year **2008**
volume **31**
part **2**

PAAT

Programme Against African Trypanosomiasis

ISSN 1812-2442

TSETSE AND TRYPANOSOMIASIS INFORMATION

INSTITUTE OF TROPICAL MEDICINE ANTWERP
DFID Department for International Development
CIRAD
FAO FIAT PANIS

year **2008**
volume **31**
part **2**

PAAT
Programme
Against
African
Trypanosomiasis

TSETSE AND TRYPANOSOMIASIS INFORMATION

Numbers 14539–14800

Edited by
James Dargie
Bisamberg
Austria

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2009

TSETSE AND TRYPANOSOMIASIS INFORMATION

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

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

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

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

Distribution dates and copy deadlines

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

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

ABBREVIATIONS USED IN *TTI*

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

Organizations

AfDB	African Development Bank
ANDE	Agence Nationale de Développement de l'Elevage
AU	African Union
AU/STRC	African Union/Scientific, Technical and Research Commission
BICOT	Biological Control of Tsetse by the Sterile Insect Technique
BMZ	German Federal Ministry for Economic Cooperation and Development
CEBV	Communauté Economique du Bétail et de la Viande

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

OCEAC	Organisation de Coordination pour la Lutte contre les Endémies en Afrique Centrale
OGAPROV	Office Gabonais pour l'Amélioration de la Production de la Viande
OIE	Office International des Epizooties
OMVG	Organisation pour la Mise en Valeur du Fleuve Gambie
PAAT	Programme against African Trypanosomiasis
PATTEC	Pan-African Tsetse and Trypanosomiasis Eradication Campaign
PRCT	Projet de Recherches Cliniques sur la Trypanosomiase
PROCORDEL	Programme de Recherche et Développement
RDI	Rural Development International
RUCA	Rijksuniversitair Centrum Antwerpen
SADC	Southern African Development Community
SIDA	Swedish International Development Authority
SODEPRA	Société pour le Développement des Productions Animales
TDR	UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases
TDRC	Tropical Diseases Research Centre
TPRI	Tropical Pesticides Research Institute
TTRI	Tsetse and Trypanosomiasis Research Institute
UCLT	Unité Centrale de Lutte contre la Trypanosomiase
UNDP	United Nations Development Programme
UNEP	United Nations Environment Programme
UNIDO	United Nations Industrial Development Organization
UNTFHS	United Nations Trust Fund for Human Security
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
UTCC	Uganda Trypanosomiasis Control Council
UTRO	Uganda Trypanosomiasis Research Organisation
WHO	World Health Organization

CONTENTS

SECTION A – NEWS

PROGRAMME AGAINST AFRICAN TRYPANOSOMIASIS (PAAT): REPORT OF THE 13th MEETING OF THE PAAT ADVISORY GROUP (PAG) COORDINATORS, LUANDA, ANGOLA, 27-28 SEPTEMBER 2007.

The meeting was hosted by the Ministry of Agriculture and Rural Development. Mr A. A. Ilemobade, Chairman of PAAT, chaired the meeting which was attended by 26 participants from international organizations (FAO, IAEA, WHO), African-based (ILRI, ICIPE, CIRDES) and European-based (IRD, ITM) research institutions and representatives of ten African countries (Angola, Benin, Botswana, Burkina Faso, Ethiopia, Ghana, Kenya, Mali, Uganda, Zimbabwe), including National PATTEC Coordinators. Representatives of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) Secretariat and Executive Committee were also in attendance.

The meeting was officially opened by the Vice-Minister of Agriculture and Rural Development, in the presence of the Vice-Minister of the Ministry of Health and the Representatives of FAO and of WHO in Angola.

The importance of Human African Trypanosomiasis (HAT) and of African Animal Trypanosomiasis (AAT) as major hindrances to the development of agriculture in Africa in general, and in Angola, in particular, was highlighted. Angola is one of the three countries in sub-Saharan Africa where still more than 1, 000 cases of HAT are detected per year. The Vice-Minister called for "speaking less and doing more" in the fight against trypanosomiasis and hoped that the PAG meeting would contribute in this regard.

In his address, Mr A. A. Ilemobade emphasised that the focus of PAAT was to reduce rural poverty and hunger, improve livelihoods, to ensure food security and sustainable agriculture and rural development (SARD). The PAG meetings therefore have to be seen as moments of reflection and opportunities for setting priorities and measures to assist Africa Union Member States to overcome the scourge of tsetse and trypanosomiasis (T&T), thus promoting poverty reduction, enhancing food security and ensuring healthy livelihoods for African people. Also, PAAT provides the international catalytic environment to promote beneficial interactions between policy advisors, planners, researchers and field workers for an increased coordinated and harmonized action against African trypanosomiasis.

The Vice-Minister of the Ministry of Agriculture and Rural Development declared the meeting officially opened.

