & Bourtzis, K., 2008**. *Wolbachia* symbiosis and insect immune response. *Insect Science*, **15**: 89-100.

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Bacterial intracellular symbiosis is very common in insects, having significant consequences in promoting the evolution of life and biodiversity. The bacterial group that has recently attracted particular attention is *Wolbachia pipientis* which probably represents the most ubiquitous endosymbiont on the planet. *W. pipientis* is a gram-negative obligatory intracellular and maternally transmitted α-proteobacterium that is able to establish symbiotic associations with arthropods and nematodes. In arthropods, *Wolbachia pipientis* infections have been described in Arachnida, in Isopoda and mainly in Insecta. They have been reported in almost all major insect orders including Diptera, Coleoptera, Hemiptera, Hymenoptera, Orthoptera and Lepidoptera. To enhance its transmission, *W. pipientis* can manipulate host reproduction by inducing parthenogenesis, feminization, male killing and cytoplasmic incompatibility. Several polymerase chain reaction surveys have indicated that up to 70 percent of all insect species may be infected with *W. pipientis.* How does *W. pipientis* manage to get established in diverse insect host species? How is this intracellular bacterial symbiont species so successful in escaping the host immune response? The present review presents recent advances and ongoing scientific efforts in the field. The current body of knowledge in the field is summarized, revelations from the available genomic information are presented and as yet unanswered questions are discussed in an attempt to present a comprehensive picture of the unique ability of *W. pipientis* to establish symbiosis and to manipulate reproduction while evading the host's immune system.

14365. **Zézé, G.D., N'dri, L., Coulibaly, B., & Sane, B. 2007.** Ongoing research and perspectives on the heterogeneity of the populations of *Glossina palpalis palpalis* in Côte d'Ivoire. *Belgian Journal of Entomology*, **9**: 29-39

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The comparison of the inferior claspers of male genitalia of *G. p. palpalis* originating in different places of its distribution area showed that the species is represented by three morphologic types ("rounded palette" type, "hammer" type and "intermediate" type) in the forest zone of Côte d'Ivoire. Nevertheless, these morphological types don't constitute different subpopulations. Genetically, the clusters, identified as composing *G. p. palpalis* species in the same geographical area (i.e. focus of Bonon, Middle-West of Côte d'Ivoire) seem to be differentiated populations. But complementary investigations are necessary to determinate effectively these reproductive units or subpopulations of *G. p. palpalis*.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also **31:** 14342, 14344, 14350, 14351]

14366. **Green, K. K. & Venter, G. J., 2007**. Evaluation and improvement of sticky traps as monitoring tools for *Glossina austeni* and *G. brevipalpis* (Diptera: *Glossinidae*) in north-eastern KwaZulu-Natal, South Africa. *Bulletin of Entomological Research,* **97** (6): 545-553.

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The attractiveness of various colours, colour combinations and sizes of sticky traps of the 3-dimensional trap (3DT), cross-shaped target (XT), rectangular screen (RT) and monopanels were evaluated for their efficacy to capture *Glossina austeni* Newstead and *G. brevipalpis* Newstead in north-eastern KwaZulu-Natal, South Africa. The 3-dimensional shapes of the XT and 3DT in light blue (l.blue) and white were significantly (ca. 3.1-6.9 times) better than the RT for *G. austeni*. On bicoloured XTs, *G. austeni* landed preferentially on electric blue (e.blue) (58 percent) and black (63 percent) surfaces when used with white; while for *G. brevipalpis*, significantly more landed on e.blue (60-66 percent) surfaces when used with l.blue, black or white surfaces. Increased trap size increased the catches of *G. brevipalpis* females and both sexes of *G. austeni* significantly. Temoocid and polybutene sticky materials were equally effective and remained durable for 2-3 weeks. The glossy shine of trap surfaces did not have any significant effect on the attraction and landing responses of the two species. The overall trap efficiency of the e.blue/l.blue XT was 23 percent for *G. brevipalpis* and 28 percent for *G. austeni*, and that of the e.blue/black XT was 16 percent for *G. brevipalpis* and 51 percent for *G. austeni*. Larger monopanels, painted e.blue/black on both sides, increased the catches of *G. austeni* females significantly by up to four times

compared to the standard e.blue/black XT. This monopanel would be recommended for use as a simple and cost effective survey tool for both species in South Africa.

14367. **Kinung'hi, S. M., Malele, II, Kibona, S. N., Matemba, L. E., Sahani, J. K., Kishamawe, C. & Mlengeya, T. D., 2006**. Knowledge, attitudes and practices on tsetse and sleeping sickness among communities living in and around Serengeti National Park, Tanzania. *Tanzanian Health Research Bulletin,* **8** (3): 168-172.

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A study was undertaken to investigate knowledge, attitudes and practices about sleeping sickness (human African trypanosomiasis) among communities living in and around Serengeti National Park (SENAPA). Structured questionnaires were administered to a total of 1490 consenting participants. Of the respondents, 924 (62 percent) knew sleeping sickness, and 807 (87.3 percent) knew the right place to seek healthcare. Of 924 who knew sleeping sickness, 386 (42 percent) said the disease was present in the areas they live. Most respondents (85.4 percent) knew that sleeping sickness infections were acquired in the bush and forest. The most common (69.3 percent) sources of information about sleeping sickness were relatives and friends. Symptoms of sleeping sickness mentioned included abnormal sleep (45.2 percent), fever (35.3 percent), body malaise (14.5 percent), headache (7.6 percent) and lymph node enlargement (6.1 percent). Of 1490 people interviewed 90.4 percent knew tsetse flies and 89.8 percent had been bitten by tsetse flies. The majority (86.6 percent) of the respondents knew that sleeping sickness is transmitted through a tsetse bite. Activities that exposed people to tsetse bites included working in tsetse infested bushes/forests, grazing livestock in tsetse infested areas and hunting game animals. In conclusion, communities living in and around SENAPA were knowledgeable about tsetse and sleeping sickness. The communities can thus understand and support community based tsetse and sleeping sickness control programmes to ensure success.

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

[See also **31**: 14353, 14404]

14368. **Cano, J., Descalzo, M. A., Ndong-Mabale, N., Ndong-Asumu, P., Bobuakasi, L., Nzambo-Ondo, S., Benito, A. & Roche, J., 2007**. Predicted distribution and movement of *Glossina palpalis palpalis* (Diptera: *Glossinidae*) in the wet and dry seasons in the Kogo trypanosomiasis focus (Equatorial Guinea). *Journal of Vector Ecology,* **32** (2): 218-225.

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The aim of this study was to predict the distribution and movement of populations of the tsetse fly, *Glossina palpalis palpalis* (Diptera: *Glossinida*e), in the wet and dry seasons and to analyze the impact of the use of mono-pyramidal traps on fly populations in the Kogo

focus in 2004 and 2005. Three *Glossina* species are present in Kogo: *Glossina palpalis palpalis*, the major HAT vector in West-Central Africa, *Glossina caliginea*, and *Glossina tabaniformis*. The apparent density (AD) of *G. p. palpalis* clearly fell from 1.23 tsetse/trap/day in July 2004 to 0.27 in December 2005. A significant reduction in the mean AD for this species was noted between seasons and years. The diversity of *Glossina* species was relatively low at all the sampling points; *G. p. palpalis* clearly predominated over the other species and significantly dropped as a consequence of control activities. The predictive models generated for the seasonal AD showed notable differences not only in the density but in the distribution of the *G. p. palpalis* population between the rainy and dry season. The mono-pyramidal traps have proven to be an effective instrument for reducing the density of the tsetse fly populations, although given that the Kogo trypanosomiasis focus extends from the southern Equatorial Guinea to northern Gabon, interventions need to be planned on a larger scale, involving both countries, to guarantee the long-term success of control.

14369. **Coustou, V., Biran, M., Breton, M., Guegan, F., Riviere, L., Plazolles, N., Nolan, D., Barrett, M. P., Franconi, J. M. & Bringaud, F., 2008.** Glucose-induced remodelling of intermediary and energy metabolism in procyclic *Trypanosoma brucei*. *Journal of Biological Chemistry*. **In press; corrected proof**.

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The procyclic form of *Trypanosoma brucei* is a parasitic protozoan that normally dwells in the midgut of its insect vector. *In vitro*, this parasite prefers D-glucose to L-proline as a carbon source, although this amino acid is the main carbon source available in its natural habitat. Here we investigate how L-proline is metabolized in glucose-rich and glucose-depleted conditions. Analysis of the excreted end products of ^{13}C-enriched L-proline metabolism showed that the amino acid is converted into succinate or L-alanine depending on the presence or the absence of D-glucose, respectively. The fact that the pathway of L-proline metabolism was truncated in glucose-rich conditions was confirmed by the analysis of 13 separate RNAi-harbouring or knockout cell lines affecting different steps of this pathway. For instance, RNA interference studies revealed the loss of succinate dehydrogenase activity to be conditionally lethal, only in the absence of D-glucose, confirming that in glucose-depleted conditions, L-proline needs to be converted beyond succinate. In addition, depletion of the F0/F1-ATP synthase activity by RNA interference led to cell death in glucose-depleted medium, but not in glucose-rich conditions. This implies that, in the presence of D-glucose, the importance of the F0/F1-ATP synthase is diminished and ATP is produced by substrate level phosphorylation. We conclude that, trypanosomes develop an elaborate adaptation of their energy production pathways in response to carbon source availability.

14370. **Fridberg, A., Olson, C. L., Nakayasu, E. S., Tyler, K. M., Almeida, I. C. & Engman, D. M., 2008**. Sphingolipid synthesis is necessary for kinetoplast segregation and cytokinesis in *Trypanosoma brucei*. *Journal of Cell Science,* **121** (4): 522-535.

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Sphingolipids and their metabolites have been thought crucial for cell growth and cell cycle progression, membrane and protein trafficking, signal transduction, and formation of lipid rafts; however, recent studies in trypanosomes point to the dispensability of sphingolipids in some of these processes. In this study, we explore the requirements for *de novo* sphingolipid biosynthesis in the insect life cycle stage of the African trypanosome *Trypanosoma brucei* by inhibiting the enzyme serine palmitoyltransferase (SPT2) by using RNA interference or treatment with a potent SPT2 inhibitor myriocin. Mass spectrometry revealed that upon SPT2 inhibition, the parasites contained substantially reduced levels of inositolphosphorylceramide. Although phosphatidylcholine and cholesterol levels were increased to compensate for this loss, the cells were ultimately not viable. The most striking result of sphingolipid reduction in procyclic *T. brucei* was aberrant cytokinesis, characterized by incomplete cleavage-furrow formation, delayed kinetoplast segregation and emergence of cells with abnormal DNA content. Organelle replication continued despite sphingolipid depletion, indicating that sphingolipids act as second messengers regulating cellular proliferation and completion of cytokinesis. Distention of the mitochondrial membrane, formation of multilamellar structures within the mitochondrion and near the nucleus, accumulation of lipid bodies and, less commonly, disruption of the Golgi complex were observed after prolonged sphingolipid depletion. These findings suggest that some aspects of vesicular trafficking may be compromised. However, flagellar membrane targeting and the association of the flagellar membrane protein calflagin with detergent-resistant membranes were not affected, indicating that the vesicular trafficking defects were mild. Our studies indicate that sphingolipid biosynthesis is vital for cell cycle progression and cell survival, but not essential for the normal trafficking of flagellar membrane-associated proteins or lipid raft formation in procyclic *T. brucei*.

14371. **Gibson, W., Peacock, L., Ferris, V., Williams, K. & Bailey, M., 2008**. The use of yellow fluorescent hybrids to indicate mating in *Trypanosoma brucei*. *Parasites and Vectors,* **1** (1): 4.

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Trypanosoma brucei undergoes genetic exchange in its insect vector, the tsetse fly, by an unknown mechanism. The difficulties of working with this experimental system of genetic exchange have hampered investigation, particularly because the trypanosome life cycle stages involved cannot be cultured *in vitro* and therefore must be examined in the insect. Searching for small numbers of hybrid trypanosomes directly in the fly has become possible through the incorporation of fluorescent reporter genes, and we have previously carried out a successful cross using a reporter-repressor strategy. However, we could not be certain that all fluorescent trypanosomes observed in that cross were hybrids, due to mutations of the repressor leading to spontaneous fluorescence, and we have therefore developed an alternative strategy. To visualize the production of hybrids in the fly, parental trypanosome clones were transfected with a gene encoding Green Fluorescent Protein (GFP) or Red Fluorescent Protein (RFP). Co-infection of flies with red and green fluorescent parental

trypanosomes produced yellow fluorescent hybrids, which were easily visualized in the fly salivary glands. Yellow trypanosomes were not seen in midgut or proventricular samples and first appeared in the glands as epimastigotes as early as 13 days after fly infection. Cloned progeny originating from individual salivary glands had yellow, red, green or no fluorescence and were confirmed as hybrids by microsatellite, molecular karyotype and kinetoplast (mitochondrial) DNA analyses. Hybrid clones showed biparental inheritance of both nuclear and kinetoplast genomes. While segregation and reassortment of the reporter genes and microsatellite alleles were consistent with Mendelian inheritance, flow cytometry measurement of DNA content revealed both diploid and polyploid trypanosomes among the hybrid progeny clones. The strategy of using production of yellow hybrids to indicate mating in trypanosomes provides a robust and unequivocal system for analysis of genetic exchange. Mating occurred with high frequency in these experimental crosses, limited only by the ability of both parental trypanosomes to invade the salivary glands. Yellow hybrids appeared as soon as trypanosomes invaded the salivary glands, implicating the short, unattached epimastigote as the sexual stage. The recovery of diploid, triploid and tetraploid hybrids in these crosses was surprising as genetic markers appeared to have been inherited according to Mendelian rules. As the polyploid hybrids could have been produced from fusion of unreduced gametes, there is no fundamental conflict with a model of genetic exchange involving meiosis.

14372. **Hu, C., Rio, R. V., Medlock, J., Haines, L. R., Nayduch, D., Savage, A. F., Guz, N., Attardo, G. M., Pearson, T. W., Galvani, A. P. & Aksoy, S., 2008**. Infections with immunogenic trypanosomes reduce tsetse reproductive fitness: potential impact of different parasite strains on vector population structure. *PLoS Neglected Tropical Diseases,* **2** (3): e192.

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The parasite *Trypanosoma brucei rhodesiense* and its insect vector *Glossina morsitans morsitans* were used to evaluate the effect of parasite clearance (resistance) as well as the cost of midgut infections on tsetse host fitness. Tsetse flies are viviparous and have a low reproductive capacity, giving birth to only 6-8 progeny during their lifetime. Thus, small perturbations to their reproductive fitness can have a major impact on population densities. We measured the fecundity (number of larval progeny deposited) and mortality in parasite-resistant tsetse females and untreated controls and found no differences. There was, however, a typanosome-specific impact on midgut infections. Infections with an immunogenic parasite line that resulted in prolonged activation of the tsetse immune system delayed intrauterine larval development resulting in the production of fewer progeny over the fly's lifetime. In contrast, parasitism with a second line that failed to activate the immune system did not impose a fecundity cost. Coinfections favoured the establishment of the immunogenic parasites in the midgut. We show that a decrease in the synthesis of *Glossina* milk gland protein (*Gmm*Mgp), a major female accessory gland protein associated with larvagenesis, likely contributed to the reproductive lag observed in infected flies. Mathematical analysis of our empirical results indicated that infection with the immunogenic trypanosomes reduced tsetse fecundity by 30 percent relative to infections with the non-immunogenic strain. We estimate that a moderate infection prevalence of about 26 percent with immunogenic

parasites has the potential to reduce tsetse populations. Potential repercussions for vector population growth, parasite-host coevolution, and disease prevalence are discussed.

14373. **Konnai, S., Mekata, H., Odbileg, R., Simuunza, M., Chembensof, M., Witola, W. H., Tembo, M. E., Chitambo, H., Inoue, N., Onuma, M. & Ohashi, K., 2008.** Detection of *Trypanosoma brucei* in field-captured tsetse flies and identification of host species fed on by the infected flies. *Vector Borne and Zoonotic Diseases.* **In press; corrected proof**

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The prevalence of trypanosome infections in tsetse flies in the Chiawa area of Lower Zambezi in Zambia, with endemic trypanosomosis, was determined by a polymerase chain reaction (PCR) method that allowed the detection of trypanosome DNA and determination of the type of animal host fed on by the tsetse fly *Glossina pallidipes*, using tsetse-derived DNA extracts as templates. Ninety *G. pallidipes* (82 females and 8 males; 18.3 percent) of the 492 flies captured by baited biconical traps tested positive for the presence of *Trypanosoma brucei* species genomic DNA. Of the 90 *T. brucei*-positive flies, 47 (52.2 percent) also tested positive for vertebrate mitochondrial DNA. Sequence analysis of the vertebrate mitochondrial DNA amplicons established that they originated from 8 different vertebrate species, namely, human (*Homo sapiens*), African elephant (*Loxodonta cyclotis*), African buffalo (*Syncerus caffer*), waterbuck (*Kobus ellipsiprymnus*), roan antelope (*Hippotragus equinus*), greater kudu (*Tragelaphus strepsiceros*), warthog (*Phacochoerus africanus*), and goat (*Capra hircus*). Furthermore, to investigate the prevalence of trypanosome infections in domestic goats in the same area where trypanosomes had been detected in tsetse flies, a total of 86 goats were randomly selected from six different herds. Among the selected goats, 36 (41.9 percent) were found to be positive for *T. brucei* species. This combined detection method would be an ideal approach not only for mass screening for infection prevalence in tsetse populations, but also for the prediction of natural reservoirs in areas endemic for trypanosomosis.

14374. **MacLeod, E. T., Maudlin**, I., & **Welburn, S. C., 2008**. Effects of cyclic nucleotides on midgut infections and maturation of *T. b. brucei* in *G. m. morsitans. Parasites and Vectors,* **1***: 5.*

Centre for Infectious Diseases, The University of Edinburgh, Edinburgh, EH25 9RG, UK.

Cyclic nucleotide signalling through cyclic adenosine monophosphate (cAMP) is thought to play an important role in the transformation of the long slender (dividing) form to the short-stumpy (arrested) form in the mammalian bloodstream but the role of cyclic nucleotides in the tsetse-based part of the trypanosome life cycle is unknown. In a series of *in vivo* experiments, it was found that cyclic guanosine monophosphate (cGMP) but not cAMP could induce significantly higher rates of midgut infection in tsetse. Continuous feeding of either cGMP or cAMP to tsetse had no effect on rates of maturation of established midgut infections suggesting that these two parts of the life cycle in tsetse are not linked.

14375. **Malele, II, Kinung'hi, S. M., Nyingilili, H. S., Matemba, L. E., Sahani, J. K., Mlengeya, T. D., Wambura, M. & Kibona, S. N., 2007**. *Glossina* dynamics in and around the sleeping sickness endemic Serengeti ecosystem of northwestern Tanzania. *Journal of Vector Ecology,* **32** (2): 263-268.

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We investigated the dynamics of *Glossina* species and their role in the transmission of trypanosomiasis in the sleeping sickness endemic Serengeti ecosystem, northwestern Tanzania. The study investigated *Glossina* species composition, trap density, trypanosome infection rates, and the diversity of trypanosomes infecting the species. Tsetse were trapped using monopyramidal traps in the mornings between 06:00 to 11:00 and transported to the veterinary laboratory in Serengeti National Park where they were sorted into species and sex, and dissected microscopically to determine trypanosome infection rates. Age estimation of dissected flies was also conducted concurrently. Tsetse samples positive for trypanosomes were subjected to PCR to determine the identity of the detected trypanosomes. Out of 2,519 tsetse trapped, 1,522 (60.42 percent) were *G. swynnertoni*, 993 (39.42 percent) were *G. pallidipes*, three (0.12 percent) were *G. m. morsitans*, and one (0.04 percent) was *G. brevipalpis*. The trap density for G. swynnertoni was between 1.40 and 14.17 while that of *G. pallidipes* was between 0.23 and 9.70. Out of 677 dissected *G. swynnertoni*, 63 flies (9.3 percent) were infected, of which 62 (98.4 percent) were females. A total of 199 *G. pallidipes* was also dissected but none was infected. There was no significant difference between the apparent densities of *G. swynnertoni* compared to that of *G. pallidipes* ($t = 1.42$, $p = 0.18$). Molecular characterization of the 63 infected *G. swynnertoni* midguts showed that 19 (30.2 percent) were trypanosomes associated with suid animals while nine (14.3 percent) were trypanosomes associated with bovid animals and five samples (7.9 percent) had *T. brucei s.l* genomic DNA. Thirty (47.6 percent) tsetse samples could not be identified. Subsequent PCR to differentiate between *T. b. brucei* and *T. b. rhodesiense* showed that all five samples that contained the *T. brucei s.l* genomic DNA were positive for the SRA molecular marker indicating that they were *T. b. rhodesiense*. These results indicate that *G. swynnertoni* plays a major role in the transmission of trypanosomiasis in the area and that deliberate and sustainable control measures should be initiated and scaled up.

14376. **Mamoudou, A., Zoli, A., Hamadama, H., Abah, S., Geerts, S., Clausen, P. H., Zessin, K. H., Kyule, M. & van den Bossche, P., 2008**. Seasonal distribution and abundance of tsetse flies (*Glossina* spp.) in the Faro and Deo Division of the Adamaoua Plateau in Cameroon. *Medical and Veterinary Entomology,* **22** (1): 32-36.

Faculty of Agronomy and Agricultural Sciences, University of Dschang, Dschang, Cameroon, and Institute for Parasitology and Tropical Veterinary Medicine, Free University of Berlin, Berlin, Germany.

Ten years after the large-scale tsetse control campaigns in the important cattle rearing areas of the Faro and Deo Division of the Adamaoua Plateau in Cameroon, the seasonal distribution and abundance of tsetse flies (*Glossina* spp.) were determined. During a period of

12 consecutive months (January-December 2005), the tsetse population was monitored along four trap transects consisting of a total of 32 traps and two flyround transects traversing the study area, which comprised the tsetse-infested valley, a buffer zone and the supposedly tsetse-free plateau. Throughout the study period, a total of 2,195 *Glossina morsitans submorsitans* and 23 *Glossina tachinoides* were captured in the traps and 1007 *G. m. submorsitans* (78.8 percent male flies) were captured along the flyround transects. All *G. tachinoides* and almost all *G. m. submorsitans* were captured in the valley. Five *G. m. submorsitans* were captured in traps located in the buffer zone, whereas no flies were captured in traps located on the plateau. The index of apparent abundance (IAA) of *G. m. submorsitans* was substantially higher in the areas close to game reserves. In the remaining part of the valley, where wildlife is scarce and cattle are present during transhumance (dry season), the IAA of tsetse was substantially lower. In this part of the valley, the abundance of tsetse seemed to be associated with the presence of cattle, with the highest IAA during transhumance when cattle are present and the lowest apparent abundance during the rainy season when cattle have moved to the plateau. It is concluded that the distribution of tsetse in a large part of the valley undergoes substantial seasonal changes depending on the presence or absence of cattle. The repercussions of those findings for the control of tsetse in the valley and the probability of reinvasion of the plateau are discussed.

14377. **Otranto, D., Stevens, J. R., Cantacessi, C. & Gasser, R. B., 2008**. Parasite transmission by insects: a female affair? *Trends in Parasitology,* **24** (3): 116-120.

Department of Veterinary Public Health, University of Bari, 70010, Valenzano (Bari), Italy. [d.otranto@veterinaria.uniba.it].

Understanding the relationship between the gender of insects and their ability to act as vectors of insect-borne diseases (IBDs) could provide clues as to the origin of the intimate interplay among insect, pathogen and vertebrate hosts. The vector activity of several species of blood-feeding insects is linked to adult females. Interestingly, the only known exception is the transmission of canine and human thelaziosis by a male Dipteran fly. This biological difference raises the question as to whether the parasitic behaviour of male and female insects transmitting IBDs is an expression of a co-evolution of vectors and pathogens.

14378. **Simo, G., Njiokou, F., Mbida Mbida, J. A., Njitchouang, G. R., Herder, S., Asonganyi, T. & Cuny, G., 2008**. Tsetse fly host preference from sleeping sickness foci in Cameroon: epidemiological implications. *Infection, Genetics and Evolution,* **8** (1): 34-39.

Medical Research Centre, Institute of Medical Research and Medicinal Plants Studies (IMPM/MINRESI), P.O. Box 6163 Yaounde, Cameroon. [gustavsca@yahoo.fr].

To determine the tsetse fly host preferences in two sleeping sickness foci of southern Cameroon, four entomological surveys (two in each focus) were carried out. For the whole study, 4,929 tsetse flies were caught: 3,933 (79.8 percent) *Glossina palpalis palpalis*, 626 (12.7 percent) *Glossina pallicera pallicera*, 276 (5.6 percent) *Glossina nigrofusca* and 94 (1.9 percent) *Glossina caliginea.* One hundred and thirty-eight blood meals were collected and the

origin of 118 (85.5 percent) meals was successfully identified: 38.4 percent from man, 23.9 percent from pig, 20.3 percent from sitatunga (*Tragelaphus spekeii*), 2.2 percent from sheep and 0.7 percent from golden cat (*Profilis aurata*). The number of *Glossina palpalis palpalis* with man blood meals is more important in the Human African Trypanosomiasis (HAT) focus showing endemic evolution (Campo) than in the focus (Bipindi) presenting a flare up of the disease. The consideration of both results of the prevalence of *Trypanosoma brucei gambiense* in vertebrate hosts and those of the tsetse fly host preferences indicates a wild animal reservoir of Gambian sleeping sickness and three transmission cycles (human, domestic and wild animals' cycles) in southern Cameroon HAT foci.