1. Recommendations

1. On guidelines for (i) collection of entomological baseline data, (ii) mass rearing of tsetse fly, (iii) socio-economic and environmental base-line data, PAG recommends:

    - To circulate among PAG members the draft guidelines that have been developed for possible comments and refinement.

Action: FAO/IAEA, ILRI.

2. On the use of the sequential aerosol technique (SAT) for tsetse suppression, PAG recommends that:

   - Those countries that have experience with SAT should provide input on proposed PAAT position paper on this technology. Emphasis should be placed on new technological developments and environmental impact assessment. The document "Tools and strategies for area-wide tsetse suppression", that was prepared by the IAEA, should be used as basis for the position paper.

   Action: FAO/IAEA.

3. On the PAAT position paper for declaring an area free of T&T, the PAG recommends:

   - To include simplified guidelines that will enable decision makers to make informed decisions based upon a logical sequence of steps.

   Action: PAAT and mandated organizations.

4. On Sleeping Sickness (SS) interventions, the PAG recommends:

   - To involve as many health systems as possible (in particular primary health care systems) in the surveillance and control of HAT. National Sleeping sickness programmes or specialised bodies at national level should be maintained to monitor and provide appropriate support to the integration process.

   Action: WHO and Member States.

5. On collection of essential baseline data, the PAG recommends:

   - To take into account historical data sets. For those areas where recent data are not available, detailed work / action plans should be developed in order to enable the implementation of grid-based surveys using the representative sampling approach.

   Action: PATTEC countries.

6. On regional training, the PAG recommends:

   - To develop and organize regional training courses on the use of GIS, Web-GIS, Data Base Management Systems (DBMS), GIS data and metadata sharing tools (e.g. GeoNetwork open source). Countries that have initiated T&T intervention, within the PATTEC initiative, should make efforts to make their GIS data sets available on the web for sharing with other Member States.

Action: PATTEC Coordination Office, PATTEC countries, PAAT.

7. On standardization and harmonization of T&T intervention(s), the PAG recommends:

   - That all steps involved in T&T intervention(s), i.e. entomological, parasitological, socio-economic, environmental baseline data collection and monitoring, land cover mapping should be standardized.

   Action: PAAT and PATTEC countries.

8. On regional approach for intervention, the PAG recommends:

   - Ongoing national T&T interventions in West and East Africa should adopt a regional approach. This should be reflected in common, standardized country reports, coordinated actions, more frequent technical meetings and increased use of available data sharing tools, i.e. PAAT website.

   Action: PATTEC countries involved in ongoing T&T interventions.

9. On modifications/adaptation of ongoing T&T intervention work plans, the PAG recommends:

   - That those countries with ongoing T&T interventions funded by AfDB and within the framework of the PATTEC initiative, and wishing to adapt their working plan to the field situation, should coordinate their requests and approaches to AfDB as a united group.

   Action: PATTEC and PATTEC countries with ongoing AfDB funded T&T interventions.

10. On the effects of climate change on the T&T problem, the PAG recommends:

    - To further pursue studies on climate change effects on T&T.

    Action: PATTEC and PAAT.

2. Review and adoption of the last PAG meeting report – A. A. Ilemobade

The PAAT Chairman reviewed the report of the past PAG meeting, held in Kasane, Botswana, October 2006. The report was adopted unanimously. Consequently, the conclusions and recommendations of the last meeting, held in Addis Ababa, Ethiopia, September 2006, were discussed and endorsed.

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

Participants were informed on the FAO and PAAT activities since the last PAG meeting. Normative, technical and logistic assistance were provided to PAAT partner countries.

FAO has initiated a study concerning mapping the benefits of tsetse and trypanosomiasis intervention in East Africa. This study completes a similar one carried out in West Africa. At the national level, FAO/PAAT contributed to the PPLPI study entitled "Comparable costings of alternatives for dealing with tsetse: estimates for Uganda", and the PPLPI Policy Brief "Choice of techniques for creating tsetse-free zones in Africa: the cost dimension". Additional normative work related to the development of guidelines to assess the feasibility of creating T&T free zones (FAO/IAEA), and the provision of harmonized spatial datasets for the management of the trypanosomiasis problem from an environmental perspective. In an advanced stage is a study which includes and links sustainable human and animal African trypanosomiasis control with rural development strategies. Another normative initiative in progress is preparation of "Guidelines for declaring areas free of tsetse flies and tsetse-transmitted trypanosomiasis". Sections of this paper deal with entomological and parasitological/serological sampling and surveillance criteria. The "FAO/IAEA Standard Operating Procedures for mass rearing of tsetse flies" were finalized as a folder that permits exchanging chapters in case technical developments necessitate updates. The PAAT Technical and Scientific paper No. 8 "Standardizing land cover mapping for T&T decision making" has been finalized and was published recently (see Abstract No. 14544, this Volume). 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). The customized land cover datasets for eight East African countries were made available for downloading through FAO-GeoNetwork, where also an interactive Web Mapping Service (WMS) was created. The work was presented as a poster at the GISVET 2007 Conference, Copenhagen, Denmark, August 2007, where it was awarded the prize for "The most interesting poster".

As part of its contribution to the Millennium Development Goals (MDGs), PAAT finalized a brochure entitled "On target Against Poverty – The Programme Against African Trypanosomiasis 1997 -2007". The brochure highlights the role and contributions of PAAT to supporting the eight MDGs.

FAO organized an interactive Training Workshop on "Harmonization of GIS-based decision support systems and information systems in T&T intervention" and participated in the Regional Meeting of National Coordinators held in July 2007 at the IAEA headquarters. The meeting focused on reviewing progress made by Burkina Faso, Ethiopia, Ghana, Kenya, Mali and Uganda in implementing the AfDB funded projects, strengthening regional coordination and improving the implementation of the above-mentioned projects. FAO, together with IAEA, continued to provide assistance and guidance to the Ethiopian Government and the STEP project for the implementation of the UNTFHS funded project (GCP/ETH/072/UNJ) in the Southern Rift Valley of the country. During technical visits made by FAO to the six AfDB beneficiary countries, it became very clear that an area of

major training is to increase African capacity building and analysis on the use of GIS/RS as tool for T&T decision making and support.

During the discussions it was pointed out that a comprehensive summary document of various baseline data manuals would be useful. In this respect, it was proposed to establish a committee to develop such a document. Another point of discussion was the necessity to develop a simplified version of the position paper "Criteria to declare the status of eradication of T&T". Also discussed was the issue of which authority (the country, international bodies such as OIE) should declare achievement of the status of eradication. Further consultations are needed on this matter.

4. Report from IAEA – M. Vreysen

Mr Vreysen of the Joint FAO/IAEA Programme in Food and Agriculture presented an overview of the activities of the IAEA in support of the PATTEC Plan of Action, and major achievements in the three main areas (normative activities, research and development, and technical cooperation) were highlighted.

During the period 2006-7, three workshops were held on the development of a detailed action plan for the collection of essential entomological baseline data in Uganda, Burkina Faso and Senegal. In August 2007, IAEA participated in a stakeholders meeting in South Africa where a feasibility study document for T&T intervention in the country and the Southern part of Mozambique was endorsed.

Regarding technical cooperation, IAEA continued to provide technical support to the STEP project in Ethiopia, particularly in the field of tsetse mass rearing and the use of the sterile insect technique (SIT). Additional technical cooperation activities, developed under the United Nations Fund for International Partnership and USA, concerned the development of GIS based maps related to T&T, generating standardised entomological baseline data, designing tsetse mass rearing facilities and development of subregional intervention strategies.

Training is a major activity of the Joint FAO/IAEA Programme. The Agency produced a computer-based training package on GIS with a set of "flash" presentations demonstrating the use of various software applications including GIS and database and focusing on pest management projects. IAEA co-sponsored a PATTEC/FAO/IAEA Regional Training Course on "Standardised collection and processing of tsetse flies for population genetics studies and morphometrics", held in Tororo, Uganda, November 2007. Several other regional training events are already planned in the course of 2008.

Regarding publications, in June 2007 IAEA published a new textbook on "Area-wide control of insect pests. From research to field implementation", based mainly on papers presented at the 2nd International Conference on area-wide control of insect pests, held at IAEA headquarters, Vienna. The textbook aims at being a comprehensive compilation of the various components of AW-IPM, i.e. basic research, modelling and methods development, feasibility studies, commercialization and regulatory issues, pilot and operational programmes. The book's final chapter provides an analysis of the lessons learned.

In relation to research activities, the focus has been on improving tsetse holding and feeding units and the tsetse diet, using X-rays as an alternative to gamma rays for sterilising flies, better understanding the Salivary Gland Hypertrophy Virus, assessing the impact of antiviral drugs on the virus and the use of UV light to sterilise the blood diet. In addition, Coordinated Research Projects were initiated or further implemented on (i) the use of GIS and population genetics as a tool for better planning AW-IPM programmes, (ii) tsetse symbionts and pathogens, and (iii) improved harmonized control quality for expanded tsetse production, sterilization and field application.

5. Report from WHO – P. Simarro

Main activities of WHO related to HAT are summarized as follows:

- supporting control activities in active foci;
- gathering historical HAT data and updating historical foci;
- updating distribution of disease data;
- improving reporting; and
- defining populations at risk.

The increased HAT surveillance, from 1.3 million people screened in 1997 to more than 3 million in 2006, resulted in a decrease in the number of positive HAT cases reported, i.e. from 36 000 in 1997 to 11 868 cases in 2006. Out of 36 tsetse affected countries, no cases are reported from 16 countries, 12 countries reported less than 100 cases, five countries between 100 and 1 000 cases and only three countries (Angola, Democratic Republic of Congo and Sudan) reported more than 1, 000 cases (exhaustive list available with WHO).