14379. **Torr, S. J., Prior, A., Wilson, P. J. & Schofield, S., 2007**. Is there safety in numbers? The effect of cattle herding on biting risk from tsetse flies. *Medical and Veterinary Entomology,* **21** (4): 301-311.

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In sub-Saharan Africa, tsetse (*Glossina* spp.) transmit species of *Trypanosoma* which threaten 45-50 million cattle with trypanosomiasis. These livestock are subject to various herding practices which may affect biting rates on individual cattle and hence the probability of infection. In Zimbabwe, studies were made of the effect of herd size and composition on individual biting rates by capturing tsetse as they approached and departed from groups of one to 12 cattle. Flies were captured using a ring of electrocuting nets and bloodmeals were analysed using DNA markers to identify which individual cattle were bitten. Increasing the size of a herd from one to 12 adults increased the mean number of tsetse visiting the herd four-fold and the mean feeding probability from 54 percent to 71 percent; the increased probability with larger herds was probably a result of fewer flies per host, which, in turn, reduced the hosts' defensive behaviour. For adults and juveniles in groups of four to eight cattle, > 89 percent of bloodmeals were from the adults, even when these comprised just 13 percent of the herd. For groups comprising two oxen, four cows/heifers and two calves, a grouping that reflects the typical composition of communal herds in Zimbabwe, approximately 80 percent of bloodmeals were from the oxen. Simple models of entomological inoculation rates suggest that cattle herding practices may reduce individual trypanosomiasis risk by up to 90 percent. These results have several epidemiological and practical implications. First, the gregarious nature of hosts needs to be considered in estimating entomological inoculation rates. Secondly, heterogeneities in biting rates on different cattle may help to explain why disease prevalence is frequently lower in younger/smaller cattle. Thirdly, the cost and effectiveness of tsetse control using insecticide-treated cattle may be improved by treating older/larger hosts within a herd. In general, the patterns observed with tsetse appear to apply to other genera of cattle-feeding Diptera (*Stomoxys*, *Anopheles*, *Tabanidae*) and thus may be important for the development of strategies for controlling other diseases affecting livestock.

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE

[See also **31**: 14345, 14348, 14359]

14380. **Courtin, F., Jamonneau, V., Duvallet, G., Garcia, A., Coulibaly, B., Doumenge, J. P., Cuny, G. & Solano, P., 2008**. Sleeping sickness in West Africa (1906-2006): changes in spatial repartition and lessons from the past. *Tropical Medicine and International Health,* **13** (3): 334-344.

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The objective of this article is to review the geography and history of sleeping sickness (Human African trypanosomiasis; HAT) over the past 100 years in West Africa, to identify priority areas for sleeping sickness surveillance and areas where HAT no longer seems active. The history and geography of HAT were summarized based on a review of old reports and recent publications and on recent results obtained from medical surveys conducted in West Africa up to 2006. Active HAT foci seem to have moved from the North to the South. Endemic HAT presently appears to be limited to areas where annual rainfall exceeds 1,200 mm, although the reasons for this remain unknown. There has also been a shift towards the south of the isohyets and of the northern distribution limit of tsetse. Currently, the most severely affected countries are Guinea and Ivory Coast, whereas the northern countries seem less affected. However, many parts of West Africa still lack information on HAT and remain to be investigated. Of particular interest are the consequences of the recent political crisis in Ivory Coast and the resulting massive population movements, given the possible consequences on HAT in neighbouring countries.

14381. **Elrayah, I. E., Rhaman, M. A., Karamalla, L. T., Khalil, K. M. & Buscher, P., 2007**. Evaluation of serodiagnostic tests for *T. b. gambiense* human African trypanosomiasis in southern Sudan. *Eastern Mediterranean Health Journal,* **13** (5): 1098-1107.

Trypanosomiasis Unit, Tropical Medicine Research Institute, Khartoum, Sudan. [intisar62@yahoo.com].

A survey was conducted in a low-endemic and in a non-endemic area of Sudan to evaluate the specificity and efficiency of different serological antibody detection techniques for *Trypanosoma brucei gambiense*. Comparisons were made of the card agglutination test for trypanosomiasis (CATT) on diluted blood, on diluted plasma and on eluates from blood dried on filter paper, the LATEX test on diluted plasma and an ELISA on diluted plasma and filter paper. The specificities of all the serological tests were not significantly different from CATT on diluted blood (99.5 percent). The specificity of CATT on diluted blood was similar (99.3 percent). The highest sensitivities (100 percent) were observed with CATT on diluted

blood and with CATT and LATEX on diluted plasma. CATT on diluted blood was more cost-efficient than the classic test, CATT on whole blood.

14382. **Fevre, E. M., Odiit, M., Coleman, P. G., Woolhouse, M. E. & Welburn, S. C., 2008**. Estimating the burden of *rhodesiense* sleeping sickness during an outbreak in Serere, eastern Uganda. *BMC Public Health,* **8**: 96.

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Zoonotic sleeping sickness, or HAT (Human African Trypanosomiasis), caused by infection with *Trypanosoma brucei rhodesiense*, is an under-reported and neglected tropical disease. Previous assessments of the disease burden expressed as Disability-Adjusted Life Years (DALYs) for this infection have not distinguished *T. b. rhodesiense* from infection with the related, but clinically distinct *Trypanosoma brucei gambiense* form. *T. b. rhodesiense* occurs focally, and it is important to assess the burden at the scale at which resource-allocation decisions are made. The burden of *T. b. rhodesiense* was estimated during an outbreak of HAT in Serere, Uganda. We identified the unique characteristics affecting the burden of *rhodesiense* HAT such as age, severity, level of under-reporting and duration of hospitalisation, and use field data and empirical estimates of these to model the burden imposed by this and other important diseases in this study population. While we modelled DALYs using standard methods, we also modelled uncertainty of our parameter estimates through a simulation approach. We distinguish between early and late stage HAT morbidity, and used disability weightings appropriate for the *T. b. rhodesiense* form of HAT. We also use a model of under-reporting of HAT to estimate the contribution of un-reported mortality to the overall disease burden in this community, and estimate the cost-effectiveness of hospital-based HAT control. The results showed that under-reporting accounts for 93 percent of the DALY estimate of *rhodesiense* HAT. The ratio of reported malaria cases to reported HAT cases in the same health unit was 133:1, however, the ratio of DALYs was 3:1. The age productive function curve had a close correspondence with the HAT case distribution, and HAT cases occupied more patient admission time in Serere during 1999 than all other infectious diseases other than malaria. The DALY estimate for HAT in Serere shows that the burden is much greater than might be expected from its relative incidence. Hospital based control in this setting appears to be highly cost-effective, highlighting the value of increasing coverage of therapy and reducing under-reporting. The results demonstrate the utility of calculating DALYs for neglected diseases at the local decision making level, and emphasize the importance of improved reporting systems for acquiring a better understanding of the burden of neglected zoonotic diseases.

14383. **Lutumba, P., Meheus, F., Robays, J., Miaka, C., Kande, V., Buscher, P., Dujardin, B. & Boelaert, M., 2007**. Cost-effectiveness of algorithms for confirmation test of Human African Trypanosomiasis. *Emerging Infectious Diseases,* **13** (10): 1484-1490.

Programmed National de Lutte contre la Trypanosomiase Humaine Africaine, Kinshasa, Democratic Republic of Congo; Institute of Tropical Medicine,

Antwerp, Belgium; Royal Tropical Institute, Amsterdam, the Netherlands; and Ecole de Santé Publique, Universit*é* Libre de Bruxelles, Brussels, Belgium. [mboelaert@itg.be].

The control of *Trypanosoma brucei gambiense* human African trypanosomiasis (HAT) is compromised by low sensitivity of the routinely used parasitologic confirmation tests. More sensitive alternatives, such as mini-anion exchange centrifugation technique (mAECT) or capillary tube centrifugation (CTC), are more expensive. We used formal decision analysis to assess the cost-effectiveness of alternative HAT confirmation algorithms in terms of cost per life saved. The effectiveness of the standard method, a combination of lymph node puncture (LNP), fresh blood examination (FBE), and thick blood film (TBF), was 36.8 percent; the LNP-FBE-CTC-mAECT sequence reached almost 80 percent. The cost per person examined ranged from Euro 1.56 for LNP-FBE-TBF to Euro 2.99 for LNP-TBF-CTC-mAECT-CATT (card agglutination test for trypanosomiasis) titration. LNP-TBF-CTC-mAECT was the most cost-effective in terms of cost per life saved. HAT confirmation algorithms that incorporate concentration techniques are more effective and efficient than the algorithms that are currently and routinely used by several *T. b. gambiense* control programmes.

14384. **Maclean, L., Odiit, M., Macleod, A., Morrison, L., Sweeney, L., Cooper, A., Kennedy, P. G. & Sternberg, J. M., 2007**. Spatially and genetically distinct African trypanosome virulence variants defined by host interferon-gamma response. *Journal of Infectious Diseases,* **196** (11): 1620-1628.

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We describe two spatially distinct foci of human African trypanosomiasis in eastern Uganda. The Tororo and Soroti foci of *Trypanosoma brucei rhodesiense* infection were genetically distinct as characterized by six microsatellite and one minisatellite polymorphic markers and were characterized by differences in disease progression and host-immune response. In particular, infections with the Tororo genotype exhibited an increased frequency of progression to and severity of the meningoencephalitic stage and higher plasma interferon (IFN)- gamma concentration, compared with those with the Soroti genotype. We propose that the magnitude of the systemic IFN- gamma response determines the time at which infected individuals develop central nervous system infection and that this is consistent with the recently described role of IFN- gamma in facilitating blood-brain barrier transmigration of trypanosomes in an experimental model of infection. The identification of trypanosome isolates with differing disease progression phenotypes provides the first field-based genetic evidence for virulence variants in *T. brucei rhodesiense*.

14385. **Zézé, G.D., N'dri, L., Coulibaly, B. & Sane, B., 2007**. Entomological assessment of sleeping sickness in the focus of Bonon (Côte d'Ivoire)**.** *Belgian Journal of Entomology***, 9: 15-28.**

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Human African Trypanosomosis or sleeping sickness represents a problem of public health in Africa. In Côte d'Ivoire, Human African Trypanosomosis occurs in more or less active focuses located in the forest zone, among which the Bonon one shows a worrying situation. During two seasonal periods (rainy season in November 2000, dry season in January 2001), *Glossina* specimens were collected by the means of Vavoua traps within 320 geo-referenced sites selected in different biotopes (places of activities, water supplying places, habitation places, access ways, hamlets) used by sick persons. *Glossina palpalis palpalis* was the only species of *Glossina* observed in the captures, irrespective of the biotope. The species was the most abundant in the access ways (8.5 flies/trap/day) and showed the lowest densities in places of habitation (1.4 flies/trap/day) located mainly in the town. With each of the biotypes *Glossina palpalis palpalis* could become infected with trypanosomes as evidenced by the high proportion of young patterns (34.29 percent to 45.33 percent) observed in these biotopes. However, the places for supplying water and for other activities seemed to be transmission areas of the sleeping sickness in the focus of Bonon.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **31**: 14348, 14354]

14386. **Bentivoglio, M. & Kristensson, K., 2007**. Neural-immune interactions in disorders of sleep-wakefulness organization. *Trends in Neuroscience,* **30** (12): 645-652.

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Novel findings on the effects of inflammatory molecules on neuronal circuits, and on molecular interactions between immunity and sleep, in health and disease, shed light on the pathogenesis of disorders of past (encephalitis lethargica) and present concern (human African trypanosomiasis and narcolepsy), which share alterations in sleep-wakefulness transitions. Although these three disorders differ in etiology, synaptic interactions with immune-response-derived molecules could play a pathogenetic role. Knowledge obtained on neural-immune interplay during senescence also has implications for age-related sleep dysregulation, which is common in the elderly population. Altogether, the data indicate that cell groups implicated in the regulation of sleep and wakefulness, circadian timing, and their interactions could be sensitive to synaptic effects of immune molecules.

14387. **Checchi, F., Filipe, J. A., Haydon, D. T., Chandramohan, D. & Chappuis, F., 2008**. Estimates of the duration of the early and late stage of *gambiense* sleeping sickness. *BMC Infectious Diseases,* **8**: 16.

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The durations of untreated stage 1 (early stage, haemo-lymphatic) and stage 2 (late stage, meningo-encephalitic) human African trypanosomiasis (sleeping sickness) due to *Trypanosoma brucei gambiense* are poorly quantified, but key to predicting the impact of screening on transmission. Here, we outline a method to estimate these parameters. We first model the duration of stage 1 through survival analysis of untreated serological suspects detected during Médecins Sans Frontières interventions in Uganda and Sudan. We then deduce the duration of stage 2 based on the stage 1 to stage 2 ratio observed during active case detection in villages within the same sites. Survival in stage 1 appears to decay exponentially (daily rate = 0.0019; mean stage 1 duration = 526 days [95 percent CI 357 to 833]), possibly explaining past reports of abnormally long duration. Assuming epidemiological equilibrium, we estimate a similar duration of stage 2 (500 days [95 percentCI 345 to 769]), for a total of nearly three years in the absence of treatment. In conclusion robust estimates of these basic epidemiological parameters are essential to formulating a quantitative understanding of sleeping sickness dynamics, and will facilitate the evaluation of different possible control strategies.

14388. **Dauvilliers, Y., Bisser, S., Chapotot, F., Vatunga, G., Cespuglio, R., Josenando, T. & Buguet, A., 2008.** Hypocretin and human African trypanosomiasis. *Sleep,* **31** (3): 348-354.

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The objectives of this study were to detail clinical and polysomnographic characteristics in patients affected with *Trypanosoma brucei gambiense* (*T b. g*.) human African trypanosomiasis (HAT) at different stages of evolution and to measure and compare cerebrospinal fluid (CSF) levels of hypocretin-1 with narcoleptic patients and neurologic controls. Twenty-five untreated patients affected with *T. b. g*. HAT were included. The patients were evaluated using a standardized clinical evaluation and a specific interview on sleep complaints. Diagnosis of stages I and II and intermediate stage was performed by CSF cell count and/or presence of trypanosomes: four patients were classified as stage II, 13 stage I, and eight "intermediate" stage. Seventeen untreated patients completed continuous 24-hour polysomnography. We measured CSF levels of hypocretin-1 in all patients at different stages and evolutions, and we compared the results with 26 patients with narcolepsy-cataplexy and 53 neurologic controls. CSF hypocretin-1 levels were significantly higher in *T.b.g*. HAT (423.2 +/- 119.7 pg/mL) than in narcoleptic patients (40.16 +/- 60.18 pg/ mL) but lower than in neurologic controls (517.32 +/- 194.5 pg/mL). One stage I patient had undetectable hypocretin levels and 1 stage II patient showed intermediate levels, both patients (out of three patients) reporting excessive daytime sleepiness but without evidence for an association with narcolepsy. No differences were found in CSF hypocretin levels between patients with HAT stages; however, the presence of major sleep-wake cycle disruptions was significantly associated with lower CSF hypocretin-1 level with a same tendency for the number of sleep-onset rapid eye movement periods. The present investigation is not in favour of a unique implication of the hypocretin system in *T. b. g.* HAT. However, we propose that dysfunction of the hypothalamic hypocretin region may participate in sleep disturbances observed in African trypanosomiasis.

14389. **Kaur, R., Gupta, V. K., Dhariwal, A. C., Jain, D. C. & Shiv, L., 2007**. A rare case of trypanosomiasis in a two month old infant in Mumbai, India. *Journal of Communicable Diseases,* **39** (2): 71-74.

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Human Trypanosomiasis is a rare occurrence in India. In the cases reported so far the disease causative species have been the species infective to animals viz., *Trypanosoma lewisi* and *Trypanosoma evansi*. These animal species usually non pathogenic in humans can acquire the desired virulence and emerge as human pathogens causing serious disease, in the right combination of environmental, host related and organism related factors. We report here a case of trypanosomiasis caused by the rodent parasite *T. lewisi* in a two months old infant in urban Mumbai. Under the fastly changing environmental scenario there is an urgent need to be prepared for the emerging zoonoses. Any unusual disease occurrence in a given geographical area acquires a special significance in this context and should be reported to assess its public health importance and be prepared to deal with the consequent challenges posed, if any.

14390. **Kennedy, P. G., 2008**. Diagnosing central nervous system trypanosomiasis: two stage or not to stage? *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **102** (4): 306-307.

Department of Neurology, Division of Clinical Neurosciences, University of Glasgow, Institute of Neurological Sciences, Southern General Hospital, Glasgow G51 4TF, UK.

One of the most difficult issues in the management of patients with human African trypanosomiasis (HAT) is the reliable distinction between early-stage disease and late-stage disease where the trypanosomes have traversed the blood-brain barrier to enter the central nervous system (CNS). Currently, there is no universal consensus for the cerebrospinal fluid (CSF) findings that define late-stage HAT and provide the rationale for treatment with toxic drugs for CNS disease. Whilst some clinicians use the WHO CSF criteria, others use alternative findings to define late-stage disease. Novel analyses in CSF of patients are urgently required for more accurate diagnostic staging.

14391. **Perez-Morga, D., 2007**. Resistance to infection by African trypanosomes. *Bulletin et mémoires de l'Académie royale de médecine de Belgique*, **162** (7-9): 381-386.

Laboratoire de Parasitologie Moléculaire, Institute de Biologie et Médecine Moléculaires IBMM, ULB, Gosselies, Belgium. [David.Perez-Morga@ulb.ac.be].

African trypanosomes (prototype: *Trypanosoma brucei*) are protozoan flagellates that infect a wide range of different mammals. In humans, these parasites have to counteract innate immunity because human serum possesses efficient trypanolytic activity. Resistance to this activity has arisen in two *T. brucei* subspecies, termed *T. b. rhodesiense* and *T. b.*

gambiense, allowing them to infect humans where they cause sleeping sickness in East and West Africa respectively. The study of the mechanism by which *T. b. rhodesiense* escapes lysis by human serum led to the identification of the trypanolytic factor, which turned out to be an ionic pore-forming apolipoprotein associated with some HDL particles.

(c) TREATMENT

[See also **31**: 14359, 14360, 14534]

14392. **Chappuis, F., 2007**. Melarsoprol-free drug combinations for second-stage Gambian sleeping sickness: the way to go. *Clinical Infectious Diseases,* **45** (11): 1443-1445.

Travel and Migration Medicine Unit, Geneva University Hospital, and Médecins Sans Frontières, Swiss Section, 1202 Geneva, Switzerland. [francois.chappuis@hcuge.ch].

No abstract available.

14393. **Checchi, F. & Barrett, M. P., 2008**. African sleeping sickness. *Bmj,* **336** (7646): 679-680.

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, Division of Infection and Immunity, Institute of Biomedical and Life Sciences, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow G12 8TA., UK. [francesco.checchi@lshtm.ac.uk].

No abstract available.

14394. **Checchi, F., Piola, P., Ayikoru, H., Thomas, F., Legros, D. & Priotto, G., 2007**. Nifurtimox plus eflornithine for late-stage sleeping sickness in Uganda: A Case Series. *PLoS Neglected Tropical Diseases,* **1** (2): e64.

Epicentre, Paris, France.

We report on the efficacy and safety outcomes from a prospective case series of 31 late-stage *T. b. gambiense* sleeping sickness (Human African Trypanosomiasis, HAT) patients treated with a combination of nifurtimox and eflornithine (N+E) in Yumbe, northwest Uganda in 2002-2003, following on a previously reported terminated trial in nearby Omugo, in which 17 patients received the combination under the same conditions. Eligible sequential late-stage patients received 400 mg/kg/day eflornithine (Ornidyl, Sanofi-Aventis) for seven days plus 15 mg/kg/day (20 mg for children <15 years old) nifurtimox (Lampit, Bayer AG) for ten days. Efficacy (primary outcome) was monitored for 24 months post discharge. Clinical and laboratory adverse events (secondary outcome) were monitored during treatment. All 31 patients were discharged alive, but two died post-discharge of non-HAT and non-treatment causes, and one was lost to follow-up. Efficacy ranged from 90.3 percent to 100.0 percent according to analysis approach. Five patients experienced major

adverse events during treatment, and neutropenia was common (9/31 patients). Combined with the previous group of 17 trial patients, this case series yields a group of 48 patients treated with N+E, among whom no deaths judged to be treatment- or HAT-related, no treatment terminations and no relapses have been noted, a very favourable outcome in the context of late-stage disease. N+E could be the most promising combination regimen available for sleeping sickness, and deserves further evaluation.

14395. **Priotto, G., Kasparian, S., Ngouama, D., Ghorashian, S., Arnold, U., Ghabri, S. & Karunakara, U., 2007**. Nifurtimox-eflornithine combination therapy for second-stage *Trypanosoma brucei gambiense* sleeping sickness: a randomized clinical trial in Congo. *Clinical Infectious Diseases,* **45** (11): 1435-1442.

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Human African trypanosomiasis caused by *Trypanosoma brucei gambiense* is a fatal disease. Current treatment options for patients with second-stage disease are either highly toxic or impracticable in field conditions. We compared the efficacy and safety of the nifurtimox-eflornithine drug combination with the standard eflornithine regimen for the treatment of second-stage disease. A randomized, open-label, active-control, phase III clinical trial comparing two arms was conducted at the Sleeping Sickness Treatment Center, which was run by Médecins Sans Frontières, in Nkayi, Bouenza Province, Republic of Congo. Patients were screened for inclusion and randomly assigned to receive eflornithine alone (400 mg/kg/day given intravenously every 6 h for 14 days) or eflornithine (400 mg/kg/day given intravenously every 12 h for 7 days) plus nifurtimox (15 mg/kg/day given orally every 8 h for 10 days). Patients were observed for 18 months. The study's outcomes were cure and adverse events attributable to treatment. A total of 103 patients with second-stage disease were enrolled. Cure rates were 94.1 percent for the eflornithine group and 96.2 percent for the nifurtimox-eflornithine group. Drug reactions were frequent in both arms, and severe reactions affected 25.5 percent of patients in the eflornithine group and 9.6 percent of those in the nifurtimox-eflornithine group, resulting in 2 and 1 treatment suspensions, respectively. There was 1 death in the eflornithine arm and no deaths in the nifurtimox-eflornithine arm. The nifurtimox-eflornithine combination appears to be a promising first-line therapy for second-stage sleeping sickness. If our findings are corroborated by ongoing findings from additional sites (a multicenter extension of this study), the new nifurtimox-eflornithine combination therapy will mark a major and multifaceted advance over current therapies.

14396. **Priotto, G., Pinoges, L., Fursa, I. B., Burke, B., Nicolay, N., Grillet, G., Hewison, C. & Balasegaram, M., 2008**. Safety and effectiveness of first line eflornithine for *Trypanosoma brucei gambiense* sleeping sickness in Sudan: cohort study. *Bmj,* **336** (7646): 705-708.

Epicentre, 75011 Paris, France.

To assess the safety and effectiveness of eflornithine as first line treatment for human African trypanosomiasis, 1,055 adults and children newly diagnosed with second stage disease in a 16 month period were studied in Ibba, southern Sudan. The main outcome measures used were deaths, severe drug reactions, and cure at 24 months. All patients

received eflornithine for 14 days (400 mg/kg/day in adults and 600 mg/kg/day in a subgroup of 96 children). Overall, 2,824 drug reactions (2.7 per patient) occurred during hospital stay, 1 219 (43.2 percent) after the first week. Severe reactions affected 138 (13.1 percent) patients (mainly seizures, fever, diarrhoea, and bacterial infections), leading to 15 deaths. Risk factors for severe reactions included cerebrospinal fluid leucocyte counts > or =100x10(9)/l (adults: odds ratio 2.6, 95 percent confidence interval 1.5 to 4.6), seizures (adults: 5.9, 2.0 to 13.3), and stupor (children: 9.3, 2.5 to 34.2). Children receiving higher doses did not experience increased toxicity. Follow-up data were obtained for 924 (87.6 percent) patients at any follow-up but for only 533 (50.5 percent) at 24 months. Of 924 cases followed, 16 (1.7 percent) died during treatment, 70 (7.6 percent) relapsed, 15 (1.6 percent) died of disease, 403 (43.6 percent) were confirmed cured, and 420 (45.5 percent) were probably cured. The probability of event free survival at 24 months was 0.88 (0.86 to 0.91). Most (65.8 percent, 52/79) relapses and disease related deaths occurred after 12 months. Risk factors for relapse included being male (incidence rate ratio 2.42, 1.47 to 3.97) and cerebrospinal fluid leucocytosis: 20-99x10(9)/l (2.35, 1.36 to 4.06); > or =100x10(9)/l (1.87, 1.07 to 3.27). Higher doses did not yield better effectiveness among children (0.87 v 0.85, P=0.981). In conclusion, eflornithine shows acceptable safety and effectiveness as a first line treatment for human African trypanosomiasis. Relapses did occur more than 12 months after treatment. Higher doses in children were well tolerated but showed no advantage in effectiveness.

14397. **Robays, J., Raguenaud, M. E., Josenando, T. & Boelaert, M., 2008**. Eflornithine is a cost-effective alternative to melarsoprol for the treatment of second-stage human West African trypanosomiasis in Caxito, Angola. *Tropical Medicine and International Health,* **13** (2): 265-271.

Institute of Tropical Medicine, Antwerp, Belgium.