There are a number of challenges remaining: (i) maintaining support using existing tools, (ii) supporting each country with cost-effective surveillance and control methods, (iii) setting up efficient evaluation and monitoring systems, (iv) improving the knowledge about disease burden and distribution, (v) maintaining awareness of the problem of HAT, (vi) increasing coordination of all actors, and (vii) facilitating links between control and research activities. The inefficiency of controlling the disease in the 1970s was mainly due to the failure to integrate the vertical approach (surveillance and control) with the primary health care systems. With the new approach, elimination of the disease appears feasible, provided that the trend in the reduction of the number of cases is maintained and that surveillance and control measures are sustainable, cost-effective and adapted to each specific epidemiological situation.

As for AAT, the issue of climate change and its effect on HAT and its vectors is a factor that merits due and serious consideration.

WHO expressed its willingness to continue to support PAAT and to co-fund part of the PAAT Information System activities.

6. PAAT Information System update: harmonization and standardization for decision making – G. Cecchi

Recent PAAT Information System (PAAT IS) developments concerned mainly (i) web mapping services for PAAT IS GIS datasets, (ii) collaboration between WHO and FAO/PAAT IS on mapping HAT, and (iii) the development, in collaboration with the FAO PPLPI project of livelihood zones and profiles of countries of the IGAD region. Livelihood zones and profiles are areas within which people share broadly the same pattern of livelihood, i.e. same production system (e.g. agriculture or pastoralism) and the same patterns of trade and exchange. Livelihood analysis can be regarded as the "lens" through which to interpret a number of questions ranging from emergency response to disaster mitigation or long term development. Hence, mapping livelihoods and associated production systems provides a support for socio-economic assessments and project impact evaluation and eventually to strategic decision making (e.g. selection of intervention areas).

The new structure and functionalities of the PAAT IS were presented:

- PAAT web site;
- PAAT Technical and Scientific Series;
- Tsetse and Trypanosomiasis Information (TTI) bulletin;
- FAO GeoNetwork;
- Network and harmonization.

Detailed explanations of each of the above sections were illustrated to the participants, with emphasis on Web GIS in PAAT IS and the FAO GeoNetwork for the PAAT community. Concerning the FAO/WHO collaboration on HAT data management and mapping, the aim is to improve the management of HAT datasets, focusing on the spatial component for better targeting of interventions. The proposed database is designed to map HAT occurrence in space and time. The database can be directly linked to the GIS databank available at WHO headquarters for mapping purposes.

Another initiative undertaken within the PAAT IS relates to global datasets for managing the trypanosomiasis problem from an environmental perspective. This initiative provides a review of state-of-the-art global datasets relevant for T&T intervention and is available in the public domain. Sample analyses and applications are depicted/displayed.

7. Update on guidelines for assessing the feasibility of creating tsetse and trypanosomiasis (T&T) free zones – M. Vreysen

This presentation emphasised the complexity of area-wide integrated pest management (AW-IPM) projects and their intensive management requirements. However, important benefits can be derived from these projects provided a certain number of prerequisites are met. These are:

- the availability of a set of accurate, recent baseline data to develop an appropriate intervention strategy adapted to local conditions;

- in case the SIT is a component of the integrated approach, optimal competitiveness of the sterile insect is required with strict quality control procedures in place, both in the rearing facility and in the field;
- an autonomous and independent management structure;
- strict continuity in the implementation of all project components;
- full commitment of all stakeholders;
- adequate funding, personnel and logistics; and
- regular independent peer reviews of the programme.

The phased conditional approach and the flow chart on the project feasibility were presented and discussed.

8. Field experience in applying the guidelines for assessing the feasibility of creating T&T free zones

Burkina Faso – I. Sidibe

The report focused on activities under Phase 1 of the AfDB funded project and the current ongoing activities under Phase 2, e.g. development of a detailed plan of action for the collection of entomological baseline data, establishment of field teams and the development of detailed maps of vegetation.

Concerning data collection, it was reported that standardized methodologies have been developed and agreed among the six countries benefiting from AfDB funds for T&T intervention for collection of parasitological, socio-economic, environmental, entomological and land use data. Background studies have been conducted with the support of FAO and IAEA to select priority areas using the PAAT-FAO/IAEA criteria. The national plans of action under the AfDB funds aim at initiating tsetse eradication on about 40 000 km^2 out of 100 000 km^2 of the total intervention area. The area has been sub-divided in blocks and baseline data collection started in Block 1, the northern limit of the tsetse distribution in Burkina Faso. A sleeping sickness survey is also being carried out in the same area by a joint CIRDES/IRD/PATTEC team.

Factors contributing to a successful T&T campaign in the intervention area can be listed as follows:

- the area has high potential for crop production and livestock development (mixed farming is already increasing);
- the northern limit of tsetse distribution is seasonally favourable for suppression and final eradication;
- national facilities and efforts have been concentrated in the intervention area;
- human activities are developing and would eventually serve as natural barriers (cotton fields, utilization of pesticides).

Various agreements have been signed with concerned national institutions to obtain support and collaboration on land use / natural resource management, environmental impact survey, information and sensitization of local communities.

A short term consultancy is planned to assess the feasibility of applying SAT for tsetse fly suppression and the eventual creation of T&T free zones.

Ghana – C. Mahama

The presenter illustrated the tsetse fly situation and distribution in Ghana and the statistics of the T&T project area intervention. This has a surface of 20 000 km^2 with a human population of, approximately, 200 000 (70 percent) engaged in agricultural activities. Cattle, pig and small ruminant populations are estimated respectively at 300 000, 700 000 and 800 000. The production system is classified as low input with less than one USD per day. Three tsetse species are present (*Glossina palpalis gambiensis*, *G. tachinoides* and *G. morsitans submorsitans*) and the prevalence of bovine trypanosomiasis (parasitological diagnostic test – buffy coat technique) is between 5 percent and 25 percent. Baseline studies that are ongoing concern collection of additional epidemiological data, socio-economics, data on environment and land cover/land use. Formats for data collection at the various levels of implementation have been developed and indicators have been fine tuned during the monitoring and evaluation activity. Also, a matrix for outputs and outcomes, including output indicators, has been produced. The T&T intervention strategy has been discussed and harmonized with Burkina Faso and Mali.

The national Parliament has approved the loan agreement with AfDB for the sum of US$11 million and has endorsed, in principle, the extension of Phase 2 and Phase 3. The Government has assured mobilisation of resources from the Poverty Alleviation Fund and from Ghana's traditional development partners for subsequent phases. However, the continued support from the Government is subject to the success of Phase 1.

Mali – A. Djiteye

In Mali, 2.5 million people and 2.7 million cattle are exposed to the risk of trypanosomiasis. More than one million trypanocidal treatments are administered every year. Trypanocides represent more than 50 percent of sales of all veterinary drugs.

Mali has an historical tradition in tsetse and trypanosomiasis intervention and the relatively new AfDB funded project is a continuation of previous tsetse control campaigns. The project has an area of 37 000 km^2: 15 000 km^2 in the Niger river basin, the peri-urban area of Bamako and 22 000 km^2 in the Bani river basin from the northern limit of the tsetse distribution to the border with Burkina Faso. The eradication concerns 15 000 km^2.

Good quality data have been collected in the river Niger basin over the past five years, while data from the Bani river basin are lacking. The Niger river basin suffers from tsetse reinvasion; therefore 3 900 insecticide impregnated traps were re-deployed resulting in 91 percent reduction in flies over four months. Additional actions for planning T&T intervention include the involvement of farmers' communities and the private sector, and training of farmers and field technicians. Data on socio-economics and environmental impact assessment will also be collected.

Under the AfDB funded project (loan US$10.5 million and grant US$422 000), the establishment of a colony of *G.p. gambiensis* is foreseen with the aim at of producing males for an SIT campaign. The location of the facility is still under discussion. The financial contribution of the Malian Government to the T&T intervention is approximately US$1.7 million.

Kenya – P. Olet

The tsetse infested area covers 25 percent of the total country area (587 000 km^2). Five main tsetse belts have been identified: (i) Lake Victoria, (ii) Rift Valley, (iii) Coast region, (iv) Central region, and (v) Eastern region. Eight tsetse species are distributed over these five regions. In tsetse infested areas over 52 percent of the total of Kenya's livestock is present (23 percent of the national cattle population estimated at 13 million head). Calf mortality ranges between 10 percent and 40 percent. Approximately five million Kenyans live in sleeping sickness foci areas. It is estimated that in tsetse infested areas, food security is reduced by between 40 percent and 50 percent. More than US$3.5 million is spent by the Government to import trypanocides. At homestead level, 25 percent of income derived from milk sales is used to purchase trypanocidal drugs.

A large amount of data has been collected in the past in the Lambwe Valley and this knowledge should not be lost. All activities would need to be more focussed and comprehensive.

The AfDB loan provided to the Kenyan Government for T&T intervention amounted to USD 11 million, with a grant of about US$480 000. However, a total amount of US$45 million is estimated to be necessary to achieve the goal of T&T eradication from the envisaged area. Main activities foreseen in the action plan focus on (i) strengthening surveillance, (ii) community training, (iii) baseline data collection, and (iv) sensitization of rural communities. A main concern for the eradication of T&T in Kenya is the fact that the tsetse populations in targeted areas (e.g. Ruma National Park identified as Zone 1 of the project area) might not be isolated. Concern was expressed regarding the need for high quality sterile flies for tsetse eradication. In addition, available funds are not sufficient to cover the cost for baseline data collection in the project area of Zone 2. On this issue, it was commented that the US$11 million provided by AfDB would be sufficient to apply SAT over the entire project area of 24 000 km^2 (cost estimate for SAT application US$7.5 million). Kenya was urged to use available funding for a feasibility study on SAT application.