To compare the cost-effectiveness of eflornithine and melarsoprol in the treatment of human African trypanosomiasis, we used data from a Médecins Sans Frontières treatment project in Caxito, Angola to do a formal cost-effectiveness analysis, comparing the efficiency of an eflornithine-based approach with melarsoprol. Endpoints calculated were: cost per death avoided; incremental cost per additional life saved; cost per years of life lost (YLL) averted; incremental cost per YLL averted. Sensitivity analysis was done for all parameters for which uncertainty existed over the plausible range. We did an analysis with and without cost of trypanocidal drugs included. Effectiveness was 95.6 percent for melarsoprol and 98.7 percent for eflornithine. Cost/patient was US$ 504.6 for melarsoprol and US$ 552.3 for eflornithine, cost per life saved was US$ 527.5 for melarsoprol and US$ 559.8 for eflornithine without cost of trypanocidal drugs but it increases to US$ 600.4 and US$ 844.6 per patient saved and US$ 627.6 and US$ 856.1 per life saved when cost of trypanocidal drugs are included. Incremental cost-effectiveness ratio is US$ 1,596 per additional life saved and US$ 58 per additional life year saved in the baseline scenario without cost of trypanocidal drugs but it increases to US$ 8,169 per additional life saved and US$ 299 per additional life year saved if costs of trypanocidal drugs are included. In conclusion, eflornithine saves more lives than melarsoprol, but melarsoprol is slightly more cost-effective. Switching from melarsoprol to eflornithine can be considered as a cost-effective option according to the WHO choice criteria.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

14398. **Burreson, E. M., 2007**. Hemoflagellates of Oregon marine fishes with the description of new species of *Trypanosoma* and *Trypanoplasma*. *Journal of Parasitology,* **93** (6): 1442-1451.

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Of 2,122 marine fishes representing 36 species collected in the north-eastern Pacific Ocean in the vicinity of Newport, Oregon from 1971 to 1973, 541 individuals (25.5 percent) representing 8 species (22.2 percent) were infected with haemoflagellates. Four morphologically distinct trypanosomes and 3 distinct trypanoplasms were found in fishes collected offshore, but no haemoflagellates were observed in fishes from Yaquina Bay estuary. *Trypanosoma pacifica* was found in English sole *Parophrys vetulus*, Pacific sanddab *Citharichthys sordidus*, and slender sole *Lyopsetta exilis*, and survived in 5 other species after intraperitoneal injection. *Trypanosoma gargantua* was found in big skate *Raja binoculata*, and the leech *Orientobdella confluens* was able to transmit the trypanosome in experimental conditions. *Trypanosoma khani* n. sp. occurred in *P. vetulus*, petrale sole *Eopsetta jordani*, and Dover sole *Microstomus pacificus*. *Trypanosoma murmanense* was found in *L. exilis* collected from a depth of 200 metres, but not in *L. exilis* collected from 80 metres. *Trypanoplasma beckeri* parasitized the cabezon *Scorpaenichthys marmoratus*. *Trypanoplasma bobolsoni* n. sp. was found in *E. jordani*, *L. exilis*, and *P. vetulus*, and survived in two other species after intraperitoneal injection. A distinct, but unnamed trypanoplasm, was found in *P. vetulus*.

14399. **Jittapalapong, S., Inpankaew, T., Sarataphan, N., Herbreteau, V., Hugot, J. P., Morand, S. & Stich, R. W., 2007**. Molecular detection of divergent trypanosomes among rodents of Thailand. *Infection, Genetics and Evolution, e publication August*.

Department of Parasitology, Kasetsart University, Bangkok, Thailand.

Herpetosoma is a homogenous subgenus of several dozen named species that are often described as morphologically indistinguishable *T. lewisi*-like parasites. These trypanosomes normally infect rodents and utilize fleas as vectors. Although this trypanosome subgenus is considered non-pathogenic to normal hosts, some of them are on rare occasion reported in association with human disease. Recently, a *T. lewisi*-like infection was detected in a sick Thai infant, thus the objective of this study was to investigate the prevalence of *T. lewisi* infections among different rodents indigenous to Thailand in order to identify possible sources of human cases. Blood was collected from a total of 276 rodents trapped from urban and rural areas of three Thai provinces between 2006 and 2007. These samples were processed for DNA isolation and tested with a PCR assay universal for the genus *Trypanosoma*, followed by internal transcribed spacer 1 (ITS-1) sequence analysis to identify infections in positive samples. *Herpetosoma* known as *T. lewisi*-like trypanosomes were present among *Rattus* (14.3 percent) and *Bandicota* (18.0 percent) rodent species and

salivarian trypanosomes closely related to *T. evansi* were detected in *Leopoldamys (*20 percent) and *Rattus* (2.0 percent) species. *Herpetosoma* were prevalent among rodents associated with both human and sylvatic habitats, while three of the four salivaria-positive rodents were from a forest biotope. A *Herpetosoma* ITS-1 sequence amplified from one of these samples was 97.9 percent identical to that reported for *T. lewisi* in an experimentally infected rat and 96.4 percent identical to the sequence amplified from blood from a Thai infant. Habitats where rodents were collected significantly affect rodent infection, at least for *T. lewisi*, suggesting that the degree of anthropization may influence the transmission of *Trypanosoma* spp. These results suggest that multiple *Herpetosoma* species or strains are enzootic to Thailand, and that *Rattus* and *Bandicota* species are possible sources of human exposure to these parasites.

14400. **Konnai, S., Mingala, C. N., Sato, M., Abes, N. S., Venturina, F. A., Gutierrez, C. A., Sano, T., Omata, Y., Cruz, L. C., Onuma, M. & Ohashi, K., 2008.** A survey of abortifacient infectious agents in livestock in Luzon, the Philippines, with emphasis on the situation in a cattle herd with abortion problems. *Acta Tropica,* **105** (3): 269-273.

Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido 060-0818, Japan.

In the Philippines, insufficient consideration has been given to the implementation of systematic control measures against major abortion-associated infectious agents in livestock. To elucidate the epidemiology of abortion-causing infectious agents in livestock, the prevalence of four abortifacient agents was assessed. Initially, a total of 96 cattle including 17 cows with history of abortion were examined in a herd in Luzon at the request of the farm owner. Six (35.3 percent) of the 17 aborting cows were found to be serologically positive for *Neospora caninum* whereas the seroprevalence in non-aborting cows was 15.9 percent (10/63). Four of the 6 serologically positive aborting cows were also RT-PCR-positive for bovine viral diarrhoea virus (BVDV). Two (12.5 percent) of the 16 bulls examined were also found to be infected with BVDV, suggesting a putative risk factor of transmission via semen. Based on sequence analysis, the isolates detected belong to BVDV type 1b group. Furthermore, an epidemiological survey of abortifacient infectious agents was conducted with various species of livestock from herds located in Luzon. Out of the 105 water buffalo samples collected, 4 (3.8 percent) were indicated positive to *N. caninum*, 2 (1.9 percent) to *Toxoplasma gondii* and 2 (1.9 percent) to *Trypanosoma evansi.* The overall seroprevalence of *N. caninum* in goat and sheep were 23.6 percent (21/89) and 26.3 percent (10/38), respectively. BVDV was not detected in these herds. The findings of this exploratory study indicate a relationship between infection and bovine abortion and that a larger study is required to statistically confirm this relationship.

14401. **Leppert, L. L., Dufty, A. M., Jr., Stock, S., Oleyar, M. D. & Kaltenecker, G. S., 2008**. Survey of blood parasites in two forest owls, Northern Saw-whet owls and Flammulated owls, of Western North America. *Journal of Wildlife Diseases,* **44** (2): 475-479.

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Except for a few studies in the eastern United States, little has been published on haemoparasites in owls. We surveyed the blood parasites of 108 Northern Saw-whet owls (*Aegolius acadicus*) and 24 Flammulated owls (*Otus flammeolus*) in Idaho during autumn migration in 1999 and 2000. We also surveyed 15 Flammulated owls (FLOW) during breeding season in Utah from 2000. *Leucocytozoon ziemanni*, *Haemoproteus syrnii*, *Haemoproteus noctuae*, and *Trypanosoma avium* were identified. The overall prevalence of infection was 53 percent (78/147) and for the combined species, prevalences of *Haemoproteus*, *Leucocytozoon,* and *Trypanosoma* species were 20 percent, 39 percent, and 4 percent, respectively. Northern Saw-whet owls (NSWO) had an overall prevalence of 51 percent (55/108), with prevalences of 6 percent, 47 percent, and 4 percent by haemoparasite genus, respectively. Flammulated owls had an overall prevalence of 59 percent (23/39), with prevalences of 56 percent, 18 percent, and 5 percent by genus, respectively. This study provides baseline haematozoa information for two boreal owl species.

14402. **Miruk, A., Hagos, A., Yacob, H. T., Asnake, F. & Basu, A. K., 2008**. Prevalence of bovine trypanosomosis and trypanocidal drug sensitivity studies on *Trypanosoma congolense* in Wolyta and Dawero zones of southern Ethiopia. *Veterinary Parasitology,* **152** (1-2): 141-147.

Department of Pathology and Parasitology, Faculty of Veterinary Medicine, Addis Ababa University, P.O. Box 34, Debre Zeit, Ethiopia.[asokebasu@gmail.com].

Cross-sectional studies were conducted in tsetse and non-tsetse-controlled areas of the Southern Nation Nationalities and Peoples Regional State (SNNPRS) of Ethiopia to determine the prevalence of bovine trypanosomosis as well as drug sensitivity tests on *Trypanosoma congolense* in both naturally and experimentally infected cattle and mice, respectively. A total trypanosome prevalence of 4.8 percent (95 percent CI: 1.8-7.5) and 20.4 percent (95 percent CI: 14-26.8) were recorded in the tsetse-controlled study area of Humbo district and the non-tsetse-controlled area of Mareka district, respectively, indicated statistically significant difference between the two areas ($P<0.001$). The mean PCV value for Humbo and Mareka was 26.2 (95 percent CI: 25.7-26.7) and 22.7 (95 percent CI: 22.1-23.3), respectively, which were also statistically significant ($P<0.001$). The prophylactic activity of isometamidium chloride (ISMM) was observed in Humbo on nine naturally positive zebu cattle. Breakthrough infections were recorded in (6/9) 66.7 percent of the cases in less than 5 weeks. A qualitative assay on mice was conducted on two *T. congolense* isolates obtained from the breakthrough cases with ranges of doses of ISMM and diminazene diaceturate (DA). Thereafter the mice were followed for relapse infection. ISMM at doses 0.5-4 mg/kg body weight (bw) and DA at doses of 3.5-28 mg/kg bw failed completely to cure *T. congolense* infections in any of the mice. A quantitative assay on mice was conducted on four *T. congolense* isolates obtained from Mareka. The four isolates were pooled into two pools (Pool-1 and Pool-2) for the quantitative assay on mice. The pooled isolates were tested with the same trypanocidal drugs and ranges of doses as it was used for the qualitative assay on mice. The minimum curative dose (MCD) of ISMM that cleared *T. congolense* infected mice

was 4 and 2mg/kg bw for Pool-1 and Pool-2, respectively, whereas MCD of DA was 28 and 14 mg/kg bw, in Pool-1 and Pool-2, respectively. Although cloned populations were not used to prove whether the observed resistance was at the individual level or not, the results show that there is resistance to both ISMM and DA; failure of the "sanative pair".

14403. **Pinchbeck, G. L., Morrison, L. J., Tait, A., Langford, J., Meehan, L., Jallow, S., Jallow, J., Jallow, A. & Christley, R. M., 2008**. Trypanosomosis in The Gambia: prevalence in working horses and donkeys detected by whole genome amplification and PCR, and evidence for interactions between trypanosome species. *BMC Veterinary Research,* 4: 7.

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The Gambia has an increasing population of *Equidae* largely used for agriculture and transportation. A review of cases at The Gambian Horse and Donkey Trust (GHDT) indicated that a common reason for presentation is a poorly defined medical condition often attributed to trypanosomosis. There are few reports describing the prevalence or the range of clinical signs associated with infection with different species of trypanosomes in horses and donkeys, but given the importance of these animals, the role of trypanosomosis requires investigation. In total 241 animals from the Central River Division in The Gambia (183 horses and 58 donkeys) were screened using Whole Genome Amplification (WGA) followed by trypanosome species identification using the polymerase chain reaction (PCR). The results indicated overall trypanosome prevalence of 91 percent; with an infection rate of 31 percent for *Trypanosoma congolense,* 87 percent for *Trypanosoma vivax* and 18 percent for *Trypanosoma brucei* sp. Multiple species were present in 43 percent of infections. Microscopy had a good specificity (100 percent) and positive predictive value (100 percent) for trypanosome detection, but the sensitivity (20 percent) and negative predictive value (10.5 percent) were low relative to PCR-based diagnosis. Infection with *T congolense* showed the greatest negative effect on packed cell volume (PCV), while infection with *T. brucei* sp also had a significant, although lesser, negative effect on PCV. In addition, cases positive by microscopy were associated with significantly lower PCV. However, concurrent infection with *T. vivax* appeared to cause less effect on PCV, compared to animals infected with *T. congolense* alone. In conclusion, the prevalence of Trypanosomosis was high in both horses and donkeys. Infection with *T. congolense* appeared to have the greatest clinical significance, while *T. vivax* infection may be of limited clinical significance in this population. Indeed, there is evidence of *T. vivax* co-infection ameliorating the pathology caused by *T. congolense*. WGA and PCR allowed a more comprehensive analysis of field infections with the detection of infections below the threshold of microscopy, and provided indications of interactions between parasite species that would otherwise remain undetected. The study raises important questions about the epidemiology of trypanosome infection in relation to disease that require a full scale longitudinal analysis.

14404. **Stenberg, P. L. & Bowerman, W. J., 2008**. Hemoparasites in Oregon Spotted Frogs (*Rana pretiosa*) from Central Oregon, USA. *Journal of Wildlife Diseases,* **44** (2): 464-468.

Sunriver Nature Center & Observatory, Box 3533, Sunriver, Oregon 97707, USA.

Between 2001 and 2003, we screened blood smears of 156 Oregon spotted frogs (*Rana pretiosa*) from three populations in central Oregon for blood parasites. A *Lankesterella* sp. and a *Trypanosoma* sp. were detected in 31 percent and 35 percent of the frogs, respectively. Parasite loads were generally light, with *Lankesterella* sporozoites in 1-2 percent of erythrocytes, and extracellular trypanosomes were seen at rates of about one parasite per 200 fields of view at 1,000x. Little work has been published on haemoparasites of ranids in the western USA in the past 30 years. Because of the recent taxonomic division of the *Rana pretiosa* complex, this may be the first published report of blood parasites for *R. pretiosa sensu stricto*. Both parasites reported here differed in morphologic features and morphometric comparisons from previous descriptions of anuran haemoparasites. Much work remains to sort out the taxonomy of haemoparasites among western USA ranids and to determine the ecological significance of these parasites; both tasks are important steps in understanding and managing these, and related, sensitive and threatened species.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **31**: 14400]

14405. **Nurulaini, R., Jamnah, O., Adnan, M., Zaini, C. M., Khadijah, S., Rafiah, A. & Chandrawathani, P., 2007**. Mortality of domesticated java deer attributed to Surra. *Tropical Biomedicine,* **24** (2): 67-70.

Veterinary Research Institute, 59, Jalan Sultan Azlan Shah, 3400, Ipoh, Perak, Java.

This paper reports an outbreak of trypanosomiasis due to *Trypanosoma evansi* in Java deer (*Cervus timorensis*) on a government deer farm in Lenggong, Perak. Seventeen adult female Java deer were found dead within a week. Symptoms of dullness, inappetence, anaemia, anorexia, respiratory distress and recumbency were seen prior to death in the infected Java deer. Beside trypanosomiasis, other parasitic infections such as theileriosis, helminthiasis and ectoparasite infestation were also recorded. Post mortem results showed generalized anaemia in most animals with isolated cases of jaundice. There was no significant finding with respect to bacteriological and viral investigations.

14406. **Osorio, A. L., Madruga, C. R., Desquesnes, M., Soares, C. O., Ribeiro, L. R. & Costa, S. C., 2008**. *Trypanosoma* (*Duttonella*) *vivax*: its biology, epidemiology, pathogenesis, and introduction in the New World - a review. *Memórias do Instituto Oswaldo Cruz,* **103** (1): 1-13.

Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, 79070-900, Brasil.

The biology, epidemiology, pathogenesis, diagnostic techniques, and history of the introduction of *Trypanosoma* (*Duttonella) vivax* in the New World are reviewed. The two main immunological responses of trypanosome-infected animals - antibody production and immunodepression - are discussed in the context of how these responses play a role in disease tolerance or susceptibility. Isolation and purification of *T. vivax* are briefly discussed. The recent reports of bovine trypanosomiasis diagnosed in cattle on farms located in the Pantanal region of the states of Mato Grosso do Sul and Mato Grosso, Brazil, are also discussed.

(c) TRYPANOTOLERANCE

[See also **31**: 14416]

14407. **Courtin, D., Berthier, D., Thevenon, S., Dayo, G. K., Garcia, A. & Bucheton, B., 2008**. Host genetics in African trypanosomiasis. *Infection, Genetics and Evolution,* **8** (3): 229-238.

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

(d) TREATMENT

[See **31**: 14402]

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

[See also **31**: 14381, 14383, 14399, 14400]

14408. **Taylor, T. K., Boyle, D. B. & Bingham, J., 2008**. Development of a TaqMan PCR assay for the detection of *Trypanosoma evansi*, the agent of surra. *Veterinary Parasitology,* **153** (3-4): 255-264.

CSIRO Livestock Industries, Australian Animal Health Laboratory, Private Bag 24, Geelong, Victoria 3220, Australia.

A TaqMan PCR assay was developed for the detection of *Trypanosoma evansi*. The assay targets the internal transcribed spacer 1 (ITS-1) region of rRNA. The ITS-1 region of eleven strains of *T. evansi* from widely separated geographical regions was sequenced and alignments compared. Primers and probe for the test were designed from these sequence data. The assay was tested using blood from infected rats and was found to be sensitive, detecting less than one genomic equivalent of *T. evansi*. The assay has been tested against 10 different species of trypanosomes found in native animals in Australia and did not detect any of these trypanosome species. Time course experiments using rats infected with *T. evansi* were performed to compare the TaqMan assay with the haematocrit centrifugation test (HCT) and the mouse inoculation (MI) assay. The assay was more sensitive than the HCT but not as sensitive as the MI. The TaqMan assay has the ability to rapidly detect *T. evansi* and determine the number of organisms present in a blood sample from an infected animal. This is the first time a TaqMan assay has been developed for the detection of *T. evansi*.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **31:** 14342, 14349, 14356, 14388, 14496]

14409. **Barkhuizen, M., Magez, S., Atkinson, R. A. & Brombacher, F., 2007**. Interleukin-12p70-dependent interferon- gamma production is crucial for resistance in African trypanosomiasis. *Journal of Infectious Diseases*, **196** (8): 1253-1260.

Division of Immunology, Institute of Infectious Diseases and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa. [fbrombac@uctgsh1.uct.ac.za].

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

14410. **Bosschaerts, T., Guilliams, M., Noel, W., Herin, M., Burk, R. F., Hill, K. E., Brys, L., Raes, G., Ghassabeh, G. H., De Baetselier, P. & Beschin, A., 2008.** Alternatively activated myeloid cells limit pathogenicity associated with African Trypanosomiasis through the IL-10 inducible gene selenoprotein P. *Journal of Immunology,* **180** (9): 6168-6175.

Department of Molecular and Cellular Interactions and †Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Cell and Tissue Laboratory, Unité de Recherche en Physiologie Moléculaire, Facultés Universitaires Notre-Dame de la Paix, Namur, Belgium, Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232 [abeschin@vub.ac.be].

Uncontrolled inflammation is a major cause of tissue injury/pathogenicity often resulting in death of a host infected with African trypanosomes. Thus, comparing the immune response in hosts that develop different degrees of disease severity represents a promising approach to discover processes contributing to trypanosomiasis control. It is known that limitation of pathogenicity requires a transition in the course of infection, from an IFN-gamma-dependent response resulting in the development of classically activated myeloid cells (M1), to a counterbalancing IL-10-dependent response associated with alternatively activated myeloid cells (M2). Herein, mechanisms and downstream effectors by which M2 contribute to lower the pathogenicity and the associated susceptibility to African trypanosomiasis have been explored. Gene expression analysis in IL-10 knockout and wild-type mice, that are susceptible and relatively resistant to *Trypanosoma congolense* infection, respectively, revealed a number of IL-10-inducible genes expressed by M2, including Sepp1 coding for selenoprotein P. Functional analyses confirm that selenoprotein P contributes to limit disease severity through anti-oxidant activity. Indeed, Sepp1 knockout mice, but not Sepp1(Delta)(240-361) mice retaining the anti-oxidant motif but lacking the selenium transporter domain of selenoprotein P, exhibited increased tissue injury that associated with increased production of reactive oxygen species and increased apoptosis in the liver immune cells, reduced parasite clearance capacity of myeloid cells, and decreased survival. These data validate M2-associated molecules as functioning in reducing the impact of parasite infection on the host.

14411. **Engstler, M., Pfohl, T., Herminghaus, S., Boshart, M., Wiegertjes, G., Heddergott, N. & Overath, P., 2007**. Hydrodynamic flow-mediated protein sorting on the cell surface of trypanosomes. *Cell,* **131** (3): 505-515.

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The unicellular parasite *Trypanosoma brucei* rapidly removes host-derived immunoglobulin (Ig) from its cell surface, which is dominated by a single type of glycosylphosphatidylinositol-anchored variant surface glycoprotein (VSG). We have

determined the mechanism of antibody clearance and found that Ig-VSG immune complexes are passively sorted to the posterior cell pole, where they are endocytosed. The backward movement of immune complexes requires forward cellular motility but is independent of endocytosis and of actin function. We suggest that the hydrodynamic flow acting on swimming trypanosomes causes directional movement of Ig-VSG immune complexes in the plane of the plasma membrane, that is, immunoglobulins attached to VSG function as molecular sails. Protein sorting by hydrodynamic forces helps to protect trypanosomes against complement-mediated immune destruction in culture and possibly in infected mammals but likewise may be of functional significance at the surface of other cell types such as epithelial cells lining blood vessels.

14412**. Guirnalda, P., Murphy, N. B., Nolan, D. & Black, S. J., 2007**. Anti-*Trypanosoma brucei* activity in Cape buffalo serum during the cryptic phase of parasitemia is mediated by antibodies. *International Journal of Parasitology*, **37** (12): 1391-1399.

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Cape buffalo are reservoir hosts of African trypanosomes. They rapidly suppress population growth of the highly antigenically variable extracellular haemoprotozoa and subsequently maintain a cryptic infection. Here we use *in vitro* cultures of trypanosomes cloned from Cape buffalo blood during cryptic infection, as well as related and unrelated trypanosomes, to identify anti-trypanosome components present in cryptic-phase infection serum. Trypanosome clone-specific complement-dependent trypanolytic IgM and IgG arose after appearance of target trypanosomes during cryptic infection. Serum collected late in the cryptic phase of infection contained complement-independent growth-inhibitory IgG which varied in activity among target trypanosomes. Removal of protein A/G-binding IgG from the serum restored its capacity to support trypanosome growth *in vitro*. Recovered growth-inhibitory IgG reacted with the variable surface glycoprotein (VSG) of parasites most affected by it, and reacted with trypanosome common antigens, notably the endosome-restricted tomato lectin-binding glycoproteins (TL-antigens). The inclusion of purified TL-antigens in culture medium did not affect the trypanosome growth-inhibitory activity of immune Cape buffalo serum. In addition, hyperimmune rabbit IgG against TL-antigens showed little or no binding to intact trypanosomes and did not affect trypanosome growth *in vitro* although it did react strongly with TL-antigens and trypanosome endosomes. We conclude that antibodies, particularly clone-specific (putatively VSG-specific) antibodies are responsible for the anti-trypanosome activity of cryptic phase infection serum consistent with a dominant role in parasite control in Cape buffalo.

14413. **Holzmuller, P., Biron, D. G., Courtois, P., Koffi, M., Bras-Goncalves, R., Daulouede, S., Solano, P., Cuny, G., Vincendeau, P. & Jamonneau, V., 2008.** Virulence and pathogenicity patterns of *Trypanosoma brucei gambiense* field isolates in experimentally infected mouse: differences in host immune response modulation by secretome and proteomics. *Microbes and Infection*, **10** (1): 79-86.

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Human African trypanosomiasis is characterised by an important clinical diversity. Although *Trypanosoma brucei gambiense* field stocks isolated from patients in the same focus did not exhibit apparent genetic variability, they showed marked differences in terms of virulence (capacity to multiply inside a host) and pathogenicity (ability of producing mortality) in experimental murine infections. Two strains exhibiting opposite pathogenic and virulence properties in mouse were further investigated through their host-parasite interactions. *In vitro*, parasite bloodstream forms or soluble factors (or secretome) from both strains induced macrophage arginase as a function of their virulence. Arginase expression, a hallmark of macrophage alternative activation pathway, favours trypanosome bloodstream forms development. Moreover, a comparative proteomic study of the trypanosome stocks' secretomes evidenced both a differential expression of common molecules and the existence of stock specific molecules. This highlighted the potential involvement of the differential expression of the same genome in the diverse infectious properties of trypanosomes.

14414. **Katzenback, B. A., Plouffe, D. A., Haddad, G. & Belosevic, M., 2008**. Administration of recombinant parasite beta-tubulin to goldfish (*Carassius auratus L.*) confers partial protection against challenge infection with *Trypanosoma danilewskyi* Laveran and Mesnil, 1904. *Veterinary Parasitology*, **151** (1): 36-45.