Uganda – L. Semakula

A comprehensive plan of action for collection of baseline data has been developed and current activities are focussing on Block one of the identified priority area. The Government of Uganda has expressed its intention to allocate US$3 million for the use of SAT. The National PATTEC Coordinator (Mr L. Semakula) suggested using part of the US$4.2 million AfDB loan (foreseen to purchase sterile flies from Ethiopia) for SAT. This would bring the total amount available for SAT to US$6 million and would leave US$1.2 million for the construction of the first two modules for the production of sterile flies in Uganda. The main challenge remains the approval of the AfDB for this change in budget line

allocation and the endorsement of the Ugandan Government for the use of SAT. It was suggested that the six countries benefiting from AfDB loans and grants use a unified strategy to approach AfDB to request changes in various project components and budget lines. This would be a much better approach than each country negotiating individually with AfDB.

On this proposal, the attention of PAG was drawn to country specific issues, i.e. different countries deal with different tsetse species, different geographical settings and agro-ecological zone(s). In addition, the data available and progress made in data collection are at different stages in different countries. Therefore, the need for increasing coordination and harmonization (hence, more regional concertation) was reiterated and more detailed work plans, including detailed budgeting for the various project components are needed.

9. Human and Animal Trypanosomiasis in Angola – T. Josenando

In Angola both human and animal trypanosomiases are endemic.

Sleeping sickness cases have been recorded in seven provinces where approximately one third of the total population is at risk from contracting the disease. Therefore, a national institution – Institute for the Control of Trypanosomiasis (ICCT) was created with the aim of setting and implementing human national trypanosomiasis control measures. The six pillars on which the HAT control strategy is based are:

- surveillance and monitoring;
- treatment;
- follow up;
- tsetse fly control;
- information, education and communication;
- training and supervision.

In 2007, out of 134, 000 persons examined 428 (0.3 percent) were found to be positive.

Animal trypanosomiasis is concentrated mainly in the southern part of the country where about 70 percent of the total national cattle stock lives. Disease prevalence data are not reliable or absent. Hence, it is envisaged, with the assistance of the international community, to conduct a survey in this respect. However, preliminary data in the Bengo province indicated an annual mortality rate of about 15 percent in the cattle population due to the disease.

Various international, regional and national partners are supporting field activities related to T&T intervention(s) in Angola. These are WHO, AU-PATTEC, IAEA, NGOs and the Portuguese Institute of Preventive Medicine. Research institutes are also providing scientific assistance.

The implementation of T&T intervention field activities faces some difficulties. The past civil disturbance has brought consequences for accessing infested/endemic areas. In addition, paucity of funds has consequences on the whole chain of field actions. However, the

National Reconstruction Programme provides good prospects for an improvement in the current situation.

10. Capacity of PAAT partners (research and scientific organizations/institutes) to support national and regional ongoing and future interventions (CIRDES, ICIPE, ILRI, and ITM)

CIRDES – I. Sidibe

The Centre, based in Bobo Dioulasso, Burkina Faso, covers seven West African countries (Benin, Burkina Faso, Côte d'Ivoire, Guinea Bissau, Mali, Niger and Togo). The current research activities focus on:

- regional epidemiology of trypanosomiasis (AfDB funded project within the PATTEC initiative);
- tsetse fly control, including tsetse population genetics, and control of sleeping sickness (funded by EU);
- vector-based control of HAT using bait technology allied to a better understanding of vector population structures (funded by the Bill and Melinda Gates Foundation);
- landscape fragmentation in the Mouhoun river: impact on tsetse habitats (funded by the Wellcome Trust);
- quality control of tsetse and sterile males (funded by IAEA);
- utilisation of a tsetse production unit (TPU) 3 for mass production of tsetse flies (funded by IAEA);
- improving the management of trypanocide resistance in the cotton zone of West Africa: a coordinated regional study (funded by BMZ/ILRI).

Human resource development and capacity building are other key activities of the Centre. In 2006, CIRDES organized eight training courses for a total of 47 participants from nine West African countries and France. In 2007, eight technicians from Senegal and four from Mali were trained on T&T control techniques. Diagnostic techniques (i.e. ELISA) have been transferred to national laboratories (e.g. Burkina Faso, Mali, Ghana). The Centre also provides expert services to member and associated countries on matters related to vector (ticks and tsetse flies) and parasite control technologies. On request, it also provides tsetse flies for the application of SIT and targets for tsetse suppression.

ICIPE – R. Saini

The ICIPE representative highlighted the strategic current and future research plans, which focus on five main research areas:

- vectors of trypanosomiasis, both human and animal, and tick-borne diseases;
- extension of research to other arthropods of medical and zoonotic importance in order to develop technologies for integrated management of these vectors and the diseases they cause;

- use of genomics and bio-informatics, and behaviour and chemical ecology for technology development and implementation;
- investigations of the effects of climate changes on the range and efficiency of vectors;
- development of holistic, site-specific packages for sustainable animal health management and production, and test packages at farmer level in different production systems and agro-ecological zones for adoption and wider dissemination.

As a general approach, the Centre is developing more holistic projects in collaboration with other collaborators in order to catalyse sustainable agriculture and rural development, improve livestock and human health, food security and reduce poverty. The Centre's strategy also includes increased capacity building activities with a view to creating cadres of research, vector control specialists and managers in livestock integrated pest and vector management (IPVM). The human resource development action embraces also the technical empowerment of communities to ensure sustainability of control efforts.

Current projects and new initiatives are:

- Development of baits for riverine tsetse species, vectors of human trypanosomiasis (funded by the Bill and Melinda Gates Foundation);
- Tsetse/trypanosomiasis rollback initiative in Ethiopia – Sustainable community-based management of T&T using strategic deployment of improved odour-baited monitoring and control traps (funded by the Swiss BioDivision Foundation);
- Community based tsetse control in the interface between agricultural land and game reserve (Mwea) in Kenya in order to reduce human-wildlife conflicts through effective tsetse control and improvement of livestock health and productivity (funded by the Swiss BioDivision Foundation);
- characterisation of odour binding proteins and receptors of tsetse for optimizing existing baits and for development of new ones (funded by WHO-TDR);
- further optimisation and validation of the repellent technology developed by ICIPE: development of dispensers for the identified waterbuck repellent blend in order to evaluate the belt's efficacy and to transform cattle into animals with "waterbuck clothing" (funded by IFAD).

ILRI – J. Maitima

The Centre's mandate is to reduce poverty and make sustainable development possible through livestock-related research and innovation in research to improve food security in Africa. ILRI's research themes focus on:

- enhancing access to market opportunities;
- securing assets through biotechnology;
- production systems (people, livestock and the environment).

Specific research on T&T concerns:

- resistance to trypanocides in the cotton zones of West Africa (Mali, Guinea, Ghana and Benin) and evaluation of trypanosomiasis control strategies in the context of drug resistance;
- molecular genetics and breeding in cattle (production traits in N'Dama cattle, Ethiopian Sheko zebu and conservation of endemic breeds in Guinea, Mali, Senegal and Gambia);
- community-led livestock disease control (promotion of animal health cooperatives, farmer to farmer knowledge transfer);
- socio-economics and environmental monitoring of T&T control projects;
- sustainable land management of tsetse areas;
- impact of climate changes on tsetse systems.

ILRI has developed "Environmental and socio-economic impact assessment framework and guidelines" for integrated impact assessment of trypanosomiasis interventions. The framework and guidelines include a selected set of indicators and the type of data to be collected for impact assessment. Diagrams of relationships and interactions, and methods for impact analysis are illustrated. Another initiative concerns the development of a dynamic ecological simulation model of tsetse transmitted trypanosomiasis in Kenya. Research actions within this initiative focus on the analysis of spatial and temporal trends in climate variables and the responses of people, livestock and wildlife to changes in rainfall and temperature. The aim is to analyze the linkages between climate, land use cover and tsetse-trypanosomiasis dynamics.

ITM – S. Geerts

Research and teaching (training) are the two main activities at the Institute for Tropical Medicine. Research related to T&T concerns drug resistance and development of collaboration with African based research institutes, like CIRDES and ITC. Teaching activities focus on (i) a module on "Vector-borne diseases" and (ii) a Web-based module "Tsetse and trypanosomiasis".

Investigations on drug resistance revealed the phenomenon is rapidly expanding in many African countries and tsetse infested areas, with populations/strains of trypanosomes possessing a multi drug resistance trait. Tests used to detect drug resistance are based on conventional methods (test in ruminants, mice, field test or block treatment) and molecular tools (PCR). Through the application of molecular technology, the Institute has been able to identify trypanosome genes responsible for drug resistance in *Trypanosoma brucei* and *T. congolense*. In addition, it appears that drug resistance mechanisms are not uniform but differ in different trypanosome populations. Once the molecular tools for drug resistance are validated, they will provide the opportunity, *inter alia*, to develop better strategies for delaying the development of drug resistance.

Collaboration with CIRDES concentrates on a new project "Strengthening of CIRDES as a regional reference centre for the diagnosis and control of trypanosomiasis and trypanocidal drug resistance". This cooperation implies the transfer of technology from ITM to CIRDES and training of technicians and PhD students.

The Belgian Government and ITM have strongly supported ITC both financially and scientifically. ITC is now in a difficult financial situation and actions have been taken by the Council to reduce expenditures and running costs. It is almost a general opinion that a merger of CIRDES and ITC is necessary in order to create a stronger livestock research centre for West Africa. The challenge ahead for ITC is to ensure core funding for maintaining a unique breeding stock of global significance, keeping a critical mass of qualified human resources and progressing with the restructuring process. ITC hosts the AfDB-GEF funded project "Sustainable management of endemic ruminant livestock in West Africa" covering Mali, Gambia, Senegal, Guinea. The project is executed by ITC and ILRI and has a budget of US$42 million (GEF US$10 million over 10 years; AfDB US$30 million over six years).