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The infection of carp and other cyprinid fish with *Trypanosoma danilewskyi* was reported to cause significant morbidity and mortality in aquaculture. Tubulin is a component of parasite excretory/secretory (ES) products recognized by antibodies present in the serum of recovered hosts. To assess the role of parasite tubulin in the induction of a protective immune response in the goldfish, recombinant *T. danilewskyi* beta-tubulin was produced in *Escherichia coli* and used to immunize goldfish against challenge with live parasites. Affinity purified rabbit anti-recombinant tubulin IgG bound to both surface and internal structures of trypanosomes, and when added to parasite cultures caused a dose-dependent inhibition of their growth *in vitro*. Immunization of goldfish i.p. with either 40 µg or 80 µg of endotoxin-free beta-tubulin+Freund's complete adjuvant (FCA) caused a significant decrease in parasitaemia during the establishment phase of the infection (days 3 and 7) and increased the time required to reach the maximal mean number of parasites compared to non-immunized sham-injected control fish. The serum from immune fish contained antibodies that recognized trypanosomes as determined by confocal immunofluorescence microscopy and specific antibodies that recognized recombinant tubulin as measured by ELISA. Thus, the immunization of goldfish with recombinant parasite beta-tubulin conferred partial antibody-mediated protection against a challenge infection with live trypanosomes. This is a first report that parasite tubulin is immunogenic in poikilothermic vertebrates.

14415. **Koudande, O. D., Thomson, P. C., Bovenhuis, H., Iraqi, F., Gibson, J. P. & van Arendonk, J. A., 2008.** Biphasic survival analysis of trypanotolerance QTL in mice. *Heredity,* **100** (4): 407-414.

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A marker-assisted introgression (MAI) experiment was conducted to transfer trypanotolerance quantitative trait loci (QTL) from a donor mouse strain, C57BL/6, into a recipient mouse strain, A/J. The objective was to assess the effect of three previously identified chromosomal regions on mouse chromosomes 1 (MMU1), 5 (MMU5) and 17 (MMU17) in different genetic backgrounds on the survival pattern following infection with *Trypanosoma congolense*. An exploratory data analysis revealed a biphasic pattern of time to death, with highly distinct early and late mortality phases. In this paper, we present survival analysis methods that account for the biphasic mortality pattern and results of reanalyzing the data from the MAI experiment. The analysis with a Weibull mixture model confirmed the biphasic pattern of time to death. Mortality phase, an unobserved variable, appears to be an important factor influencing survival time and is modelled as a binary outcome variable using logistic regression analysis. Accounting for this biphasic pattern in the analysis reveals that a previously observed sex effect on average survival is rather an effect on proportion of mice in the two mortality phases. The C57BL/6 (donor) QTL alleles on MMU1 and MMU17 act dominantly in the late mortality phase while the A/J (recipient) QTL allele on MMU17 acts dominantly in the early mortality phase. From this study, we found clear evidence for a biphasic survival pattern and provided models for its analysis. These models can also be used when studying defence mechanisms against other pathogens. Finally, these approaches provide further information on the nature of gene actions.

14416. **Laha, R. & Sasmal, N. K., 2008.** Characterization of immunogenic proteins of *Trypanosoma evansi* isolated from three different Indian hosts using hyperimmune sera and immune sera. *Research in Veterinary Science*, **e Publication, March.**

Indian Veterinary Research Institute, Eastern Regional Station, 37, Belgachia Road, Kolkata 700 037, West Bengal, India.

The western blot analysis for identification of immunogenic proteins in whole cell lysate (WCL) antigens (Ags) prepared from the *Trypanosoma evansi* of buffalo, horse and cattle origins using hyperimmune sera (HIS) showed 11 immunogenic proteins and naturally *T. evansi* infected immune sera (IS) of horse detected 19 immunogenic proteins. HIS and IS of horse recognized five common immunogenic proteins of relative molecular weight (M(r)) ranges 61-64, 44-47, 33-34, 25-26 and 14-16 kilo Dalton (kDa). HIS rose against WCL Ags of *T. evansi* of buffalo origin and immune sera of horse cross reacted with WCL Ags of *T. evansi* of different host origin. It can be concluded that in comparison to HIS, IS of horse could able to detect more numbers of immunogenic proteins and five common immunogenic proteins in WCL Ags of *T. evansi* of different hosts origin. The evidence of higher reactivity of IS in comparison to HIS against *T. evansi* is being reported for the first time.

14417. **Lemos, K. R., Marques, L. C., Deaquino, L. P., Alessi, A. C. & Machado, R. Z., 2007**. Immunohistochemical characterization of mononuclear cells and MHC II expression in the brain of horses with experimental chronic *Trypanosoma evansi* infection. *Revista Brasileira de Parasitologia Veterinária*, **16** (4): 186-192.

Departamento de Medicina Veterinária, Centro Politecnico, UNICENTRO. Rua Simeao Camargo Varela de Sa, 03, Guarapuava, PR, 85040-080, Brasil. [krlemos@yahoo.com.br].

An histochemical and immunohistochemical study was carried out to evaluate the mechanisms of immune response of horses experimentally infected by *Trypanosoma evansi.* For this purpose the HE histochemical stain and the avidin biotin peroxidase method were used. To determine the presence and immunoreactivity of immune cells we used anti-major histocompatibility complex II antibodies. Cellular infiltration phenotype was characterized with the aid of anti-CD3 antibody for T lymphocytes and by anti-BLA 36 antibodies for B lymphocytes. Macrophages were marked with an antibody against myeloid/histyocites antigen (clone Mac387). Lesions in the CNS of experimentally infected horses were those of a wide spread non suppurative encephalomyelitis and meningomyelitis. The severity of lesions varied in different parts of the nervous system, reflecting an irregular distribution of inflammatory vascular changes. Lymphoid perivascular cuffs and meningeal infiltrations were of predominantly composed of T and B cells. The parasite, *T. evansi*, was not identified in these horses' tissues.

14418. **Mansfield, J. M. & Paulnock, D. M., 2008.** Genetic manipulation of African trypanosomes as a tool to dissect the immunobiology of infection. *Parasite Immunology,* **30** (4): 245-253.

Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, USA. [mansfield@bact.wisc.edu].

The variant surface glycoprotein (VSG) coat of African trypanosomes exhibits immunobiological functions distinct from its prominent role as a variant surface antigen. In order to address questions regarding immune stealth effects of VSG switch-variant coats, and the innate immune system activating effects of shed VSG substituents, several groups have genetically modified the ability of trypanosomes to express or release VSG during infection of the mammalian host. The role of mosaic surface coats expressed by VSG switch-variants (VSG double-expressors) in escaping early immune detection, and the role of VSG glycosylphosphatidylinositol (GPI) anchor substituents in regulating host immunity have been revealed, respectively, by stable co-expression of an exogenous VSG gene in trypanosomes expressing an endogenous VSG gene, and by knocking out the genetic locus for GPI-phospholipase C (PLC) that releases VSG from the membrane. Both approaches to genetic modification of African trypanosomes have suggested interesting and unexpected immunobiological effects associated with surface coat molecules.

14419. **Masocha, W., Rottenberg, M. E. & Kristensson, K., 2007**. Migration of African trypanosomes across the blood-brain barrier. *Physiology and Behavior*, **92** (1-2): 110-114.

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

14420. **Ngure, R. M., Eckersall, P. D., Jennings, F. W., Mburu, J., Burke, J., Mungatana, N. & Murray, M., 2008**. Acute phase response in mice experimentally infected with *Trypanosoma congolense*: a molecular gauge of parasite-host interaction. *Veterinary Parasitology*, **151** (1): 14-20.

Department of Veterinary Clinical Biochemistry, Faculty of Veterinary Medicine, University of Glasgow, Glasgow, UK.

Mice infected with *Trypanosoma congolense* developed a severe anaemia 1 week after infection, which persisted till treatment with diminazene aceturate when the packed cell volume (PCV) recovered to pre-infection levels. This was accompanied by a marked increase in the plasma levels of the acute phase proteins (APP), serum amyloid P-component (SAP) and haptoglobin (Hp). The initial peak levels of Hp and SAP were attained 7 and 12 days post-infection (DPI), respectively. Thereafter SAP levels decreased significantly to near pre-infection levels, but later increased even after treatment to give a second peak 34 DPI after which there was a decline till the study was terminated. The Hp levels on the other hand decreased to an intermediate level after the initial peak increasing to a second peak 22 DPI. Thereafter Hp decreased significantly following diminazene aceturate treatment to reach pre-infection levels within 5 days post-treatment. This indicates that *T. congolense*-infected mice develop severe anaemia accompanied by an acute phase response leading to an increase in SAP and Hp but that following treatment divergent responses occurred indicating differences in the pathways for stimulation of the APP. Haptoglobin was shown to be an earlier indicator of infection and a better marker in monitoring the response to treatment.

14421. **Nishimura, K., Sakakibara, S., Mitani, K., Yamate, J., Ohnishi, Y. & Yamasaki, S., 2008.** Inhibition of interleukin-12 production by *Trypanosoma brucei* in rat macrophages. *Journal of Parasitology*, **94** (1): 99-106.

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The immune response of a host infected with *Trypanosoma brucei* is modulated by trypomastigotes. We examined the changes in cytokine production in *T. brucei gambiense* (Wellcome strain; WS) infected rats and the influence on production of interleukin (IL)-12 by macrophages. The blood concentration of interferon-gamma, tumour necrosis factor-alpha, and IL-10 increased beginning the second day after infection. However, an increase in IL-12p40 was not observed until 4 days after infection. When spleen macrophages and Kupffer cells harvested from uninfected rats and HS-P cells (a rat macrophage-like cell line) were cocultured with WS, IL-12p40 production did not change. When HS-P cells were cultured with WS, transport of nuclear factor-kappaB into the nucleus increased. Levels of macrophage colony-stimulating factor (M-CSF) and granulocyte macrophage colony-stimulating factor mRNA in the spleens and livers of WS-infected rats were high in comparison with uninfected rats, suggesting that the WS promotes macrophage proliferation. The level of IL-12p40 mRNA in HS-P cells cocultured with WS increased in response to transfection with a small interfering RNA against M-CSF or addition of anti-M-CSF antibody. These results suggest that the WS inhibits IL-12p40 mRNA production by promoting production of macrophage colony-stimulating factor by macrophages.

14422. **Nishimura, K., Yagi, M., Ohnishi, Y. & Yamasaki, S., 2008**. Cytokine and nitric oxide production by *Trypanosoma brucei* infection in rats fed polyamine-deficient chow. *Journal of Parasitology*, **94** (1): 107-113.

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Feeding polyamine-deficient chow (PDC) to rats decreases blood polyamines, increases the activity of ornithine decarboxylase as an index of polyamine production, and increases resistance to *Trypanosoma brucei gambiense* (Wellcome strain) (WS) infection. In this study, we investigated the influence on cytokine and nitric oxide (NO) production of feeding PDC to rats infected with WS. At 4 days postinfection with WS, serum concentration of interleukin (IL)-12, tumour necrosis factor-alpha, interferon-gamma, IL-10, and NO increased in PDC-fed rats; however, IL-12 concentration in normal chow (NC)-fed rats did not increase. In spleen cells cocultured with WS, levels of IL-12 and inducible NO synthase (NOS) mRNA expression were higher in PDC-fed rats than in NC-fed rats. Proliferation of WS in coculture with spleen cells from PDC-fed rats was inhibited, but inhibition of WS proliferation was not observed when an NOS inhibitor was added into the culture media. Ornithine decarboxylase (ODC) activity increased in NC-fed rats after WS infection, but decreased in PDC-fed rats. These results show that feeding WS-infected rats PDC influences the production of cytokines such as IL-12 and the regulation of NO and polyamine production, and also leads to an increase in resistance against WS.

14423. **Saerens, D., Stijlemans, B., Baral, T. N., Nguyen Thi, G. T., Wernery, U., Magez, S., De Baetselier, P., Muyldermans, S. & Conrath, K., 2008.** Parallel selection of multiple anti-infectome nanobodies without access to purified antigens. *Journal of Immunological Methods*, **329** (1-2): 138-150.

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

A strategy was developed to isolate nanobodies, camelid-derived single-domain antibody fragments, against the parasite infectome without a priori knowledge of the antigens nor having access to the purified antigens. From a dromedary, infected with *T. evansi*, we cloned a pool of nanobodies and selected after phage display 16 different nanobodies specific for a single antigen, i.e. variant surface glycoprotein of *T. evansi*. Moreover 14 nanobodies were isolated by panning on different total parasite lysates. Thus, this anti-infectome experiment generated nanobodies, monospecific for one *Trypanosoma* species, whereas others were pan-reactive to various *Trypanosoma* species. Several nanobodies could label specifically the coat of a set of *Trypanozoon* species. The recognized target(s) are present in GPI-linked membrane fractions of bloodstream- and fly-form parasites. Due to the omnipresence of these targets on different parasite species and forms, these antibody fragments are a valuable source for validation of novel, not yet identified targets to design new diagnostics and therapeutics.

14424. **Wang, Y., Utzinger, J., Saric, J., Li, J. V., Burckhardt, J., Dirnhofer, S., Nicholson, J. K., Singer, B. H., Brun, R. & Holmes, E., 2008.** Global metabolic responses of mice to *Trypanosoma brucei brucei* infection. *Proceedings of the National Academy of Sciences USA*, **105** (16): 6127-6132.

Department of Biomolecular Medicine, Division of Surgery, Oncology, Reproductive Biology and Anaesthetics, Faculty of Medicine, Imperial College London, London SW7 2AZ, UK.

Human African trypanosomiasis (HAT) is transmitted by tsetse flies and, if untreated, is fatal. Treatment depends on infection stage, and early diagnosis is crucial for effective disease management. The systemic host biochemical changes induced by HAT that enable biomarker discovery or relate to therapeutic outcome are largely unknown. We have characterized the multivariate temporal responses of mice to *Trypanosoma brucei brucei* infection, using (1)H nuclear magnetic resonance (NMR) spectroscopic metabolic phenotyping of urine and plasma. Marked alterations in plasma metabolic profiles were detected already 1 day postinfection. Elevated plasma concentrations of lactate, branched chain amino acids, and acetylglycoprotein fragments were noted. *T. brucei brucei*-infected mice also had an imbalance of plasma alanine and valine, consistent with differential gluconeogenesis (parasite)-ketogenesis (host) pathway counterflux, involving stimulated host glycolysis, ketogenesis, and enhanced lipid oxidation in the host. Histopathologic evidence of *T. brucei brucei*-induced extramedullary hepatic haemopoiesis, renal interstitial nephritis, and a provoked inflammatory response was also noted. Metabolic disturbance of gut microbiotal activity was associated with infection, as indicated by changes in the urinary concentrations of the microbial co-metabolites, including hippurate. Concluding, parasite infection results in multiple systemic biochemical effects in the host and disturbance of the symbiotic gut microbial metabolic interactions. Investigation of these transgenomic metabolic alterations may underpin the development of new diagnostic criteria and metrics of therapeutic efficacy.

14425. **Wei, G. & Tabel, H., 2008**. Regulatory T cells prevent control of experimental African trypanosomiasis. *Journal of Immunology*, **180** (4): 2514-2521.

Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

African trypanosomes are single-cell, extra-cellular blood parasites causing profound immunosuppression. Susceptible BALB/c mice infected s.c. into a footpad with 10(4) *Trypanosoma congolense* die with fulminating parasitaemia within 10 days. We injected BALB/c mice 2 days before such an infection with different doses of a depleting mAb specific for CD25, a surface marker of regulatory T cells (Tregs). Pretreatment with a low, optimal dose of anti-CD25 resulted in a dramatic effect, in that the infected mice did not develop parasitaemia, as well as eliminated all parasites and showed no signs of disease. Their spleens showed a 100 percent reduction of CD4(+)CD25(high) T cells and overall a 70 percent reduction of CD4(+)CD25(+)Foxp3(+) T cells 7 days postinfection. The protective effect of treatment with an optimal dose of anti-CD25 could be reversed by administration of l-N6-(1-imminoethyl) lysine, a specific inhibitor of inducible NO synthase or administration of anti-CD8 Ab. Analysis of the cytokine patterns and cell surface marker in infected mice pretreated with anti-CD25 Abs pointed to a potential NKT cell response. We then conducted infections in CD1d(-/-) mice. From our observations, we conclude that CD4(+)CD25(high)Foxp3(+) Tregs prevent, in normal infected susceptible mice, an early protective response mediated by CD8(+) NKT cell-dependent activation of macrophages to kill parasites by production of NO. Our results also indicate that different populations of NKT cells have protective or suppressive effects. Our observations lead us to propose a hypothesis of cross-regulation of NKT cells and Tregs in trypanosome infections.

(c) CHEMOTHERAPEUTICS

[See also **31**: 14343, 14357, 14500, 14501, 14536]

14426. **Akpa, P. O., Ezeokonkwo, R. C., Eze, C. A. & Anene, B. M., 2008**. Comparative efficacy assessment of pentamidine isethionate and diminazene aceturate in the chemotherapy of *Trypanosoma brucei brucei* infection in dogs. *Veterinary Parasitology,* **151** (2-4): 139-149.

Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.

The chemotherapeutic efficacy of diminazene aceturate (Berenil)-a standard veterinary trypanocide and pentamidine isethionate (PMI)--a human trypanocide was compared in dogs experimentally infected with *Trypanosoma brucei brucei*. Also, the activities of the drugs on some serum liver enzymes were evaluated before and after treatment to ascertain the relative safety of the drugs. Fifteen local dogs (mongrels) were used for the study. Three of the dogs were uninfected controls, and twelve were infected with a stock of *T. brucei brucei*. Three of the infected dogs were untreated controls, three were given diminazene aceturate (DA) at 7 mg/kg body weight intramuscularly (i/m), another three received pentamidine isethionate (PMI) at 4 mg/kg i/m on days 14, 17, 19, 27, 29, and 31 post infection (PI) and the remaining three dogs were also given same dose of PMI on days 14, 16, 18, 20, 22, 24 and 26 PI. Both

trypanocides effectively cleared the parasites from the blood of the infected treated dogs. However, the infection subsequently relapsed at day 42 PI in one of the dogs in the DA treated group which later died at day 70 PI. Relapse infection was not recorded with the PMI treated groups although two dogs died in the PMI treated group II (treatment at days 14, 17, 19, 27, 29, and 31 PI) without showing relapsed parasitaemia. The packed cell volume (PCV), red blood cell (RBC) count, and haemoglobin (Hb) level which decreased significantly following infection, were reversed by the trypanocidal treatment. The reversal in the red cell values was faster in the PMI treated groups than in the DA treated group. The serum alkaline phosphate (SAP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels increased following infection and drug administration. The increase in the enzyme levels was greater in the DA treated groups than PMI treated groups. It was thus concluded that PMI given at 4 mg/kg i/m at days 14, 16, 18, 20, 22, 24, and 26 PI constituted a safe and efficient trypanocide and exhibited a superior trypanocidal action than DA in *T. brucei brucei* infected dogs.

14427. **Amin, D. N., Masocha, W., Ngan'dwe, K., Rottenberg, M. & Kristensson, K., 2008**. Suramin and minocycline treatment of experimental African trypanosomiasis at an early stage of parasite brain invasion. *Acta Tropica,* **106** (1): 72-74.

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The effect of treatment on relapses of *Trypanosoma brucei* (*T. b.*) *brucei* infections in mice in relation to passage of the parasites across the blood-brain barrier (BBB) as visualized by immunohistochemistry was studied. Three daily intraperitoneal injections of 20mg/kg suramin starting at 15 days post-infection (p.i.), when trypanosomes had begun to traverse the BBB, were curative, but not when starting at 21 days p.i. when parasite brain invasion was more pronounced. Relapses occurred in all mice after one or two daily injections of suramin starting at 15 days p.i., but they were delayed when treatment was supplemented with minocycline, which impedes penetration of *T. b. brucei* into the brain. This study supports the notion that suramin may be effective even when minor parasite neuroinvasion has appeared in African trypanosomiasis and it shows that minocyline can affect relapses of the disease.

14428. **Bolognesi, M. L., Lizzi, F., Perozzo, R., Brun, R. & Cavalli, A., 2008**. Synthesis of a small library of 2-phenoxy-1,4-naphthoquinone and 2-phenoxy-1,4-anthraquinone derivatives bearing anti-trypanosomal and anti-leishmanial activity. *Bioorganic and Medicinal Chemistry Letters,* **18** (7): 2272-2276.

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Taking advantage of the structural features of natural products showing anti-trypanosomatid activity, we designed and synthesized a small library of 2-phenoxy-1,4-naphthoquinone and 2-phenoxy-1,4-anthraquinone derivatives. The library was obtained following a parallel approach and using readily available synthons. All the derivatives showed inhibitory activity toward either *Trypanosoma* or *Leishmania* species, with 8, 10, and

16 being the most active compounds against *Trypanosoma brucei rhodesiense*, *Leishmania donovani*, and *Trypanosoma cruzi* cells (IC(50)=50nM, IC(50)=0.28µM, and IC(50)=1.26µM, respectively).

14429. **Boutrin, M. C., Foster, H. A. & Pentreath, V. W., 2008**. The effects of bee (*Apis mellifera*) venom phospholipase A2 on *Trypanosoma brucei brucei* and enterobacteria. *Experimental Parasitology,* **119** *(2): 246-251*.

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14430. **Cavazzuti, A., Paglietti, G., Hunter, W. N., Gamarro, F., Piras, S., Loriga, M., Allecca, S., Corona, P., McLuskey, K., Tulloch, L., Gibellini, F., Ferrari, S. & Costi, M. P., 2008.** Discovery of potent pteridine reductase inhibitors to guide antiparasite drug development. *Proceedings of the National Academy of Sciences USA,* **105** (5): 1448-1453.

Dipartimento di Scienze Farmaceutiche, Universita di Modena e Reggio Emilia, Via Campi 183, 41100 Modena, Italy.

14431. **Chambers, J. W., Fowler, M. L., Morris, M. T. & Morris, J. C., 2008**. The anti-trypanosomal agent lonidamine inhibits *Trypanosoma brucei* hexokinase 1. *Molecular and Biochemical Parasitology,* **158** (2): 202-207.

Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634, USA.

Glycolysis is essential to the parasitic protozoan *Trypanosoma brucei*. The first step in this metabolic pathway is mediated by hexokinase, an enzyme that transfers the gamma-phosphate of ATP to a hexose. The *T. brucei* genome (TREU927/4 GUTat10.1) encodes two hexokinases (*Tb*HK1 and *Tb*HK2) that are 98 percent identical at the amino acid level. Our previous efforts have revealed that *Tb*HK2 is an important regulator of *Tb*HK1 in procyclic form parasites. Here, we have found through RNAi that TbHK1 is essential to the bloodstream form parasite. Silencing the gene for 4 days reduces cellular hexokinase approximately 60 percent and leads to parasite death. Additionally, we have found that the recombinant enzyme is inhibited by lonidamine (IC(50)=850 µM), an anti-cancer drug that targets tumour hexokinases. This agent also inhibits HK activity from whole parasite lysate (IC (50)=965 µM). Last, lonidamine is toxic to cultured bloodstream form parasites (LD (50)=50 µM) and procyclic form parasites (LD(50)=180 µM). Interestingly, overexpression of *Tb*HK1 protects PF parasites from lonidamine. These studies provide genetic evidence that *Tb*HK1 is a valid therapeutic target while identifying a potential molecular target of the anti-trypanosomal agent lonidamine.

14432. **Goebel, T., Ulmer, D., Projahn, H., Kloeckner, J., Heller, E., Glaser, M., Ponte-Sucre, A., Specht, S., Sarite, S. R., Hoerauf, A., Kaiser, A., Hauber, I., Hauber, J. & Holzgrabe, U., 2008.** In search of novel agents for therapy of

tropical diseases and human immunodeficiency virus. *Journal of Medicinal Chemistry,* **51** (2): 238-250.

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Malaria, sleeping sickness, Chagas' disease, Aleppo boil, and AIDS are among the tropical diseases causing millions of infections and cases of deaths per year because only inefficient chemotherapy is available. Since the targeting of the enzymes of the polyamine pathway may provide novel therapy options, we aimed to inhibit the deoxyhypusine hydroxylase, which is an important step in the biosynthesis of the eukaryotic initiation factor 5A. In order to identify new lead compounds, piperidines were produced and biologically evaluated. The 3,5-diethyl piperidone-3,5-dicarboxylates 11 and 13 substituted with 4-nitrophenyl rings in the 2 and 6 positions were found to be active against *Trypanosoma brucei brucei* and *Plasmodium falciparum* combined with low cytotoxicity against macrophages. The corresponding monocarboxylates are only highly active against the *T. brucei brucei*. The piperidine oximether 53 demonstrated the highest plasmodicidal activity. Moreover, compounds 11 and 53 were also able to inhibit replication of HIV-1.

14433. **Ismail, M. A., Arafa, R. K., Wenzler, T., Brun, R., Tanious, F. A., Wilson, W. D. & Boykin, D. W., 2008**. Synthesis and antiprotozoal activity of novel bis-benzamidino imidazo[1,2-a]pyridines and 5,6,7,8-tetrahydro-imidazo[1,2-a]pyridines. *Bioorganic and Medicinal Chemistry,* **16** (2): 683-691.

Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303-3083, USA.

14434. **Korkhov, V. M. & Tate, C. G., 2008**. Electron crystallography reveals plasticity within the drug binding site of the small multidrug transporter EmrE. *Journal of Molecular Biology,* **377** (4): 1094-1103.

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 0QH, UK.

14435. **Lepesheva, G. I., Ott, R. D., Hargrove, T. Y., Kleshchenko, Y. Y., Schuster, I., Nes, W. D., Hill, G. C., Villalta, F. & Waterman, M. R., 2007.** Sterol 14alpha-demethylase as a potential target for antitrypanosomal therapy: enzyme inhibition and parasite cell growth. *Chemistry and Biology*, **14** (11): 1283-1293.

Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232-0146, USA. [galina.i.lepesheva@vanderbilt.edu].

14436. **Mallari, J. P., Shelat, A., Kosinski, A., Caffrey, C. R., Connelly, M., Zhu, F., McKerrow, J. H. & Guy, R. K., 2008**. Discovery of trypanocidal thiosemicarbazone inhibitors of rhodesain and *Tbc*atB. *Bioorganic and Medicinal Chemistry Letters*, **18** (9): 2883-2885..

Graduate Program in Chemistry and Chemical Biology, University of California, San Francisco, CA 94143-2280, USA; Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis, TN 38105, USA.

Human African trypanosomiasis (HAT) is caused by the protozoan parasite *Trypanosoma brucei*. The cysteine proteases of *T. brucei* have been shown to be crucial for parasite replication and represent an attractive point for therapeutic intervention. Herein we describe the synthesis of a series of thiosemicarbazones and their activity against the trypanosomal cathepsins *Tb*catB and rhodesain, as well as human cathepsins L and B. The activity of these compounds was determined against cultured *T. brucei*, and specificity was assessed with a panel of four mammalian cell lines.

14437. **Mallari, J. P., Shelat, A. A., Obrien, T., Caffrey, C. R., Kosinski, A., Connelly, M., Harbut, M., Greenbaum, D., McKerrow, J. H. & Guy, R. K., 2008.** Development of potent purine-derived nitrile inhibitors of the trypanosomal protease *Tb*catB. *Journal of Medicinal Chemistry*, **51** (3): 545-552.

Graduate Program in Chemistry and Chemical Biology and Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA 94143-2280, USA.

Human African trypanosomiasis (HAT), a major health concern in sub-Saharan Africa, is caused by the protozoan parasite *Trypanosoma brucei*. Recent studies have shown that a cathepsin B like protease, *Tb*catB, is essential to the survival of *T. brucei in vitro* (Mackey, Z. B.; O'Brien, T. C.; Greenbaum, D. C.; Blank, R. B.; McKerrow, J. H. J. Biol. Chem. 2004, 279, 48426-48433). Herein, we describe the first inhibitors of *Tb*catB, a series of purine nitriles. The compounds are potent trypanocides, killing the parasite with a high degree of selectivity over a panel of three human cell lines. In addition, a predictive model of trypanocidal activity was developed on the basis of potency against *Tb*catB and various calculated physical property descriptors.

14438. **Merschjohann, K. & Steverding, D., 2008**. *In vitro* trypanocidal activity of the anti-helminthic drug niclosamide. *Experimental Parasitology,* **118** (4): 637-640.

Department of Parasitology, Ruprecht-Karls-University, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany.

Only a few drugs are available for chemotherapy of African trypanosomiasis and there is an urgent need for the development of new anti-trypanosomal agents. In this study, the anti-helminthic drug niclosamide was tested for its trypanocidal activity *in vitro* using culture-adapted bloodstream forms of *Trypanosoma brucei brucei* and *Trypanosoma congolense*. The concentrations of niclosamide to reduce the growth rate by 50 percent and to kill all cells were in the low- and mid micromolar ranges for *T. b. brucei* and *T. congolense*, respectively. The very low toxicity of niclosamide for mammals makes the compound interesting for drug development for African trypanosomiasis.

14439. **Mesia, G. K., Tona, G. L., Nanga, T. H., Cimanga, R. K., Apers, S., Cos, P., Maes, L., Pieters, L. & Vlietinck, A. J., 2008**. Antiprotozoal and cytotoxic screening of 45 plant extracts from Democratic Republic of Congo. *Journal of Ethnopharmacology,* **115** (3): 409-415.

University of Kinshasa, Faculty of Pharmaceutical Sciences, PO BOX 212, Kinshasa XI, People's Republic of Congo.

To evaluate *in vitro* the antiprotozoal and cytotoxic activities of a methanol extract from 45 medicinal plants collected in Sankuru (Democratic Republic of Congo) against *Trypanosoma brucei brucei*, *Trypanosoma cruzi* and the chloroquine-sensitive Ghanaian strain of *Plasmodium falciparum*, and MRC-5 cell lines respectively, extracts were obtained by maceration of each plant part used with 80 percent methanol for 24h. The mixture was filtered and evaporated *in vacuo* to give corresponding dried extract. The activity against *Trypanosoma brucei brucei* and *Trypanosoma cruzi* was tested in 96-well tissue plates each containing 10 µ mol of aqueous plant extract dilutions (100 to 0.01 µg/ml) with 10 µmol of the parasite suspension cultured in Hirumi medium supplemented with 10 percent foetal calf serum and a solution of 2 percent penicillin/streptomycin (2 percent P/S) After 4 days incubation with Almar blue solution, fluorescence was measured at 500 nm emission and 530 nm excitation and results expressed as percentage reduction in parasite compared to control wells. The antiplasmodial activity was assessed *in vitro* against the chloroquine-sensitive Ghanaian strain of *Plasmodium falciparum* cultured in RPMI-1640 medium by the lactate deshydrogenase assay in the presence of plant extracts (50 to 0.01 µg/ml). Cell-lines MRC-5 were cultured in MEM medium supplemented with 20mM l-glutamine, 16.5mM Na_2HCO_3, 5 percent foetal calf serum and 2 percent P/S solution. After 4h incubation, cell proliferation/viability was spectrophotometrically assessed at 540 nm after addition of MTT. In each assay, the IC50 value for each sample was derived by the drug concentration-response curves. The extracts from *Alcornea cordifolia* leaves, *Momordica charantia* whole plant, *Omphalocarpum glomerata* root bark and *Piptadia africanum* stem bark showed good antiprotozoal activity against *Trypanosoma brucei brucei* with IC50 values from 0.7 to 7 µg/ml. Only *Piptadenia africanum* extract showed a pronounced antiprotozoal activity against *Trypanosoma cruzi* (IC50=4.0+/-0.6 µg/ml). The extracts from *Alchornea cordifolia*, *Polyathia suavelens* stem bark, *Sapium cornutum* stem bark and *Triclisia giletii* stem bark exhibited a pronounced antiplasmodial activity against *P. falciparum* Ghanaian strain with IC50 values ranging from 0.5 to 3.0 µg/ml. *Piptadenia africanum* extract was the most cytotoxic sample (IC50=0.25 µg/ml) with poor selectivity against all selected protozoa (SI<10) while other active extracts did not show a significant cytotoxic effect against MCR-5 cell-lines with good selectivity according to the case. These active plant extracts are selected for extensive studies leading to the isolation of active constituents.

14440. **Morgan, R. E. & Werbovetz, K. A., 2008**. Selective lead compounds against kinetoplastid tubulin. *Advances in Experimental Medicine and Biology,* **625**: 33-47.

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

Kinetoplastid parasites are responsible for the potentially fatal diseases leishmaniasis, African sleeping sickness and Chagas' disease. The current treatments for these diseases are far from ideal and new compounds are needed as antiparasitic drug candidates. Tubulin is the accepted target for treatments against cancer and helminths, suggesting that kinetoplastid tubulin is also a suitable target for antiprotozoal compounds. Selective lead compounds against kinetoplastid tubulin have been identified that could represent a starting point for the development of new drug candidates against these parasites.

14441. **Murebwayire, S., Frederich, M., Hannaert, V., Jonville, M. C. & Duez, P., 2008**. Antiplasmodial and antitrypanosomal activity of *Triclisia sacleuxii* (Pierre) Diels. *Phytomedicine*. **In press; corrected proof.**

Laboratory of Pharmacognosy, Bromatology and Human Nutrition, Institute of Pharmacy, Free University of Brussels (ULB), C.P. 205/09, Bd du Triomphe, 1050 Brussels, Belgium.

14442. **Nowicki, M. W., Tulloch, L. B., Worralll, L., McNae, I. W., Hannaert, V., Michels, P. A., Fothergill-Gilmore, L. A., Walkinshaw, M. D. & Turner, N. J., 2008.** Design, synthesis and trypanocidal activity of lead compounds based on inhibitors of parasite glycolysis. *Bioorganic and Medicinal Chemistry.* **In press; corrected proof.**

School of Chemistry, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh, EH9 3JJ, UK; Structural Biochemistry Group, Institute of Structural and Molecular Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, UK.

The glycolytic pathway has been considered a potential drug target against the parasitic protozoan species of *Trypanosoma* and *Leishmania.* We report the design and the synthesis of inhibitors targeted against *Trypanosoma brucei* phosphofructokinase (PFK) and *Leishmania mexicana* pyruvate kinase (PyK). Stepwise library synthesis and inhibitor design from a rational starting point identified furanose sugar amino amides as a novel class of inhibitors for both enzymes with IC(50) values of 23mμM and 26mμM against PFK and PyK, respectively. Trypanocidal activity also showed potency in the low micromolar range and confirms these inhibitors as promising candidates for the development towards the design of anti-trypanosomal drugs.

14443. **Papanastasiou, I., Tsotinis, A., Kolocouris, N., Prathalingam, S. R. & Kelly, J. M., 2008**. Design, synthesis, and trypanocidal activity of new aminoadamantane derivatives. *Journal of Medicinal Chemistry,* **51** (5): 1496-1500.

Department of Pharmaceutical Chemistry, University of Athens, Athens, Greece.

To develop functionalized adamantanes for treating African trypanosomiasis, we report on the synthesis of new 1-alkyl-2-aminoadamantanes 1a- i, 1-alkyltricyclo [3.3.1.1

(3,7)]decan-2-guanylhydrazones 2a- g, and their congeneric thiosemicarbazones 3a, b. The potency of these compounds against *Trypanosoma brucei* was compared to that of amantadine and rimantadine and found to be substantially higher. The most active analogues, 1c, 1d, 2c, 2g, and 3b, illustrate the synergistic effect of the lipophilic character of the C1 side chain and the C2 functionality on trypanocidal activity.

14444. **Rodriguez, F., Rozas, I., Kaiser, M., Brun, R., Nguyen, B., Wilson, W. D., Garcia, R. N. & Dardonville, C., 2008**. New bis(2-aminoimidazoline) and bisguanidine DNA minor groove binders with potent *in vivo* antitrypanosomal and antiplasmodial activity. *Journal of Medicinal Chemistry,* **51** (4): 909-923.

Centre for Synthesis and Chemical Biology, School of Chemistry, Trinity College Dublin, Dublin 2, Ireland.

A series of 75 guanidine and 2-aminoimidazoline analogue molecules were assayed *in vitro* against *Trypanosoma brucei rhodesiense* STIB900 and *Plasmodium falciparum* K1. The dicationic diphenyl compounds exhibited the best activities with IC 50 values against *T. b. rhodesiense* and *P. falciparum* in the nanomolar range. Five compounds (7b, 9a, 9b, 10b, and 14b) cured 100 percent of treated mice upon ip administration at 20 mg/kg in the difficult to cure *T. b. rhodesiense* STIB900 mouse model. Overall, the compounds that bear the 2-aminoimidazoline cations benefit from better safety profiles than the guanidine counterparts. The observation of a correlation between DNA binding affinity at AT sites and trypanocidal activity for three series of compounds supported the view of a mechanism of antitrypanosomal action due in part to the formation of a DNA complex. No correlation between antiplasmodial activity and *in vitro* inhibition of ferriprotoporphyrin IX biomineralization was observed, suggesting that additional mechanism of action is likely to be involved.

14445. **Shuaibu, M. N., Wuyep, P. T., Yanagi, T., Hirayama, K., Ichinose, A., Tanaka, T. & Kouno, I., 2008**. Trypanocidal activity of extracts and compounds from the stem bark of *Anogeissus leiocarpus* and *Terminalia avicennoides*. *Parasitology Research,* **102** (4): 697-703.

Natural Product Chemistry, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki-shi, Japan. [nshuaibu@yahoo.com].

The antitrypanosomal activity of methanolic extracts of *Anogeissus leiocarpus* and *Terminalia avicennoides* was evaluated *in vitro* against four strains of *Trypanosoma* species with minimum inhibitory concentration (MIC) value range of 12.5-50 mg/ml. Successive fractionations of the two plant extracts in water, butanol and ethyl acetate gave a range of activity (MIC, 20 to >/=50 µg/ml). Activity-guided and chromatographic analysis of butanolic fractions on Sephadex LH-20 column followed by high-performance liquid chromatography, nuclear magnetic resonance analysis and both ultraviolet and thin layer chromatography revealed hydrolysable tannins with a range of activity (MIC, 7.5-27.5 µg/ml or 14-91 µM). Effect of the compounds on fibroblasts did not reveal serious toxicity at moderate concentration but is concentration dependent.

14446. **Wilson, W. D., Tanious, F. A., Mathis, A., Tevis, D., Hall, J. E. & Boykin, D. W., 2008.** Antiparasitic compounds that target DNA. *Biochimie*. **In press; corrected proof.**

Department of Chemistry, Georgia State University, Atlanta, GA 30303, USA.

Designed, synthetic heterocyclic diamidines have excellent activity against eukaryotic parasites that cause diseases such as sleeping sickness and *Leishmania* and adversely affect millions of people each year. The most active compounds bind specifically and strongly in the DNA minor groove at AT sequences. The compounds enter parasite cells rapidly and appear first in the kinetoplast that contains the mitochondrial DNA of the parasite. With time the compounds are also generally seen in the cell nucleus but are not significantly observed in the cytoplasm. The kinetoplast decays over time and disappears from the mitochondria of treated cells. At this point the compounds begin to be observed in other regions of the cell, such as the acidocalcisomes. The cells typically die in 24-48h after treatment. Active compounds appear to selectively target extended AT sequences and induce changes in kinetoplast DNA minicircles that cause a synergistic destruction of the catenated kinetoplast DNA network and cell death.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION

14447. **Chowdhury, A. R., Zhao, Z. & Englund, P. T., 2008**. Effect of hydroxyurea on procyclic *Trypanosoma brucei*: an unconventional mechanism for achieving synchronous growth. *Eukaryotic Cell,* **7** (2): 425-428.

Department of Biological Chemistry, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA.

Procyclic *Trypanosoma brucei* cells were synchronized with 0.2 mM hydroxyurea. The cells did not arrest at the G(1)/S boundary but proceeded through one round of replication and arrested near the end of S phase. The mitochondrial genome (kinetoplast DNA network) replicated, forming two progeny networks, but the repair of minicircle gaps was inhibited.

(b) TAXONOMY; CHARACTERISATION OF ISOLATES

[See also **31**: 14384, 14398, 14399, 14402, 14404]

14448. **Adams, E. R. & Hamilton, P. B., 2008**. New molecular tools for the identification of trypanosome species. *Future Microbiology,* **3**: 167-176.

School of Biological Sciences, University of Bristol, Bristol, BS8 1UG, UK. [emily.adams@bristol.ac.uk].

Trypanosomes are the causative agents of many diseases of medical and veterinary importance, including sleeping sickness and nagana in Africa, and Chagas' disease in South America. Accurate identification of trypanosome species is essential, as some species are morphologically indistinguishable, yet differ greatly in their pathogenicity. A range of molecular tools has been developed for identification of species and strains of trypanosomes. PCR, using primer sets designed to amplify a specific DNA fragment from each trypanosome species, is frequently used. More recently, generic systems have been developed that can potentially recognize all trypanosome species, such as amplification of the internal transcribed spacer and fluorescent fragment length barcoding, both of which use interspecies size variation in PCR fragments amplified from the ribosomal RNA locus. Loop-mediated isothermal amplification is a promising technique and is able to detect trypanosomes in blood, serum and cerebrospinal fluid. The advantages of these techniques for high-throughput and sensitive molecular identification will be discussed.

14449**. Adams, E. R., Hamilton, P. B., Malele, I. I. & Gibson, W.C., 2008.** The identification, diversity and prevalence of trypanosomes in field caught tsetse in Tanzania using ITS-1 primers and fluorescent fragment length barcoding. *Infection, Genetics and Evolution.* **In press; corrected proof.**

School of Biological Sciences, University of Bristol, Bristol BS8 1UG, U.K., School of Biological Sciences, University of Exeter, Exeter EX4 4PS, U.K.,Tsetse and Trypanosomiasis Research Institute, P.O. Box 1026, Tanga, Tanzania.

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

14450. **Andrade, H. M., Murta, S. M., Chapeaurouge, A., Perales, J., Nirde, P. & Romanha, A. J., 2008.** Proteomic analysis of *Trypanosoma cruzi* resistance to benznidazole. *Journal of Proteome Research.* **Web Release Date April 2008.**

Laboratório de Parasitologia Celular e Molecular, Centro de Pesquisa Rene Rachou/FIOCRUZ, Brazil, Universidade Federal do Paiui, Lab. Imunogenética e Biologia Molecular, Teresina, Piaui, Brazil, Laboratório de Toxinologia, Departamento de Fisiologia e Farmacodinamica, Instituto Oswaldo

Cruz/FIOCRUZ, Brazil, and INSERM U540, 60 rue de Navacelles, 34090 Montpellier, France [helidandrade@gmail.com].

The first proteomic analysis of *Trypanosoma cruzi* resistance to benznidazole (BZ) is presented. The differential proteome of *T. cruzi* with selected *in vivo* resistance to benznidazole (BZR and clone 27R), its susceptible pairs (BZS and clone 9S), and a pair from a population with benznidazole- *in vitro*-induced resistance (17LER) and the susceptible pair 17WTS were analyzed by two-dimensional gel electrophoresis (2-DE) followed by mass spectrometry (MS) for protein identification. Out of 137 spots analyzed through MS, 110 were identified as 56 distinct proteins. Out of the 56 distinct proteins, 36 were present in resistant, 9 in susceptible, and 11 in both phenotypes. Among the proteins identified in resistant samples, 5 were found in Cl 27R and in BZR (calpain-like cysteine peptidase, hypothetical protein conserved 26 kDa, putative peptidase, peroxiredoxin and tyrosine amino transferase) and 4 in Cl 27R and 17LER (cyclophilin A, glutamate dehydrogenase, iron superoxide dismutase and nucleoside diphosphate kinase). As for the proteins identified in benznidazole-susceptible samples, PGF-2a was found in BZS and 17WTS. A functional category analysis showed that the proteins involved with transcription and protein destination were overexpressed for the benznidazole-resistant phenotype. Thus, the present study provides large-scale, protein-related information for investigation of the mechanism of *T. cruzi* resistance to benznidazole.

14451. **Areekit, S., Singhaphan, P., Kanjanavas, P., Khuchareontaworn, S., Sriyapai, T., Pakpitcharoen, A. & Chansiri, K., 2007**. Genetic diversity of *Trypanosoma evansi* in beef cattle based on internal transcribed spacer region. *Infection, Genetics and Evolution*. **Available on line November 2007**.

Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, Sukhumvit 23, Bangkok 10110, Thailand.

This study was focused on genetic diversity of *Trypanosoma evansi* which is a widely distributed haemoflagellate of veterinary importance that infects a variety of larger mammals including horses, mules, camels, buffalo, cattle and deer. The genetic diversity of *T. evansi* of beef cattle LAM 19 was accomplished by using phylogenetic analysis based on internal transcribed spacer region (ITS). Blood samples were collected from naturally infected beef cattle LAM 19 and parasitaemia was raised by mouse inoculation. The parasites were collected and isolated by using DE 52 DEAE cellulose anion exchange column prior to DNA extraction. Upon PCR amplification of ITS region, the product of 1,300 bp in size was obtained. The ITS nucleotide sequences were analyzed and revealed that it could demonstrate the genetic diversity of *T. evansi* of beef cattle LAM19. Based on the ITS tree, beef cattle LAM 19 *T. evansi* were categorized into two main groups where the genetic diversity occurred within Group 1. The data could be applicable for the survey of parasite dynamics, epidemiological studies as well as prevention and control of the disease.

14452. **de Oliveira Lima, A. N., da Silva Santos, S., Herrera, H. M., Gama, C., Cupolillo, E., Jansen, A. M. & Fernandes, O., 2008**. *Trypanosoma evansi:* molecular homogeneity as inferred by phenetical analysis of ribosomal internal transcribed spacers DNA of an eclectic parasite. *Experimental Parasitology,* **118** (3): 402-407.

Laboratory of Tripanosomatid Biology, Oswaldo Cruz Institute, FIOCRUZ, Av. Brasil 4365, CEP. 21040-360, Rio de Janeiro/RJ, Brazil.

The protozoan *Trypanosoma evansi* is described as presenting high morphological and genetic similarities among the isolates despite its biological heterogeneity and wide geographical distribution. PCR amplification of the internal transcribed spacers of the ribosomal gene in combination with the coding region of the 5.8S ribosomal subunit further submitted to restriction enzymes digestion were carried out in DNAs extracted from 41 *T. evansi* strains isolated from horses, dogs, coatis and capybaras from two distinct regions of the Brazilian Pantanal. We also used one *T. evansi* isolate from Africa, one from Asia and one isolate of *T. b. brucei* from Africa. Analysis of the RFLP profiles yielded a unique "riboprinting" that does not vary intraspecifically. These results provide insights on the ribosomal gene organization of *T. evansi* and showed that ITS analysis by RFLP show high genetic similarity of this locus among isolates of this protozoan parasite.

14453. **Lai, D. H., Hashimi, H., Lun, Z. R., Ayala, F. J. & Lukes, J., 2008**. Adaptations of *Trypanosoma brucei* to gradual loss of kinetoplast DNA: *Trypanosoma equiperdum* and *Trypanosoma evansi* are petite mutants of *T. brucei*. *Proceedings of the National Academy of Sciences USA,* **105** (6): 1999-2004.

Biology Centre, Institute of Parasitology, Czech Academy of Sciences, 37005 Ceske Budejovice, Czech Republic.

Trypanosoma brucei is a kinetoplastid flagellate, the agent of human sleeping sickness and ruminant nagana in Africa. Kinetoplastid flagellates contain their eponym kinetoplast DNA (kDNA), consisting of two types of interlocked circular DNA molecules: scores of maxicircles and thousands of minicircles. Maxicircles have typical mitochondrial genes, most of which are translatable only after RNA editing. Minicircles encode guide RNAs, required for decrypting the maxicircle transcripts. The life cycle of *T. brucei* involves a bloodstream stage (BS) in vertebrates and a procyclic stage (PS) in the tsetse fly vector. Partial [dyskinetoplastidy (Dk)] or total [akinetoplastidy (Ak)] loss of kDNA locks the trypanosome in the BS form. Transmission between vertebrates becomes mechanical without PS and tsetse mediation, allowing the parasite to spread outside the African tsetse belt. *Trypanosoma equiperdum* and *Trypanosoma evansi* are agents of dourine and surra, diseases of horses, camels, and water buffaloes. We have characterized representative strains of *T. equiperdum* and *T. evansi* by numerous molecular and classical parasitological approaches. We show that both species are actually strains of *T. brucei*, which lost part (Dk) or all (Ak) of their kDNA. These trypanosomes are not monophyletic clades and do not qualify for species status. They should be considered two subspecies, respectively *T. brucei equiperdum* and *T. brucei evansi*, which spontaneously arose recently. Dk/Ak trypanosomes may potentially emerge repeatedly from *T. brucei.*

14454. **Mamoudou, A., Delespaux, V., Chepnda, V., Hachimou, Z., Andrikaye, J. P., Zoli, A. & Geerts, S., 2008**. Assessment of the occurrence of trypanocidal drug resistance in trypanosomes of naturally infected cattle in the Adamaoua region of

Cameroon using the standard mouse test and molecular tools. *Acta Tropica,* **106** (2) 115-118.

Université de Dschang, Faculté d'Agronomie et des Sciences Agricoles, BP 96, Dschang, Cameroon.

From May to November 2005, a study was carried out to assess the occurrence of trypanocidal drug resistance (DR) in trypanosomes of naturally infected cattle of the Adamaoua region of Cameroon. Two distinct polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) procedures were used together with an allele specific-PCR (AS-PCR) and the standardized single-dose mouse test. Using the mouse test, 3 of the 13 *Trypanosoma brucei* isolates and all 14 tested *Trypanosoma congolense* isolates were resistant to ISM. However, only 11 of the 25 *T. congolense* isolates were diagnosed as resistant to ISM using the MboII-PCR-RFLP. Resistance to DA was identified in one of the 13 *T. brucei* isolates and all 11 *T. congolense* isolates which were tested with the mouse test. Using the AS-PCR or BclI-PCR-RFLP, three of the 13 *T. brucei* isolates and all 25 *T. congolense* isolates respectively were found resistant. The data presented in this study prove that DR is widespread in the Adamaoua Department of Cameroon. The problem appears to be more serious in *T. congolense* than in *T. brucei*. Appropriate measures need to be taken in order to control bovine trypanosomosis in this area.

14455. **Nerima, B., Matovu, E., Lubega, G. W. & Enyaru, J. C., 2007**. Detection of mutant P2 adenosine transporter (*Tb*AT1) gene in *Trypanosoma brucei gambiense* isolates from northwest Uganda using allele-specific polymerase chain reaction. *Tropical Medicine and International Health,* **12** (11): 1361-1368.

National Livestock Health Research Institute, Tororo, Uganda.

To assess the application of allele-specific PCR (AS-PCR) as a fast, cheap and reliable method for detecting mutant *Tb*AT1 associated with melarsoprol relapse in *Trypanosoma brucei gambiense* isolates from northwest Uganda, 105 trypanosome isolates were analysed using SfaN1 restriction fragment length polymorphism (RFLP) and AS-PCR, the former used as the gold standard. Sensitivity, specificity, positive and negative predictive values of AS-PCR as well as agreement between the tests were determined. Eleven trypanosome isolates had mutant *Tb*AT1 while 94 exhibited the wild-type *Tb*AT1 genes. There was a highly significant agreement between SfaN1 RFLP and AS-PCR with kappa and intra-class correlation values of 1.0. The sensitivity and specificity of AS-PCR were both 100 percent, while the positive and negative predictive values were found to be equal to 1.0. Cost and time analyses were performed and AS-PCR was 4.3 times cheaper than SfaN1 RFLP, in addition to the less time required for its execution. It was concluded that AS-PCR should be the test of choice for screening for mutant *Tb*AT1 in the ever-increasing numbers of field trypanosome isolates.

14456. **Njiru, Z. K., Mikosza, A. S., Armstrong, T., Enyaru, J. C., Ndung'u, J. M. & Thompson, A. R., 2008**. Loop-mediated isothermal amplification (LAMP) method for rapid detection of *Trypanosoma brucei rhodesiense*. *PLoS Neglected Tropical Diseases,* **2** (1): e147.

School of Nursing, Murdoch University, Mandurah, Western Australia, Australia.

Loop-mediated isothermal amplification (LAMP) of DNA is a novel technique that rapidly amplifies target DNA under isothermal conditions. In the present study, a LAMP test was designed from the serum resistance-associated (SRA) gene of *Trypanosoma brucei rhodesiense*, the cause of the acute form of African sleeping sickness, and used to detect parasite DNA from processed and heat-treated infected blood samples. The SRA gene is specific to *T. b. rhodesiense* and has been shown to confer resistance to lysis by normal human serum. The assay was performed at 62^{o} C for 1 h, using six primers that recognised eight targets. The template was varying concentrations of trypanosome DNA and supernatant from heat-treated infected blood samples. The resulting amplicons were detected using SYTO-9 fluorescence dye in a real-time thermocycler, visual observation after the addition of SYBR Green I, and gel electrophoresis. DNA amplification was detected within 35 min. The SRA LAMP test had an unequivocal detection limit of one pg of purified DNA (equivalent to 10 trypanosomes/ml) and 0.1 pg (1 trypanosome/ml) using heat-treated buffy coat, while the detection limit for conventional SRA PCR was approximately 1,000 trypanosomes/ml. The expected LAMP amplicon was confirmed through restriction enzyme RsaI digestion, identical melt curves, and sequence analysis. The reproducibility of the SRA LAMP assay using a water bath and a heat-processed template, and the ease of results readout show great potential for the diagnosis of *T. b. rhodesiense* in endemic regions.

14457. **Njiru, Z. K., Mikosza, A. S., Matovu, E., Enyaru, J. C., Ouma, J. O., Kibona, S. N., Thompson, R. C. & Ndung'u, J. M., 2008**. African trypanosomiasis: sensitive and rapid detection of the sub-genus *Trypanozoon* by loop-mediated isothermal amplification (LAMP) of parasite DNA. *International Journal of Parasitology,* **38** (5): 589-599.

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Control of human African trypanosomiasis (HAT) is dependent on accurate diagnosis and treatment of infected patients. However, sensitivities of tests in routine use are unsatisfactory, due to the characteristically low parasitaemias in naturally infected individuals. We have identified a conserved sequence in the repetitive insertion mobile element (RIME) of the sub-genus *Trypanozoon* and used it to design primers for a highly specific loop-mediated isothermal amplification (LAMP) test. The test was used to analyse *Trypanozoon* isolates and clinical samples from HAT patients. The RIME LAMP assay was performed at 62^{0} C using real-time PCR and a water bath. DNA amplification was detectable within 25min. All positive samples detected by gel electrophoresis or in real-time using SYTO-9 fluorescence dye could also be detected visually by addition of SYBR Green I to the product. The amplicon was unequivocally confirmed through restriction enzyme NdeI digestion, analysis of melt curves and sequencing. The analytical sensitivity of the RIME LAMP assay was equivalent to 0.001 trypanosomes/ml while that of classical PCR tests ranged from 0.1 to 1,000 trypanosomes/ml. LAMP detected all 75 *Trypanozoon* isolates while TBR1 and two primers (specific for sub-genus *Trypanozoon*) showed a sensitivity of

86.9 percent. The SRA gene PCR detected 21 out of 40 *Trypanosoma brucei rhodesiense* isolates while *Trypanosoma gambiense*-specific glycoprotein primers (TgsGP) detected 11 out of 13 *T. b. gambiense* isolates. Using clinical samples, the LAMP test detected parasite DNA in 18 out of 20 samples which included using supernatant prepared from boiled blood, CSF and direct native serum. The sensitivity and reproducibility of the LAMP assay coupled with the ability to detect the results visually without the need for sophisticated equipment indicate that the technique has strong potential for detection of HAT in clinical settings. Since the LAMP test shows a high tolerance to different biological substances, determination of the appropriate protocols for processing the template to make it a user-friendly technique, prior to large scale evaluation, is needed.

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14458. **Abdille, M. H., Li, S. Y., Jia, Y., Suo, X. & Mkoji, G., 2008**. Evidence for the existence of paraflagellar rod protein 2 (PFR2) gene in *Trypanosoma evansi* and its conservation among other kinetoplastid parasites. *Experimental Parasitology,* **118** (4): 614-618.

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Paraflagellar rod proteins required for cell motility are unique among the kinetoplastids and their heteropolymers provide the building block of the flagellum. We investigated the existence of the paraflagellar rod protein 2 (PFR2) gene in *Trypanosoma evansi* by reverse transcription-polymerase chain reaction (RT-PCR) using primers designed based on the open reading frame of the PFR2 gene of *Trypanosoma brucei*. The PFR2 gene was cloned and the PFR2-encoded protein was expressed in bacteria. The expressed His-tag protein was purified using nickel affinity chromatography and confirmed by gel electrophoresis and Western blotting. The nucleotide sequence of the PFR2 gene of *T. evansi* showed 100 percent identity with the sequence of the PFR2 gene of *T. brucei* and 83.4 percent and 76.6 percent similarity with that of *Trypanosoma cruzi* and *Leishmania mexicana*, respectively. The conserved domain among various PFR2 genes present in kinetoplastids could be used as a target for the development of vaccines against multiple *Trypanosoma* species.

14459. **Absalon, S., Blisnick, T., Kohl, L., Toutirais, G., Dore, G., Julkowska, D., Tavenet, A. & Bastin, P., 2008**. Intraflagellar transport and functional analysis of genes required for flagellum formation in Trypanosomes. *Molecular Biology of the Cell,* **19** (3): 929-944.

Trypanosome Cell Biology Unit, Pasteur Institute and Centre National de la Recherche Scientifique, 75015 Paris, France, Dynamique et Regulation des Génomes, Muséum National d'Histoire Naturelle, Institut National de la Santé et de la Recherche Médicale and Centre National de la Recherche Scientifique, 75005 Paris, France.

Intraflagellar transport (IFT) is the bidirectional movement of protein complexes required for cilia and flagella formation. We investigated IFT by analyzing nine conventional IFT genes and five novel putative IFT genes (PIFT) in *Trypanosoma brucei* that maintain its existing flagellum while assembling a new flagellum. Immunostaining against IFT172 or expression of tagged IFT20 or green fluorescent protein GFP::IFT52 revealed the presence of IFT proteins along the axoneme and at the basal body and probasal body regions of both old and new flagella. IFT particles were detected by electron microscopy and exhibited a strict localization to axonemal microtubules 3-4 and 7-8, suggesting the existence of specific IFT tracks. Rapid (>3 mum/s) bidirectional intraflagellar movement of GFP::IFT52 was observed in old and new flagella. RNA interference silencing demonstrated that all individual IFT and PIFT genes are essential for new flagellum construction but the old flagellum remained present. Inhibition of IFTB proteins completely blocked axoneme construction. Absence of IFTA proteins (IFT122 and IFT140) led to formation of short flagella filled with IFT172, indicative of defects in retrograde transport. Two PIFT proteins turned out to be required for retrograde transport and three for anterograde transport. Finally, flagellum membrane elongation continues despite the absence of axonemal microtubules in all IFT/PIFT mutant.

14460. **Adler, A., Forster, N., Homann, M. & Goringer, H. U., 2008**. Post-SELEX chemical optimization of a trypanosome-specific RNA aptamer. *Combinatorial Chemistry and High Throughput Screening,* **11** (1): 16-23.

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African trypanosomes are the causative agent of sleeping sickness. The therapeutics used to control and treat the disease are very ineffective and thus, the development of improved drugs is urgently needed. Recently, new strategies for the design of novel trypanocidals have been put forward. Among them are techniques that rely on parasite-specific RNA aptamers. One approach involves the aptamer-directed transport of lytic compounds to the lysosome of the parasite. The aptamer has been termed 2-16 RNA and here we report the optimization of the RNA for its applications *in vivo*. To convert aptamer 2-16 into a serum-stable reagent 2'-deoxy-2'-F- and/or 2'-deoxy-2'-NH(2)-uridine- and cytidine-substituted RNAs were generated. While 2'-NH(2)-dC/dU-modified RNAs were RNase-resistant, they were functionally inactive. By contrast, 2'-F-dC/dU-substituted 2-16 RNA retained its ability to bind to live trypanosomes (K(d)=45 nM) and was routed to the lysosome identically to unmodified RNA. 2'-F-dC/dU-substituted 2-16 RNA is thermostable (T(m)=75 degrees C) and has a serum half-life of 3.4 days. Furthermore, aptamer 2-16 was site-specifically PEGylated to increase its serum retention time. Conjugation with PEG polymers < or = 10 kDa only marginally impacted the binding characteristics of the RNA, while the addition of higher molecular mass PEG molecules resulted in non-functional aptamers. Together, the data provide optimized conjugation chemistries for the large-scale production of substituted aptamer 2-16 preparations with improved *in vivo* functionality.

14461. **Alatortsev, V. S., Cruz-Reyes, J., Zhelonkina, A. G. & Sollner-Webb, B., 2008**. *Trypanosoma brucei* RNA editing: coupled cycles of U deletion reveal processive activity of the editing complex. *Molecular and Cellular Biology,* **28** (7): 2437-2445.

Department of Biological Chemistry, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205, USA.

RNA editing in *Trypanosoma brucei* is posttranscriptional uridylate removal/addition, generally at vast numbers of pre-mRNA sites, but to date, only single editing cycles have been examined *in vitro*. We here demonstrate achieving sequential cycles of U deletion *in vitro*, with editing products confirmed by sequence analysis. Notably, the subsequent editing cycle is much more efficient and occurs far more rapidly than single editing cycles; plus, it has different recognition requirements. This indicates that the editing complex acts in a concerted manner and does not dissociate from the RNA substrate between these cycles. Furthermore, the multicycle substrate exhibits editing that is unexpected from a strictly 3'-to-5' progression, reminiscent of the unexpected editing that has been shown to occur frequently in *T. brucei* mRNAs edited *in vivo*. This unexpected editing is most likely due to alternate mRNA:guide RNA (gRNA) alignment forming a hyphenated anchor; its having only a 2-bp proximal duplex helps explain the prevalence of unexpected editing *in vivo*. Such unexpected editing was not previously reported *in vitro*, presumably because the common use of artificially tight mRNA:gRNA base pairing precludes alternate alignments. The multicycle editing and unexpected editing presented in this paper bring *in vitro* reactions closer to reproducing the *in vivo* editing process.

14462. **Almo, S. C., Bonanno, J. B., Sauder, J. M., Emtage, S., Dilorenzo, T. P., Malashkevich, V., Wasserman, S. R., Swaminathan, S., Eswaramoorthy, S., Agarwal, R., Kumaran, D., Madegowda, M., Ragumani, S., Patskovsky, Y., Alvarado, J., Ramagopal, U. A., Faber-Barata, J., Chance, M. R., Sali, A., Fiser, A., Zhang, Z. Y., Lawrence, D. S. & Burley, S. K., 2007**. Structural genomics of protein phosphatases. *Journal of Structural and Functional Genomics,* **8** (2-3): 121-140.

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The New York SGX Research Center for Structural Genomics (NYSGXRC) of the NIGMS Protein Structure Initiative (PSI) has applied its high-throughput X-ray crystallographic structure determination platform to systematic studies of all human protein phosphatases and protein phosphatases from biomedically-relevant pathogens. To date, the NYSGXRC has determined structures of 21 distinct protein phosphatases: 14 from human, two from mouse, two from the pathogen *Toxoplasma gondii*, one from *Trypanosoma brucei*, the parasite responsible for African sleeping sickness, and two from the principal mosquito vector of malaria in Africa, *Anopheles gambiae*. These structures provide insights into both normal and pathophysiologic processes, including transcriptional regulation, regulation of major signaling pathways, neural development, and type 1 diabetes. In conjunction with the contributions of other international structural genomics consortia, these efforts promise to provide an unprecedented database and materials repository for structure-guided experimental and computational discovery of inhibitors for all classes of protein phosphatases.

14463. **Amaro, R. E., Swift, R. V. & McCammon, J. A., 2007**. Functional and structural insights revealed by molecular dynamics simulations of an essential RNA editing ligase in *Trypanosoma brucei*. *PLoS Neglected Tropical Diseases,* **1** (2): e68.

Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, California, USA.

RNA editing ligase 1 (TbREL1) is required for the survival of both the insect and bloodstream forms of *Trypanosoma brucei*, the parasite responsible for the devastating tropical disease African sleeping sickness. The type of RNA editing that *Tb*REL1 is involved in is unique to the trypanosomes, and no close human homolog is known to exist. In addition, the high-resolution crystal structure revealed several unique features of the active site, making this enzyme a promising target for structure-based drug design. In this work, two 20 ns atomistic molecular dynamics (MD) simulations are employed to investigate the dynamics of *Tb*REL1, both with and without the ATP substrate present. The flexibility of the active site, dynamics of conserved residues and crystallized water molecules, and the interactions between *Tb*REL1 and the ATP substrate are investigated and discussed in the context of *Tb*REL1's function. Differences in local and global motion upon ATP binding suggest that two peripheral loops, unique to the trypanosomes, may be involved in interdomain signaling events. Notably, a significant structural rearrangement of the enzyme's active site occurs during the apo simulations, opening an additional cavity adjacent to the ATP binding site that could be exploited in the development of effective inhibitors directed against this protozoan parasite. Finally, ensemble averaged electrostatics calculations over the MD simulations reveal a novel putative RNA binding site, a discovery that has previously eluded scientists. Ultimately, we use the insights gained through the MD simulations to make several predictions and recommendations, which we anticipate will help direct future experimental studies and structure-based drug discovery efforts against this vital enzyme.

14464. **Arakaki, T. L., Buckner, F. S., Gillespie, J. R., Malmquist, N. A., Phillips, M. A., Kalyuzhniy, O., Luft, J. R., Detitta, G. T., Verlinde, C. L., Van Voorhis, W. C., Hol, W. G. & Merritt, E. A., 2008**. Characterization of *Trypanosoma brucei* dihydroorotate dehydrogenase as a possible drug target; structural, kinetic and RNAi studies. *Molecular Microbiology,* **68** (1): 37-50.

Department of Biochemistry, University of Washington, Seattle, WA 98195, USA.

Nucleotide biosynthesis pathways have been reported to be essential in some protozoan pathogens. Hence, we evaluated the essentiality of one enzyme in the pyrimidine biosynthetic pathway, dihydroorotate dehydrogenase (DHODH) from the eukaryotic parasite *Trypanosoma brucei* through gene knockdown studies. RNAi knockdown of DHODH expression in bloodstream form *T. brucei* did not inhibit growth in normal medium, but profoundly retarded growth in pyrimidine-depleted media or in the presence of the known pyrimidine uptake antagonist 5-fluorouracil (5-FU). These results have significant implications for the development of therapeutics to combat *T. brucei* infection. Specifically, a combination therapy including a *T. brucei*-specific DHODH inhibitor plus 5-FU may prove to be an effective therapeutic strategy. We also show that this trypanosomal enzyme is

inhibited by known inhibitors of bacterial Class 1A DHODH, in distinction to the sensitivity of DHODH from human and other higher eukaryotes. This selectivity is supported by the crystal structure of the *T. brucei* enzyme, which is reported here at a resolution of 1.95 A. Additional research, guided by the crystal structure described herein, is needed to identify potent inhibitors of *T. brucei* DHODH.

14465. **Autio, K. J., Guler, J. L., Kastaniotis, A. J., Englund, P. T. & Hiltunen, J. K., 2008**. The 3-hydroxyacyl-ACP dehydratase of mitochondrial fatty acid synthesis in *Trypanosoma brucei*. *FEBS Letters,* **582** (5): 729-733.

Department of Biochemistry and Biocenter Oulu, University of Oulu, Oulu, Finland. [kaija.autio@oulu.fi].

14466. **Azzouz, N., Gerold, P. & Schwarz, R. T., 2008**. Metabolic labelling and structural analysis of glycosylphosphatidylinositols from parasitic protozoa. *Methods in Molecular Biology,* **446**: 183-198.

Laboratory for Organic Chemistry, Swiss Federal Institute of Technology, Zurich, Switzerland.

14467. **Barth, S., Shalem, B., Hury, A., Tkacz, I. D., Liang, X. H., Uliel, S., Myslyuk, I., Doniger, T., Salmon-Divon, M., Unger, R. & Michaeli, S., 2008**. Elucidating the role of C/D snoRNA in rRNA processing and modification in *Trypanosoma brucei*. *Eukaryotic Cell,* **7** (1): 86-101.

The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel.

14468. **Bellofatto, V. & Palenchar, J. B., 2008**. RNA interference as a genetic tool in trypanosomes. *Methods in Molecular Biology,* **442**: 83-94.

Department of Microbiology and Molecular Genetics, UMDNJ-NJ Medical School, International Center for Public Health, Newark, USA.

RNA interference (RNAi) is a cellular mechanism that is often exploited as a technique for quelling the expression of a specific gene. RNAi studies are carried out *in vivo*, making this a powerful means for the study of protein function *in situ.* Several trypanosomatids, including those organisms responsible for human and animal diseases, naturally possess the machinery necessary for RNAi manipulations. This allows for the use of RNAi in unravelling many of the pressing questions regarding the parasite's unique biology. The completion of the *Trypanosoma brucei* genome sequence, coupled with several powerful genetic tools, has resulted in widespread utilization of RNAi in this organism. The key steps for RNAi-based reduction of gene expression, including parasite cell culture, DNA transfection, RNAi expression, and experimental execution, are discussed with a focus on procyclic forms of *Trypanosoma brucei.*

14469. **Brandenburg, J., Schimanski, B., Nogoceke, E., Nguyen, T. N., Padovan, J. C., Chait, B. T., Cross, G. A. & Gunzl, A., 2007**. Multifunctional class I transcription in *Trypanosoma brucei* depends on a novel protein complex. The *Embo Journal,* **26** (23): 4856-4866.

Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT 06030-3710, USA.

The vector-borne, protistan parasite *Trypanosoma brucei* is the only known eukaryote with a multifunctional RNA polymerase I that, in addition to ribosomal genes, transcribes genes encoding the parasite's major cell surface proteins-the variant surface glycoprotein (VSG) and procyclin. In the mammalian bloodstream, antigenic variation of the VSG coat is the parasite's means to evade the immune response, while procyclin is necessary for effective establishment of trypanosome infection in the fly. Moreover, the exceptionally high efficiency of mono-allelic VSG expression is essential to bloodstream trypanosomes since its silencing caused rapid cell-cycle arrest *in vitro* and clearance of parasites from infected mice. Here we describe a novel protein complex that recognizes class I promoters and is indispensable for class I transcription; it consists of a dynein light chain and six polypeptides that are conserved only among trypanosomatid parasites. In accordance with an essential transcriptional function of the complex, silencing the expression of a key subunit was lethal to bloodstream trypanosomes and specifically affected the abundance of rRNA and VSG mRNA. The complex was dubbed class I transcription factor A.

14470. **Brenchley, R., Tariq, H., McElhinney, H., Szoor, B., Huxley-Jones, J., Stevens, R., Matthews, K. & Tabernero, L., 2007**. The TriTryp phosphatome: analysis of the protein phosphatase catalytic domains. *BMC Genomics,* **8**: 434.

Faculty of Life Sciences, Michael Smith, University of Manchester, M13 9PT, UK. [Rachel.Brenchley@postgrad.manchester.ac.uk].

14471. **Bridges, D. J., Pitt, A. R., Hanrahan, O., Brennan, K., Voorheis, H. P., Herzyk, P., de Koning, H. P. & Burchmore, R. J., 2008**. Characterisation of the plasma membrane subproteome of bloodstream form *Trypanosoma brucei*. *Proteomics,* **8** (1): 83-99.

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

Proteome analysis by conventional approaches is biased against hydrophobic membrane proteins, many of which are also of low abundance. We have isolated plasma membrane sheets from bloodstream forms of *Trypanosoma brucei* by subcellular fractionation, and then applied a battery of complementary protein separation and identification techniques to identify a large number of proteins in this fraction. The results of these analyses have been combined to generate a subproteome for the pellicular plasma membrane of bloodstream forms of *T. brucei* as well as a separate subproteome for the pellicular cytoskeleton. In parallel, we have used *in silico* approaches to predict the relative abundance of proteins potentially expressed by bloodstream form trypanosomes, and to

identify likely polytopic membrane proteins, providing quality control for the experimentally defined plasma membrane subproteome. We show that the application of multiple high-resolution proteomic techniques to an enriched organelle fraction is a valuable approach for the characterisation of relatively intractable membrane proteomes. We present here the most complete analysis of a protozoan plasma membrane proteome to date and show the presence of a large number of integral membrane proteins, including 11 nucleoside/nucleobase transporters, 15 ion pumps and channels and a large number of adenylate cyclases hitherto listed as putative proteins.

14472. **Cabrera, N., Hernandez-Alcantara, G., Mendoza-Hernandez, G., Gomez-Puyou, A. & Perez-Montfort, R., 2008**. Key residues of loop 3 in the interaction with the interface residue at position 14 in triosephosphate isomerase from *Trypanosoma brucei*. *Biochemistry,* **47** (11): 3499-3506.

Departamento de Bioquimica, Instituto de Fisiologia Celular, Universidad Nacional Autonoma de Mexico, Apartado Postal 70242, 04510 Mexico DF, Mexico.

14473. **Chambers, J. W., Kearns, M. T., Morris, M. T. & Morris, J. C., 2008**. Assembly of heterohexameric trypanosome hexokinases reveals that hexokinase 2 is a regulable enzyme. *Journal of Biological Chemistry*. **Published online April 3, 2008**

Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634-0318, USA.

14474. **Chambers, J. W., Morris, M. T., Smith, K. S. & Morris, J. C., 2008**. Residues in an ATP binding domain influence sugar binding in a trypanosome hexokinase. *Biochemical and Biophysical Research Communications,* **365** (3): 420-425.

Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634, USA.

14475. **Das, A., Banday, M. & Bellofatto, V., 2008**. RNA polymerase transcription machinery in trypanosomes. *Eukaryotic Cell,* **7** (3): 429-434.

Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, 225 Warren St., Newark, NJ 07103, USA.

14476. **Davila, A. M., Mendes, P. N., Wagner, G., Tschoeke, D. A., Cuadrat, R. R., Liberman, F., Matos, L., Satake, T., Ocana, K. A., Triana, O., Cruz, S. M., Juca, H. C., Cury, J. C., Silva, F. N., Geronimo, G. A., Ruiz, M., Ruback, E., Silva, F. P., Jr., Probst, C. M., Grisard, E. C., Krieger, M. A., Goldenberg, S., Cavalcanti, M. C., Moraes, M. O., Campos, M. L. & Mattoso, M., 2008**. ProtozoaDB: dynamic visualization and exploration of protozoan genomes. *Nucleic Acids Research,* **36** (Database issue): D547-552.

Oswaldo Cruz Institute, FIOCRUZ, CCB, Federal University of Santa Catarina (UFSC), ACBS/UNOESC/SC, COPPE, Federal University of Rio de Janeiro, Brazil. [davila@fiocruz.br].

ProtozoaDB (http://www.biowebdb.org/protozoadb) is being developed to initially host both genomics and post-genomics data from *Plasmodium falciparum*, *Entamoeba histolytica*, *Trypanosoma brucei*, *T. cruzi* and *Leishmania major*, but will hopefully host other protozoan species as more genomes are sequenced. It is based on the Genomics Unified Schema and offers a modern Web-based interface for user-friendly data visualization and exploration. This database is not intended to duplicate other similar efforts such as GeneDB, PlasmoDB, TcruziDB or even TDRtargets, but to be complementary by providing further analyses with emphasis on distant similarities (HMM-based) and phylogeny-based annotations including orthology analysis. ProtozoaDB will be progressively linked to the above-mentioned databases, focusing in performing a multi-source dynamic combination of information through advanced interoperable Web tools such as Web services. Also, to provide Web services will allow third-party software to retrieve and use data from ProtozoaDB in automated pipelines (workflows) or other interoperable Web technologies, promoting better information reuse and integration. We also expect ProtozoaDB to catalyze the development of local and regional bioinformatics capabilities (research and training), and therefore promote/enhance scientific advancement in developing countries.

14477. **Davila Lopez, M. & Samuelsson, T., 2008**. Early evolution of histone mRNA 3' end processing. *Rna,* **14** (1): 1-10.

Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, Sahlgrenska Academy at Goteborg University, SE-405 30 Goteborg, Sweden.

14478. **Ebikeme, C. E., Peacock, L., Coustou, V., Riviere, L., Bringaud, F., Gibson, W. C. & Barrett, M. P., 2008**. N-acetyl D-glucosamine stimulates growth in procyclic forms of *Trypanosoma brucei* by inducing a metabolic shift. *Parasitology,* **135** (5): 585-594.

Division of Infection and Immunity, Glasgow Biomedical Research Centre, University of Glasgow, 120 University Place, Glasgow G12 8TA, UK.

14479. **Farrera-Sinfreu, J., Espanol, Y., Geslain, R., Guitart, T., Albericio, F., Ribas de Pouplana, L. & Royo, M., 2008**. Solid-phase combinatorial synthesis of a lysyl-tRNA synthetase (LysRS) inhibitory library. *Journal of Combinatorial Chemistry,* **10** (3): 391-400.

Institute for Research in Biomedicine and Combinatorial Chemistry Unit, Barcelona Science Park, University of Barcelona, Josep Samitier 1, 08028-Barcelona, Spain, Department of Organic Chemistry, University of Barcelona, Marti i Franques 1, 08028-Barcelona, Spain, and Catalan Institution for Research and Advanced Studies (ICREA), Passeig Lluis Companys 23, 08010-Barcelona, Spain. [mroyo@pcb.ub.es].

The solid-phase combinatorial synthesis of a new library with potential inhibitory activity against the cytoplasmic lysyl-tRNA synthetase (LysRS) isoform of *Trypanosoma brucei* is described. The library has been specifically designed to mimic the lysyl adenylate complex. The design was carried out by dividing the complex into four modular parts. Proline derivatives (cis-gamma-amino- l-proline or trans-gamma-hydroxy- l-proline) were chosen as central scaffolds. After primary screening, three compounds of the library caused *in vitro* inhibition of the tRNA aminoacylation reaction in the low micromolar range.

14480. **Fenn, K. & Matthews, K. R., 2007**. The cell biology of *Trypanosoma brucei* differentiation. *Current Opinion in Microbiology,* **10** (6): 539-546.

Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Ashworth Laboratories, King's Buildings, West Mains Road, Edinburgh EH9 3JT, UK.

Developmental events in the life-cycle of the sleeping sickness parasite comprise integrated changes in cell morphology, metabolism, gene expression and signalling pathways. In each case these processes differ from the eukaryotic norm. In the past three years, understanding of these developmental processes has progressed from a description of the cytological events of differentiation to a discovery of its underlying molecular controls. With an expanding set of reagents for the identification of distinct parasite life-cycle stages in the tsetse, trypanosome differentiation is being studied from the molecular to the organismal and population level. Interestingly, the new molecular discoveries provide insights into the biology of the parasite in the field.

14481. **Field, M. C., Horn, D. & Carrington, M., 2008**. Analysis of small GTPase function in trypanosomes. *Methods in Enzymology,* **438**: 57-76.

Department of Pathology, University of Cambridge, Cambridge, UK.

Trypanosomatids are protozoan parasites, of interest due to both their disease burden and deeply divergent position within the eukaryotic lineage. The African trypanosome, *Trypanosoma brucei*, has emerged as a very amenable model system, with a considerable toolbox of methods available, including inducible overexpression, RNA interference, and a completed genome. Here we describe some of the special considerations that need to be addressed when studying trypanosome gene function, and in particular small GTPases; we provide protocols for transfection, RNA interference, overexpression and basic transport assays, in addition to an overview of available vectors, cell lines, and strategies.

14482. **Filser, M., Comini, M. A., Molina-Navarro, M. M., Dirdjaja, N., Herrero, E. & Krauth-Siegel, R. L., 2008**. Cloning, functional analysis, and mitochondrial localization of *Trypanosoma brucei* monothiol glutaredoxin-1. Journal *ofBiological Chemistry,* **389** (1): 21-32.

Biochemie-Zentrum der Universität Heidelberg, Im Neuenheimer Feld 504, D-69120 Heidelberg, Germany.

14483. **Gannavaram, S., Vedvyas, C. & Debrabant, A., 2008**. Conservation of the pro-apoptotic nuclease activity of endonuclease G in unicellular trypanosomatid parasites. *Journal of Cell Science,* **121** (Pt 1): 99-109.

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Endonuclease G is a mitochondrial protein implicated in DNA fragmentation during apoptosis in cell types ranging from fungi to mammals. Features of programmed cell death have been reported in a number of single-celled organisms, including the human trypanosomatid parasites *Leishmania* and *Trypanosoma*. However, the protozoan cell death pathways and the effector molecules involved in such processes remain to be identified. In this report, we describe the pro-apoptotic function of endonuclease G in trypanosomatid parasites. Similar to metazoans, trypanosome endoG showed intrinsic nuclease activity, is localized in mitochondria and is released from this organelle when cell death is triggered. Overexpression of endoG strongly promoted apoptotic cell death under oxidant or differentiation-related stress in *Leishmania* and, conversely, loss of endoG expression conferred robust resistance to oxidant-induced cell death in *T. brucei*. These data demonstrate the conservation of the pro-apoptotic endonuclease activity of endoG in these evolutionarily ancient eukaryotic organisms. Furthermore, nuclear DNA degradation by endoG upon release from mitochondria might represent a caspase-independent cell death mechanism in trypanosomatid parasites as genes encoding caspase-like proteins have not been identified in their genomes.

14484. **Glover, L., McCulloch, R. & Horn, D., 2008**. Sequence homology and microhomology dominate chromosomal double-strand break repair in African trypanosomes. *Nucleic Acids Research*, **36** (8): 2608-2618.

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Genetic diversity in fungi and mammals is generated through mitotic double-strand break-repair (DSBR), typically involving homologous recombination (HR) or non-homologous end joining (NHEJ). Microhomology-mediated joining appears to serve a subsidiary function. The African trypanosome, a divergent protozoan parasite, relies upon rearrangement of subtelomeric variant surface glycoprotein (VSG) genes to achieve antigenic variation. Evidence suggests an absence of NHEJ but chromosomal repair remains largely unexplored. We used a system based on I-SceI meganuclease and monitored temporally constrained DSBR at a specific chromosomal site in bloodstream form *Trypanosoma brucei*. In response to the lesion, adjacent single-stranded DNA was generated; the homologous strand-exchange factor, Rad51, accumulated into foci; a G(2)M checkpoint was activated and >50 percent of cells displayed successful repair. Quantitative analysis of DSBR pathways employed indicated that inter-chromosomal HR dominated. HR displayed a strong preference for the allelic template but also the capacity to interact with homologous sequence on

heterologous chromosomes. Intra-chromosomal joining was predominantly, and possibly exclusively, microhomology mediated, a situation unique among organisms examined to date. These DSBR pathways available to *T. brucei* likely underlie patterns of antigenic variation and the evolution of the vast VSG gene family.

14485. **Guler, J. l., Kriegova, E.; Smith, T. K., Lukeš J. & Englund P.T., 2008.** Mitochondrial fatty acid synthesis is required for normal mitochondrial morphology and function in *Trypanosoma brucei. Molecular Microbiology,* **67** (5): 1125-1142.

Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD, USA.,Institute of Parasitology, Czech Academy of Sciences, and Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic, Division of Biological Chemistry and Drug Discovery, Wellcome Trust Biocentre, College of Life Sciences, University of Dundee, Dundee, U.K. [penglund@jhmi.edu]

Trypanosoma brucei use microsomal elongases for *de novo* synthesis of most of its fatty acids. In addition, this parasite utilizes an essential mitochondrial type II synthase for production of octanoate (a lipoic acid precursor) as well as longer fatty acids such as palmitate. Evidence from other organisms suggests that mitochondrially synthesized fatty acids are required for efficient respiration but the exact relationship remains unclear. In procyclic form trypanosomes, we also found that RNAi depletion of the mitochondrial acyl carrier protein, an important component of the fatty acid synthesis machinery, significantly reduces cytochrome-mediated respiration. This reduction was explained by RNAi-mediated inhibition of respiratory complexes II, III and IV, but not complex I. Other effects of RNAi, such as changes in mitochondrial morphology and alterations in membrane potential, raised the possibility of a change in mitochondrial membrane composition. Using mass spectrometry, we observed a decrease in total and mitochondrial phosphatidylinositol and mitochondrial phosphatidylethanolamine. Thus, we conclude that the mitochondrial synthase produces fatty acids needed for maintaining local phospholipid levels that are required for activity of respiratory complexes and preservation of mitochondrial morphology and function.

14486. **Haanstra, J. R., Stewart, M., Luu, V. D., van Tuijl, A., Westerhoff, H. V., Clayton, C. & Bakker, B. M., 2008.** Control and regulation of gene expression: quantitative analysis of the expression of phosphoglycerate kinase in bloodstream form *Trypanosoma brucei*. *Journal of Biological Chemistry*, **283** (5): 2495-2507.

Vrije Universiteit, Biocentrum Amsterdam, De Boelelaan 1085, Amsterdam, The Netherlands.

Isoenzymes of phosphoglycerate kinase in *Trypanosoma brucei* are differentially expressed in its two main life stages. This study addresses how the organism manages to make sufficient amounts of the isoenzyme with the correct localization, which processes (transcription, splicing, and RNA degradation) control the levels of mRNAs, and how the organism regulates the switch in isoform expression. For this, we combined new quantitative

measurements of phosphoglycerate kinase mRNA abundance, RNA precursor stability, trans splicing, and ribosome loading with published data and made a kinetic computer model. For the analysis of regulation we extended regulation analysis. Although phosphoglycerate kinase mRNAs are present at surprisingly low concentrations (e.g. 12 molecules per cell), its protein is highly abundant. Substantial control of mRNA and protein levels was exerted by both mRNA synthesis and degradation, whereas splicing and precursor degradation had little control on mRNA and protein concentrations. Yet regulation of mRNA levels does not occur by transcription, but by adjusting mRNA degradation. The contribution of splicing to regulation is negligible, as for all cases where splicing is faster than RNA precursor degradation.

14487. **Hartley, C. L. & McCulloch, R., 2008**. *Trypanosoma brucei* BRCA2 acts in antigenic variation and has undergone a recent expansion in BRC repeat number that is important during homologous recombination. *Molecular Microbiology,* **68** (5): 1237-1251.

The Wellcome Centre for Molecular Parasitology and Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow Biomedical Research Centre, 120 University Place, Glasgow G12 8TA, UK.

Antigenic variation in *Trypanosoma brucei* has selected for the evolution of a massive archive of silent Variant Surface Glycoprotein (VSG) genes, which are activated by recombination into specialized expression sites. Such VSG switching can occur at rates substantially higher than background mutation and is dependent on homologous recombination, a core DNA repair reaction. A key regulator of homologous recombination is BRCA2, a protein that binds RAD51, the enzyme responsible for DNA strand exchange. Here, we show that *T. brucei* BRCA2 has undergone a recent, striking expansion in the number of BRC repeats, a sequence element that mediates interaction with RAD51. *T. brucei* BRCA2 mutants are shown to be significantly impaired in antigenic variation and display genome instability. By generating BRCA2 variants with reduced BRC repeat numbers, we show that the BRC expansion is crucial in determining the efficiency of *T. brucei* homologous recombination and RAD51 localization. Remarkably, however, this appears not to be a major determinant of the activation of at least some VSG genes.

14488. **Hartmann, C. & Clayton, C., 2008**. Regulation of a transmembrane protein gene family by the small RNA-binding proteins *Tb*UBP1 and *Tb*UBP2. *Molecular and Biochemical Parasitology,* **157** (1): 112-115.

Zentrum fur Molekulare Biologie der Universität Heidelberg, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany.

14489. **Hashimi, H., Zikova, A., Panigrahi, A. K., Stuart, K. D. & Lukes, J., 2008**. *Tb*RGG1, an essential protein involved in kinetoplastid RNA metabolism that is associated with a novel multiprotein complex. *Rna,* **14** (5): 970-980.

Biology Centre, Institute of Parasitology, Czech Academy of Sciences, eske Budjovice (Budweis), Czech Republic.

14490. **Helm, J. R., Wilson, M. E. & Donelson, J. E., 2008**. Different trans RNA splicing events in bloodstream and procyclic *Trypanosoma brucei*. *Molecular and Biochemical Parasitology,* **159** (2): 134-137.

Department of Biochemistry, University of Iowa, Iowa City, IA 52242, USA.

Most trypanosomatid genes are transcribed into polycistronic precursor RNAs that are processed into monocistronic mRNAs possessing a 39-nucleotide spliced leader (SL) at their 5'-ends and polyadenylation at their 3'-ends. We show here that precursor RNA derived from a luciferase gene integrated in reverse orientation at the rDNA locus of *Trypanosoma brucei* is processed into three major SL-containing RNAs in bloodstream cells and a single SL-containing RNA in procyclic RNAs. This difference in trans RNA splicing between bloodstream and procyclic cells is independent of the 5'- and 3'-UTRs flanking the luciferase coding region. Thus, bloodstream cells can recognize some sequences in precursor RNA as a SL addition site that procyclic cells do not. These alternative SL addition sites may be aberrant or they might be utilized to expand the number of gene products from individual genes. Future experiments on endogenous genes will be necessary to examine the latter possibility.

14491. **Herman, M., Perez-Morga, D., Schtickzelle, N. & Michels, P. A., 2008**. Turnover of glycosomes during life-cycle differentiation of *Trypanosoma brucei*. *Autophagy,* **4** (3): 294-308.

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

Protozoan Kinetoplastida, a group that comprises the pathogenic *Trypanosoma brucei*, compartmentalize several metabolic systems such as the major part of the glycolytic pathway, in multiple peroxisome-like organelles, designated glycosomes. Trypanosomes have a complicated life cycle, involving two major, distinct stages living in the mammalian bloodstream and several stages inhabiting different body parts of the tsetse fly. Previous studies on non-differentiating trypanosomes have shown that the metabolism and enzymatic contents of glycosomes in bloodstream-form and cultured procyclic cells, representative of the stage living in the insect's midgut, differ considerably. In this study, the morphology of glycosomes and their position relative to the lysosome were followed, as were the levels of some glycosomal enzymes and markers for other subcellular compartments, during the differentiation from bloodstream-form to procyclic trypanosomes. Our studies revealed a small tendency of glycosomes to associate with the lysosome when a population of long-slender bloodstream forms differentiated into short-stumpy forms which are pre-adapted to live in the fly. The same phenomenon was observed during the short-stumpy to procyclic transformation, but then the process was fast and many more glycosomes were associated with the dramatically enlarged degradation organelle. The observations suggested an efficient glycosome turnover involving autophagy. Changes observed in the levels of marker enzymes are consistent with the notion that, during differentiation, glycosomes with enzymatic contents specific for the old life-cycle stage are degraded and new glycosomes with different contents are synthesized, causing that the metabolic repertoire of trypanosomes is, at each stage, optimally adapted to the environmental conditions encountered.

14492. **Horn, D., 2008**. Codon usage suggests that translational selection has a major impact on protein expression in trypanosomatids. *BMC Genomics,* **9**: 2.

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Different proteins are required in widely different quantities to build a living cell. In most organisms, transcription control makes a major contribution to differential expression. This is not the case in trypanosomatids where most genes are transcribed at an equivalent rate within large polycistronic clusters. Thus, trypanosomatids must use post-transcriptional control mechanisms to balance gene expression requirements. Here, the evidence for translational selection, the enrichment of "favoured" codons in more highly expressed genes, is explored. A set of highly expressed, tandem-repeated genes display codon bias in *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major*. The tRNA complement reveals forty-five of the sixty-one possible anticodons indicating widespread use of "wobble" tRNAs. Consistent with translational selection, cognate tRNA genes for favoured codons are over-represented. Importantly, codon usage (Codon Adaptation Index) correlates with predicted and observed expression level. In addition, relative codon bias is broadly conserved among syntenic genes from different trypanosomatids. Synonymous codon bias is correlated with tRNA gene copy number and with protein expression level in trypanosomatids. Taken together, the results suggest that translational selection is the dominant mechanism underlying the control of differential protein expression in these organisms. The findings reveal how trypanosomatids may compensate for a paucity of canonical Pol II promoters and subsequent widespread constitutive RNA polymerase II transcription.

14493. **Jackson, A. P., 2007**. Evolutionary consequences of a large duplication event in *Trypanosoma brucei*: chromosomes 4 and 8 are partial duplicons. *BMC Genomics,* **8**: 432.

Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire CB10 1SA, UK. [aj4@sanger.ac.uk].

Gene order along the genome sequence of the human parasite *Trypanosoma brucei* provides evidence for a 0.5 Mb duplication, comprising the 3' regions of chromosomes 4 and 8. Here, the principal aim was to examine the contribution made by this duplication event to the *T. brucei* genome sequence, emphasising the consequences for gene content and the evolutionary change subsequently experienced by paralogous gene copies. The duplicated region may be browsed online at http://www.genedb.org/genedb/tryp/48dup_image.jsp. Comparisons of trypanosomatid genomes demonstrated widespread gene loss from each duplicon, but also showed that 47 percent of duplicated genes were retained on both chromosomes as paralogous loci. Secreted and surface-expressed genes were over-represented among retained paralogs, reflecting a bias towards important factors at the host-parasite interface, and consistent with a dosage-balance hypothesis. Genetic divergence in both coding and regulatory regions of retained paralogs was bimodal, with a deficit in moderately divergent paralogs; in particular, non-coding sequences were either conserved or entirely remodelled. The conserved paralogs included examples of remarkable sequence

conservation, but also considerable divergence of both coding and regulatory regions. Sequence divergence typically displayed strong negative selection; but several features, such as asymmetric evolutionary rates, positively-selected codons and other non-neutral substitutions, suggested that divergence of some paralogs was driven by functional change. The absence of orthologues to retained paralogues in *T. congolense* indicated that the duplication event was specific to *T. brucei*. The duplication of this chromosomal region doubled the dosage of many genes. Rather than creating "more of the same", these results show that paralogs were structurally modified according to various evolutionary trajectories. The retention of paralogs, and subsequent elaboration of both their primary structures and regulatory regions, strongly suggests that this duplication was a seminal development, stimulating functional innovation and fundamentally altering the genetic repertoire of *T. brucei* relative to other trypanosomatids.

14494. **Jaquenoud, M., Pagac, M., Signorell, A., Benghezal, M., Jelk, J., Butikofer, P. & Conzelmann, A., 2008**. The Gup1 homologue of *Trypanosoma brucei* is a GPI glycosylphosphatidylinositol remodelase. *Molecular Microbiology,* **67** (1): 202-212.

Department of Medicine/Biochemistry, University of Fribourg, Chemin du Musee 5, CH-1700, Switzerland.

14495. **Jones, C., Anderson, S., Singha, U. K. & Chaudhuri, M., 2008**. Protein phosphatase 5 is required for Hsp90 function during proteotoxic stresses in *Trypanosoma brucei*. *Parasitology Research,* **102** (5): 835-844.

Department of Biochemistry, Emory University School of Medicine, Atlanta, GA, 30322, USA.

Trypanosoma brucei, a parasitic protozoan that causes African trypanosomiasis in human and domestic animals, adapt in various environments during their digenetic life cycle. In this study, we found that Hsp90 is crucial for the survival of this parasite. Inhibition of Hsp90 activity by geldanamycin (GA) reduced cell growth and increased the level of Hsp90. Both the bloodstream and procyclic forms of *T. brucei* showed a several-fold greater sensitivity than the mammalian cells to GA and also to 17-AAG, a less toxic derivative of GA, suggesting that Hsp90 could be a potential chemotherapeutic target for African trypanosomiasis. *T. brucei* Hsp90 interacts with the protein phosphatase 5 (PP5) *in vivo*. Under normal growth conditions, *T. brucei* PP5 (TbPP5) and Hsp90 are primarily localized in the cytosol. However, with increase in growth temperature and GA treatment, these proteins translocate to the nucleus. Overproduction of *Tb*PP5 by genetic manipulation reduced the growth inhibitory effect of GA, while knockdown of *Tb*PP5 reduced cell growth more in the presence of GA, as compared to parental control. Depletion of TbPP5, however, did not prevent the induction of Hsp90 protein level during GA treatment. Together, these results suggest that *Tb*PP5 positively regulates the function of Hsp90 to maintain cellular homeostasis during proteotoxic stresses in *T. brucei*.

14496. **Jones, N. G., Nietlispach, D., Sharma, R., Burke, D. F., Eyres, I., Mues, M., Mott, H. R. & Carrington, M., 2008.** Structure of a glycosylphosphatidylinositol-anchored

domain from a trypanosome variant surface glycoprotein. *Journal of Biological Chemistry*, **283** (6): 3584-3593.

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The cell surface of African trypanosomes is covered by a densely packed monolayer of a single protein, the variant surface glycoprotein (VSG). The VSG protects the trypanosome cell surface from effector molecules of the host immune system and is the mediator of antigenic variation. The sequence divergence between VSGs that is necessary for antigenic variation can only occur within the constraints imposed by the structural features necessary to form the monolayer barrier. Here, the structures of the two domains that together comprise the C-terminal di-domain of VSG ILTat1.24 have been determined. The first domain has a structure similar to the single C-terminal domain of VSG MITat1.2 and provides proof of structural conservation in VSG C-terminal domains complementing the conservation of structure present in the N-terminal domain. The second domain, although based on the same fold, is a minimized version missing several structural features. The structure of the second domain contains the C-terminal residue that in the native VSG is attached to a glycosylphosphatidylinositol (GPI) anchor that retains the VSG on the external face of the plasma membrane. The solution structures of this domain and a VSG GPI glycan have been combined to produce the first structure-based model of a GPI-anchored protein. The model suggests that the core glycan of the GPI anchor lies in a groove on the surface of the domain and that there is a close association between the GPI glycan and protein. More widely, the GPI glycan may be an integral part of the structure of other GPI-anchored proteins.

14497. **Kowieski, T. M., Lee, S. & Denu, J. M., 2008**. Acetylation-dependent ADP-ribosylation by *Trypanosoma brucei* Sir2. *Journal of Biological Chemistry*, **283** (9): 5317-5326.

Department of Biomolecular Chemistry, University of Wisconsin, School of Medicine and Public Health, Madison, Wisconsin 53706, USA.

14498. **Landfear, S. M., 2008**. Drugs and transporters in kinetoplastid protozoa. *Advances in Experimental Medicine and Biology,* **625**: 22-32.

Department of Molecular Microbiology and Immunology, Oregon Health & Science University, Portland, Oregon 97239, USA. [landfear@ohsu.edu].

Kinetoplastid protozoa express hundreds of membrane transport proteins that allow them to take up nutrients, establish ion gradients, efflux metabolites, translocate compounds from one intracellular compartment to another, and take up or export drugs. The combination of molecular cloning, genetic approaches, and the completed genome projects for *Trypanosoma brucei*, *Leishmania major*, and *Trypanosoma cruzi* have allowed detailed functional analysis of various transporters and predictions about the likely functions of others. Thus many opportunities exist to define the biological and pharmacological properties of parasite transporters whose genes were often difficult to identify in the pregenomic era. A subset of these transporters that are essential for parasite viability could serve as targets for

novel drug therapies by identifying compounds that interfere with their uptake functions. Other permeases provide routes for uptake of selectively cytotoxic compounds and can thus be useful for delivery of drugs. Drug resistance may develop in strains where such drug uptake transporters are nonfunctional or in parasites that over-express other permeases that export a drug. A summary of recent work on *Leishmania* transporters for glucose and for purines is provided as an example of permeases that are being studied in molecular detail.

14499. **Lanteri, C. A., Tidwell, R. R. & Meshnick, S. R., 2008**. The mitochondrion is a site of trypanocidal action of the aromatic diamidine DB75 in bloodstream forms of *Trypanosoma brucei*. *Antimicrobial Agents and Chemotherapy,* **52** (3): 875-882.

Department of Pathology and Laboratory Medicine, University of North Carolina, 2102C McGavran/Greenberg Hall, Chapel Hill, NC 27599, USA.

Human African trypanosomiasis (HAT) is a fatal tropical disease caused by infection with protozoans of the species *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. An oral prodrug, DB289, is a promising new therapy undergoing phase III clinical trials for early-stage HAT. DB289 is metabolically converted to the active trypanocidal diamidine DB75 [2,5-bis(4-amidinophenyl)furan]. We previously determined that DB75 inhibits yeast mitochondrial function. The purpose of this study was to investigate if DB75 targets the mitochondrion of *T. b. brucei* bloodstream forms. DB75 rapidly accumulates within the mitochondria of living trypanosomes, as indicated by the fluorescent colocalization of DB75 with a mitochondrion-specific dye. Fluorescence-activated cell sorting analysis of rhodamine 123-stained living trypanosomes shows that DB75 and other trypanocidal diamidines (pentamidine and diminazene) collapse the mitochondrial membrane potential. DB75 inhibits ATP hydrolysis within *T. brucei* mitochondria and appears to inhibit the oligomycin-sensitive F 1 F 0-ATPase and perhaps other ATPases. DB75 is most likely not an inhibitor of electron transport within trypanosome mitochondria, since DB75 fails to inhibit mitochondrial respiration when glycerol-3-phosphate is used as the respiratory substrate. However, DB75 inhibits whole-cell respiration (50 percent inhibitory concentration, 20 microM) at drug concentrations and incubation durations that also result in the dissipation of the mitochondrial membrane potential. Taken together, these findings suggest that the mitochondrion is a target of the trypanocidal action of DB75.

14500. **Law, J. A., O'Hearn, S. F. & Sollner-Webb, B., 2008.** *Trypanosoma brucei* RNA editing protein *Tb*MP42 (band VI) is crucial for the endonucleolytic cleavages but not the subsequent steps of U-deletion and U-insertion. *Rn*a. **Published online 25 April 2008.**

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Trypanosome mitochondrial mRNAs achieve their coding sequences through RNA editing. This process, catalyzed by approximately 20S protein complexes, involves large numbers of uridylate (U) insertions and deletions within mRNA precursors. Here we analyze the role of the essential *Tb*MP42 protein (band VI/KREPA2) by individually examining each step of the U-deletional and U-insertional editing cycles, using reactions in the approximately

linear range. We examined control extracts and RNA interference (RNAi) extracts prepared soon after *Tb*MP42 was depleted (when primary effects should be most evident) and three days later (when precedent shows secondary effects can become prominent). This analysis shows TbMP42 is critical for cleavage of editing substrates by both the U-deletional and U-insertional endonucleases. However, on simple substrates that assess cleavage independent of editing features, *Tb*MP42 is similarly required only for the U-deletional endonuclease, indicating *Tb*MP42 affects the two editing endonucleases differently. Supplementing RNAi extract with recombinant *Tb*MP42 partly restores these cleavage activities. Notably, we find that all the other editing steps (the 3'-U-exonuclease [3'-U-exo] and ligation steps of U-deletion and the terminal-U-transferase [TUTase] and ligation steps of U-insertion) remain at control levels upon RNAi induction, and hence are not dependent on *Tb*MP42. This contrasts with an earlier report that *Tb*MP42 is a 3'-U-exo that may act in U-deletion and additionally is critical for the TUTase and/or ligation steps of U-insertion, observations our data suggest reflect indirect effects of *Tb*MP42 depletion. Thus, trypanosomes require *Tb*MP42 for both endonucleolytic cleavage steps of RNA editing, but not for any of the subsequent steps of the editing cycles.

14501. **Li, Z., Lindsay, M. E., Motyka, S. A., Englund, P. T. & Wang, C. C., 2008**. Identification of a bacterial-like HslVU protease in the mitochondria of *Trypanosoma brucei* and its role in mitochondrial DNA replication. *PLoS Pathogens,* **4** (4): e1000048.

Department of Pharmaceutical Chemistry, University of California, San Francisco, California, USA.

ATP-dependent protease complexes are present in all living organisms, including the 26S proteasome in eukaryotes, *Archaea,* and *Actinomycetales*, and the HslVU protease in eubacteria. The structure of HslVU protease resembles that of the 26S proteasome, and the simultaneous presence of both proteases in one organism was deemed unlikely. However, HslVU homologues have been identified recently in some primordial eukaryotes, though their potential function remains elusive. We characterized the HslVU homologue from *Trypanosoma brucei*, a eukaryotic protozoan parasite and the causative agent of human sleeping sickness. *Tb*HslVU has ATP-dependent peptidase activity and, like its bacterial counterpart, has essential lysine and N-terminal threonines in the catalytic subunit. By epitope tagging, *Tb*HslVU localizes to mitochondria and is associated with the mitochondrial genome, kinetoplast DNA (kDNA). RNAi of *Tb*HslVU dramatically affects the kDNA by causing over-replication of the minicircle DNA. This leads to defects in kDNA segregation and, subsequently, to continuous network growth to an enormous size. Multiple discrete foci of nicked/gapped minicircles are formed on the periphery of kDNA disc, suggesting a failure in repairing the gaps in the minicircles for kDNA segregation. *Tb*HslVU is a eubacterial protease identified in the mitochondria of a eukaryote. It has a novel function in regulating mitochondrial DNA replication that has never been observed in other organisms.

14502. **Long, S., Jirku, M., Mach, J., Ginger, M. L., Sutak, R., Richardson, D., Tachezy, J. & Lukes, J., 2008**. Ancestral roles of eukaryotic frataxin: mitochondrial frataxin function and heterologous expression of hydrogenosomal *Trichomonas* homologs in trypanosomes. *Molecular Microbiology*. **Available online 7 May 2008.**

Biology Centre, Institute of Parasitology, Czech Academy of Sciences, and Faculty of Natural Sciences, University of South Bohemia, ceske Budejovice (Budweis), Czech Republic.

14503. **Madej, M. J., Niemann, M., Huttenhofer, A. & Goringer, H. U., 2008**. Identification of novel guide RNAs from the mitochondria of *Trypanosoma brucei*. *RNA Biology,* **5:** Issue 2.

Innsbruck Biocenter, Division of Genomics and RNomics, Innsbruck Medical University, Fritz-Pregl-Str. 3, 6020, Innsbruck, Austria.

14504. **Mandava, V., Janzen, C. J. & Cross, G. A., 2008**. Trypanosome H2Bv replaces H2B in nucleosomes enriched for H3 K4 and K76 trimethylation. *Biochemical and Biophysical Research Communications,* **368** (4): 846-851.

Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.

Some inroads have been made into characterizing histone variants and post translational modifications of histones in *Trypanosoma brucei*. Histone variant H2BV lysine 129 is homologous to *Saccharomyces cerevisiae* H2B lysine 123, whose ubiquitination is required for methylation of H3 lysines 4 and 79. We show that *T. brucei* H2BV K129 is not ubiquitinated, but trimethylation of H3 K4 and K76, homologs of H3 K4 and K79 in yeast, was enriched in nucleosomes containing H2BV. Mutation of H2BV K129 to alanine or arginine did not disrupt H3 K4 or K76 methylation. These data suggest that H3 K4 and K76 methylation in trypanosomes is regulated by a novel mechanism, possibly involving the replacement of H2B with H2BV in the nucleosome.

14505. **Manthri, S., Guther, M. L., Izquierdo, L., Acosta-Serrano, A. & Ferguson, M. A., 2008**. Deletion of the *Tb*ALG3 gene demonstrates site-specific N-glycosylation and N-glycan processing in *Trypanosoma brucei*. *Glycobiology,* **18** (5): 367-383.

The Division of Biological Chemistry and Drug Discovery, The Wellcome Trust Biocentre, College of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK.

14506. **Mattiacio, J. L. & Read, L. K., 2008**. Roles for *Tb*DSS-1 in RNA surveillance and decay of maturation by-products from the 12S rRNA locus. *Nucleic Acids Research,* **36** (1): 319-329.

Department of Microbiology and Immunology, Witebsky Center for Microbial Pathogenesis and Immunology, SUNY Buffalo School of Medicine, Buffalo, NY 14214, USA.

14507. **McCann, A. K., Schwartz, K. J. & Bangs, J. D., 2008**. A determination of the steady state lysosomal pH of bloodstream stage African trypanosomes. *Molecular and Biochemical Parasitology,* **159** (2): 146-149.

Department of Medical Microbiology & Immunology, University of Wisconsin School of Medicine & Public Health, Microbial Sciences Building, 1550 Linden Drive, Madison, WI 53706, USA.

The lysosomal/endosomal system of African trypanosomes is developmentally regulated and is important in the pathogenesis associated with infection of the mammalian bloodstream. Long considered to be a target for drug development, the internal pH of the lysosome has been variously reported to range from <5.0 to >6.0. We have refined a flow cytometric technique using a pH-sensitive probe that specifically targets the lysosome, tomato lectin:Oregon Green 488 conjugate. The probe is delivered to the lysosome with fidelity, where it is shielded against external pH. Measurement of fluorescent output in the presence and absence of lysomotropic agent (NH(4)Cl) then allows precise titration of steady state lysosomal pH (4.84+/-0.23). Using bafilomycin A1 to inhibit acidification we demonstrate that this method is responsive to pharmacological perturbation of lysosomal physiology. This work should facilitate future studies of the lysosomal function in African trypanosomes, as well as other parasitic protozoa.

14508. **Mendoza-Palomares, C., Biteau, N., Giroud, C., Coustou, V., Coetzer, T., Authie, E., Boulange, A. & Baltz, T., 2008**. Molecular and biochemical characterization of a cathepsin B-like protease family unique to *Trypanosoma congolense*. *Eukaryotic Cell,* **7** (4): 684-697.

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Cysteine proteases have been shown to be essential virulence factors and drug targets in trypanosomatids and an attractive antidisease vaccine candidate for *Trypanosoma congolense*. Here, we describe an important amplification of genes encoding cathepsin B-like proteases unique to *T. congolense*. More than 13 different genes were identified, whereas only one or two highly homologous genes have been identified in other trypanosomatids. These proteases grouped into three evolutionary clusters: TcoCBc1 to TcoCBc5 and TcoCBc6, which possess the classical catalytic triad (Cys, His, and Asn), and TcoCBs7 to TcoCBs13, which contains an unusual catalytic site (Ser, Xaa, and Asn). Expression profiles showed that members of the TcoCBc1 to TcoCBc5 and the TcoCBs7 to TcoCBs13 groups are expressed mainly in bloodstream forms and localize in the lysosomal compartment. The expression of recombinant representatives of each group (TcoCB1, TcoCB6, and TcoCB12) as proenzymes showed that TcoCBc1 and TcoCBc6 are able to autocatalyze their maturation 21 and 31 residues, respectively, upstream of the predicted start of the catalytic domain. Both displayed a carboxydipeptidase function, while only TcoCBc1 behaved as an endopeptidase. TcoCBc1 exhibited biochemical differences regarding inhibitor sensitivity compared to that of other cathepsin B-like proteases. Recombinant pro-TcoCBs12 did not automature *in vitro*, and the pepsin-matured enzyme was inactive in tests with cathepsin B fluorogenic substrates. *In vivo* inhibition studies using CA074Me (a cell-permeable cathepsin B-specific inhibitor)

demonstrated that TcoCB are involved in lysosomal protein degradation essential for survival in bloodstream form. Furthermore, TcoCBc1 elicited an important immune response in experimentally infected cattle. We propose this family of proteins as a potential therapeutic target and as a plausible antigen for *T. congolense* diagnosis.

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Department of Parasitology, The Kuvin Center for the Study of Infectious and Tropical Diseases, Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel.

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Department of Microbiology, Immunology & Molecular Genetics, David Geffen School of Medicine, University of California, Los Angeles, California 90095, USA.

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6-Phosphogluconate dehydrogenase is a potential target for new drugs against African trypanosomiasis. Phosphorylated aldonic acids are strong inhibitors of 6-phosphogluconate dehydrogenase, and 4-phospho-d-erythronate (4PE) and 4-phospho-d-erythronohydroxamate are two of the strongest inhibitors of the *Trypanosoma brucei* enzyme. Binding of the substrate 6-phospho-d-gluconate (6PG), the inhibitors 5-phospho-d-ribonate (5PR) and 4PE, and the coenzymes NADP, NADPH and NADP analogue 3-amino-pyridine adenine dinucleotide phosphate to 6-phospho-d-gluconate dehydrogenase from *T. brucei* was studied using isothermal titration calorimetry. Binding of the substrate (K(d) = 5 microm) and its analogues (K(d) =1.3 microm and K(d) = 2.8 microm for 5PR and 4PE, respectively) is entropy driven, whereas binding of the coenzymes is enthalpy driven. Oxidized coenzyme and its analogue, but not reduced coenzyme, display a half-site reactivity in the ternary complex with the substrate or inhibitors. Binding of 6PG and 5PR poorly affects the dissociation constant of the coenzymes, whereas binding of 4PE decreases the dissociation constant of the coenzymes by two orders of magnitude. In a similar manner, the K(d) value of 4PE decreases by two orders of magnitude in the presence of the coenzymes. The results suggest that 5PR acts as a substrate analogue, whereas 4PE mimics the transition state of dehydrogenation. The stronger affinity of 4PE is interpreted on the basis of the mechanism of

the enzyme, suggesting that the inhibitor forces the catalytic lysine 185 into the protonated state.

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Wellcome Centre for Molecular Parasitology and Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK.

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Vesicle traffic to and from the surface is highly polarized in African trypanosomes. Actin is required for polarized endocytic traffic in bloodstream forms of African trypanosomes but its role in other pathways has remained equivocal. A combination of metabolic pulse chase labelling and surface biotinylation during the chase period along with the use of conditional RNA interference was employed to demonstrate that substantial loss of actin had no effect on the export of newly synthesized proteins to the surface of bloodstream and procyclic forms of *Trypanosoma brucei*. These results indicated that this trafficking pathway to the surface operates as normal even when actin levels are significantly lower than normal and endocytic activity is abolished. Taken together the data support the view that the secretory and endocytic pathways are not obligatorily coupled.

14514. **Ochsenreiter, T., Cipriano, M. & Hajduk, S. L., 2008**. Alternative mRNA editing in trypanosomes is extensive and may contribute to mitochondrial protein diversity. *PLoS ONE,* **3** (2): e1566.

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

The editing of trypanosome mitochondrial mRNAs produces transcripts necessary for mitochondrial functions including electron transport and oxidative phosphorylation. Precursor-mRNAs are often extensively edited by specific uridine insertion or deletion that is directed by small guide RNAs (gRNAs). Recently, it has been shown that cytochrome c oxidase subunit III (COXIII) mRNAs can be alternatively edited to encode a novel mitochondrial membrane protein composed of a unique hydrophilic N-terminal sequence of unknown function and the C-terminal hydrophobic segment of COXIII. To extend the analysis of alternative editing in *Trypanosoma brucei* we have constructed libraries with over 1100 full-length mitochondrial cDNAs and the sequences of over 1200 gRNA genes. Using this data, we show that alternative editing of COXIII, ATPase subunit 6 (A6), and NADH dehydrogenase subunits 7, 8 and 9 (ND7, 8, 9) mRNAs can produce novel open reading

frames (ORFs). Several gRNAs potentially responsible for the alternative editing of these mRNAs were also identified. These findings show that alternative editing of mitochondrial mRNAs is common in *T. brucei* and expands the diversity of mitochondrial proteins in these organisms.

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Seattle Biomedical Research Institute, Seattle, Washington 98109, USA.

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Faculty of Biology, Department of Botany, University of Athens, Panepistimioupolis, Athens 15781, Greece; Department of Biochemistry, Hellenic Pasteur Institute, 127 Vassilissis Sophias, 115 21 Athens, Greece.

14517. **Patrick, K. L., Luz, P. M., Ruan, J. P., Shi, H., Ullu, E. & Tschudi, C., 2008**. Genomic rearrangements and transcriptional analysis of the spliced leader-associated retrotransposon in RNA interference-deficient *Trypanosoma brucei*. *Molecular Microbiology,* **67** (2): 435-447.

Department of Epidemiology and Public Health, Yale University Medical School, 295 Congress Avenue, New Haven, CT 06536-0812, USA.

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Department of Microbiology, Immunology, and Molecular Genetics, University of California, 609 Charles E. Young Drive, Los Angeles, CA 90095, USA, Molecular Biology Institute, University of California, Los Angeles, CA 90095, USA. [kenthill@mednet.ucla.edu].

African trypanosomes, i.e. *Trypanosoma brucei* and related sub-species, are devastating human and animal pathogens that cause significant human mortality and limit sustained economic development in sub-Saharan Africa. *Trypanosoma brucei* is a highly motile protozoan parasite and coordinated motility is central to both disease pathogenesis in the mammalian host and parasite development in the tsetse fly vector. Since motility is critical for parasite development and pathogenesis, understanding unique aspects of the *T. brucei* flagellum may uncover novel targets for therapeutic intervention in African sleeping sickness. Moreover, studies of conserved features of the *T. brucei* flagellum are directly relevant to understanding fundamental aspects of flagellum and cilium function in other eukaryotes,

making *T. brucei* an important model system. The *T. brucei* flagellum contains a canonical 9 + 2 axoneme, together with additional features that are unique to kinetoplastids and a few closely-related organisms. Until recently, much of our knowledge of the structure and function of the trypanosome flagellum was based on analogy and inference from other organisms. There has been an explosion in functional studies in *T. brucei* in recent years, revealing conserved as well as novel and unexpected structural and functional features of the flagellum. Most notably, the flagellum has been found to be an essential organelle, with critical roles in parasite motility, morphogenesis, cell division and immune evasion. This review highlights recent discoveries on the *T. brucei* flagellum.

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Faculty of Life Sciences, University of Manchester, The Michael Smith Building, Oxford Road, Manchester, UK.

14520. **Robles, A. & Clayton, C., 2008**. Regulation of an amino acid transporter mRNA in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology,* **157** (1): 102-106.

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

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Department of Epidemiology & Public Health, Yale University Medical School, 295 Congress Avenue, New Haven, CT 06536-0812, USA.

14522. **Scahill, M. D., Pastar, I. & Cross, G. A., 2008**. CRE recombinase-based positive-negative selection systems for genetic manipulation in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology,* **157** (1): 73-82.

Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.

The limited repertoire of drug-resistance markers imposes a serious obstacle to genetic manipulation of *Trypanosoma brucei*. Here we describe experiments with a fusion protein that allows positive selection for genome integration followed by CRE recombinase-mediated excision of the marker cassette that can be selected by ganciclovir, although the excision event is so efficient that selection is not strictly necessary. We describe two variants of the tetracycline-inducible pLEW100-based CRE-expression vector that reduced its toxicity when stably integrated into the genome, and we demonstrate that transient transfection of circular pLEW100-CRE is highly efficient at catalyzing marker excision. We used this approach to delete the last two enzymes of the pyrimidine synthesis pathway, creating a cell line that is

resistant to fluoroorotic acid, which would allow the same enzymes (PYR6-5) to be used as an alternative negative selectable marker.

14523. **Schneider, A., Bouzaidi-Tiali, N., Chanez, A. L. & Bulliard, L., 2007**. ATP production in isolated mitochondria of procyclic *Trypanosoma brucei*. *Methods in Molecular Biology,* **372**: 379-387.

Department of Biology, University of Fribourg, Switzerland.

This paper describes a luciferase-based protocol to measure adenosine triphosphate (ATP) production in isolated mitochondria of *Trypanosoma brucei*. The assay represents an excellent method to characterize the functionality of isolated mitochondria. Comparing the ATP production induced by substrates for oxidative phosphorylation to the one induced by substrates for substrate-level phosphorylation allows conclusions regarding the integrity of the outer and inner mitochondrial membranes. Furthermore, the assay is a valuable tool for characterization of RNA interference cell lines suspected to affect mitochondrial functions.

14524. **Schneider, A., Bursac, D. & Lithgow, T., 2008**. The direct route: a simplified pathway for protein import into the mitochondrion of trypanosomes. *Trends in Cell Biology,* **18** (1): 12-18.

Department of Biology, Cell and Developmental Biology, University of Fribourg, Chemin du Musee 10, CH-1700 Fribourg, Switzerland. [andre.schneider@unifr.ch].

Trypanosoma brucei is a unicellular eukaryote that causes the deadly human African trypanosomiasis (“sleeping sickness”) in humans. The parasite has a complicated lifestyle, it developmentally changes aspects of its mitochondrial function as it alternates from forms in the tsetse fly to forms adapted for life in the human bloodstream. The single mitochondrion found in each trypanosome has to be duplicated precisely in each round of the cell cycle in order for parasites to replicate, and this depends on the import of proteins from the cytosol. Here we review what is known about the mitochondrial protein import pathway in *T. brucei*, how it compares with the process in humans, and how the distinguishing features seen in *T. brucei* and humans promise new understanding of the mitochondrial protein import process in all eukaryotes.

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Department of Biology, University of Fribourg, Switzerland.

The mitochondrion of the parasitic protozoon *Trypanosoma brucei* shows a number of unique features, many of which represent highly interesting research topics. Studies of these subjects require the purification of mitochondrial fractions. Here, we describe and discuss the two most commonly used methods to isolate mitochondria from insect stage *T. brucei*. In the first protocol, the cells are lysed under hypotonic conditions, and mitoplast vesicles are

isolated on Percoll gradients; in the second method, lysis occurs isotonically by N2 cavitation, and the mitochondrial vesicles are isolated by Nycodenz gradient centrifugation.

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Division of Clinical Pharmacology, Department of Medicine and of Pharmacology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA. [jrscocca@jhmi.edu].

Trypanosoma and *Leishmania*, protozoans that cause major human diseases, have a topologically intricate mitochondrial DNA (kinetoplast or kDNA) in the form of a network of thousands of interlocked circles. This unusual system provides a useful reporter for studying topoisomerase functions *in vivo*. We now find that these organisms have three type IA topoisomerases, one of which is phylogenetically distinctive and which we designate topoisomerase IA(mt). In *Trypanosoma brucei* topoisomerase IA(mt) immunolocalizes within the mitochondrion close to the kDNA disk in patterns that vary with the cell cycle. When expression of TOPIA(mt) is silenced by RNAi there is a striking accumulation of kDNA late theta structure replication intermediates, with subsequent loss of kDNA networks and halt in cell growth. This essential enzyme provides clear molecular evidence for the obligatory role of a type IA enzyme in the resolution of late theta structures *in vivo*. With no close orthologue in humans it also offers a target for the rational development of selectively toxic new antiprotozoal therapies.

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Laboratory of Molecular Parasitology, The Rockefeller University, New York, USA.

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Department of Microbial Pathogenesis and Immune Response, 1005 D.B. Todd Jr. Boulevard, School of Medicine, Meharry Medical College, Nashville, TN 37208, USA.

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College of Life Sciences, University of Dundee, Dundee, Angus DD1 5EH., UK. [m.a.j.ferguson@dundee.ac.uk].

14530. **Tarun, S. Z., Jr., Schnaufer, A., Ernst, N. L., Proff, R., Deng, J., Hol, W. & Stuart, K., 2008**. KREPA6 is an RNA-binding protein essential for editosome integrity and survival of *Trypanosoma brucei*. *Rna,* **14** (2): 347-358.

Seattle Biomedical Research Institute, Seattle, Washington 98109, USA

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The Mina & Everard Goodman, Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel.

14532. **Vertommen, D., Van Roy, J., Szikora, J. P., Rider, M. H., Michels, P. A. & Opperdoes, F. R., 2008**. Differential expression of glycosomal and mitochondrial proteins in the two major life-cycle stages of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology,* **158** (2): 189-201.

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Label-free semi-quantitative differential three-dimensional liquid chromatography coupled to mass spectrometry (3D-LC-MS/MS) was used to compare the glycosomal and mitochondrial proteomes of the bloodstream- and insect-form of *Trypanosoma brucei.* The abundance of glycosomal marker proteins identified in the two life-cycle stages corresponded well with the relative importance of biochemical pathways present in the glycosomes of the two stages and the peptide spectral count ratios of selected enzymes were in good agreement with published data about their enzymatic specific activities. This approach proved extremely useful for the generation of large scale proteomics data for the comparison of different life-cycle stages. Several proteins involved in oxidative stress protection, sugar-nucleotide synthesis, purine salvage, nucleotide-monophosphate formation and purine-nucleotide cycle were identified as glycosomal proteins.

14533. **Vodnala, M., Fijolek, A., Rofougaran, R., Mosimann, M., Maser, P. & Hofer, A., 2008**. Adenosine kinase mediates high affinity adenosine salvage in *Trypanosoma brucei*. *Journal of Biological Chemistry,* **283** (9): 5380-5388.

Department of Medical Biochemistry and Biophysics, Umea University, SE-901 87 Umea, Sweden.

African sleeping sickness is caused by *Trypanosoma brucei*. This extracellular parasite lacks *de novo* purine biosynthesis, and it is therefore dependent on exogenous purines such as adenosine that is taken up from the blood and other body fluids by high affinity transporters.

The general belief is that adenosine needs to be cleaved to adenine inside the parasites in order to be used for purine nucleotide synthesis. We have found that *T. brucei* also can salvage this nucleoside by adenosine kinase (AK), which has a higher affinity to adenosine than the cleavage-dependent pathway. The recombinant *T. brucei* AK (*Tb*AK) preferably used ATP or GTP to phosphorylate both natural and synthetic nucleosides in the following order of catalytic efficiencies: adenosine > cordycepin > deoxyadenosine > adenine arabinoside (Ara-A) > inosine > fludarabine (F-Ara-A). TbAK differed from the AK of the related intracellular parasite *Leishmania donovani* by having a high affinity to adenosine (K m = 0.04-0.08 microm depending on phosphate) and by being negatively regulated by adenosine (K i = 8-14 microm). These properties make the enzyme functionally related to the mammalian AKs, although a phylogenetic analysis grouped it together with the *L. donovani* enzyme. The combination of a high affinity AK and efficient adenosine transporters yields a strong salvage system in *T. brucei*, a potential Achilles' heel making the parasites more sensitive than mammalian cells to adenosine analogues such as Ara-A. Studies of wild-type and AK knockdown trypanosomes showed that Ara-A inhibited parasite proliferation and survival in an AK-dependent manner by affecting nucleotide levels and by inhibiting nucleic acid biosynthesis.

14534. **Wilkinson, S. R., Taylor, M. C., Horn, D., Kelly, J. M. & Cheeseman, I., 2008**. A mechanism for cross-resistance to nifurtimox and benznidazole in trypanosomes. *Proceedings of the National Academy of Sciences USA,* **105** (13): 5022-5027.

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Nifurtimox and benznidazole are the front-line drugs used to treat Chagas' disease, the most important parasitic infection in the Americas. These agents function as prodrugs and must be activated within the parasite to have trypanocidal effects. Despite >40 years of research, the mechanism(s) of action and resistance have remained elusive. Here, we report that in trypanosomes, both drugs are activated by a NADH-dependent, mitochondrially localized, bacterial-like, type I nitroreductase (NTR), and that down-regulation of this explains how resistance may emerge. Loss of a single copy of this gene in *Trypanosoma cruzi*, either through *in vitro* drug selection or by targeted gene deletion, is sufficient to cause significant cross-resistance to a wide range of nitroheterocyclic drugs. In *Trypanosoma brucei*, loss of a single NTR allele confers similar cross-resistance without affecting growth rate or the ability to establish an infection. This potential for drug resistance by a simple mechanism has important implications, because nifurtimox is currently undergoing phase III clinical trials against African trypanosomiasis.

14535. **Williams, S., Saha, L., Singha, U. K. & Chaudhuri, M., 2008**. *Trypanosoma brucei*: differential requirement of membrane potential for import of proteins into mitochondria in two developmental stages. *Experimental Parasitology,* **118** (3): 420-433.

Department of Microbial Pathogenesis and Immune Response, School of Medicine, Meharry Medical College, 1005 D.B. Todd Jr. Boulevard, Nashville, TN 37208, USA.

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Department of Biological Chemistry, Johns Hopkins Medical School, Baltimore, MD 21205, USA.

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Biology Centre, Institute of Parasitology, Czech Academy of Sciences, Ceske Budejovice (Budweis), Czech Republic; Faculty of Science, University of South Bohemia, Ceske Budejovice (Budweis), Czech Republic; Seattle Biomedical Research Institute, Seattle, USA.

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