11. Needs assessment for comprehensive training and capacity building in support of PATTEC projects – R. Saini

National systems lack capacity at all levels to undertake large scale integrated disease and vector control programmes; reduced funding and relatively poor infrastructure are further limitations to build the necessary human capacity. Given the magnitude of the T&T problem and its interdependency with different related special fields, multi-disciplinary teams of trained manpower are required. Strategic investments in strengthening capacity of African countries and institutions are therefore of highest priority.

A training questionnaire was developed to identify targeted training needs. A training survey was conducted in Burkina Faso, Mali, Ghana, Ethiopia, Uganda and Kenya. Analysis of the questionnaire led to the identification and prioritisation of the critical areas in which capacity is lacking and the determination of the number of cadres and technical experts that need training. This training needs assessment exercise allowed also an integrated data information management system (including GIS) to be developed and established in each country, and sub-regional training facility centres, needs for their rehabilitation and for strengthening national and regional capacities in T&T intervention and for environmental audit to be identified.

Identified training priorities were:

- GIS, data base management and networking;
- project planning, development and management;
- basic tsetse biology and ecology, and baseline entomological survey;
- environmental/land cover baseline survey and land use impact assessment,
- socio-economic baseline survey and analysis including T&T elimination impact;
- mass rearing of tsetse and SIT, including sterilization and release;
- community empowerment on T&T management;
- HAT surveillance, diagnosis and treatment;
- animal health providers, extension staff, veterinary officers training.

The foreseen goal and objectives of capacity building activities should enhance the scientific and technical capacities of mid-level personnel in T&T affected countries to enable them to plan, implement, monitor and evaluate the implementation of T&T projects/interventions. More specifically, training should concentrate on:

- Training of project managers in planning and executing the programmes;
- Training and sensitisation of communities and extension workers and their empowerment in T&T control and management issues;
- Promotion of cooperation and networking among affected countries;
- Production of training manuals and related information materials to be used at the continental scale.

Implementation of the training plan foresees that training sessions will be held over a five-year period with trainees (about 24 trainees per course) selected from different ongoing projects and geographical areas. Since it is impossible to meet the entire capacity strengthening demands of all countries, emphasis will be on training of trainees who, in turn will return to their national systems and train other staff. Since new training needs may arise from unforeseen developments while implementing the projects in the field, flexibility in the course curricula will be needed. Training materials (e.g. manuals) should be bilingual (English and French) and addressed to technical staff rather than scientists. Two specialized workshops per year (one in French and the second one in English) should also be held with a maximum 20 participants per workshop. Suggested topics include:

- Integration of T&T control within the framework of other national and regional development strategies for reducing poverty and enhancing food security and rural development;
- Specific programme/project review workshops;
- Public relations, public awareness and sensitisation/information flow to all stakeholders and donors.

Implementation and coordination will be ensured by the PATTEC Coordination Office, where a Training Coordination Unit will be established. Training centres will be identified in West and East Africa taking advantage of already existing infrastructures and advanced laboratories. Resource persons will be from ICIPE, CIRDES, ILRI, KARI-TRC, and other mandated organization such as FAO, IAEA and WHO.

12. Acknowledgement

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

INTERNATIONAL ATOMIC ENERGY AGENCY

Baseline data collection in Senegal as part of a feasibility study to create a zone free of *Glossina palpalis gambiensis*.

The Government of Senegal has embarked on a project that aims at the elimination of the tsetse fly *Glossina palpalis gambiensis* from the Niayes area (north west of Dakar) and La Petite Côte (south east of Dakar). Assistance was requested from the IAEA and since 2005,

technical support has been provided through a TC Project SEN 5029. During the last two years, excellent progress has been made in this project largely due to the commitment of the Government of Senegal and the good leadership and organisational talents of the counterparts of the Direction de l'Élevage (DIREL) and of the Institut Sénégalais de Recherches Agricoles (ISRA). In the initial phase of the project, emphasis was given on training and so far, 16 technical staff have received training at the Centre International de Recherche-Développement de l'Élevage en zone Subhumide (CIRDES), Bobo Dioulasso, Burkina Faso in tsetse biology, tsetse control methods, GIS, data analysis, etc. The team leaders also attended an FAO/IAEA/PATTEC Regional Training Course on principles of baseline data collection for integrated area-wide tsetse and trypanosomosis intervention projects with a sterile insect technique component that was organised in Dakar from 18 February to 14 March 2008.

In the last two years, the following activities have been implemented: (i) the collection and genotyping of *G. p. gambiensis* flies from different areas inside and outside the target area for population genetic studies, (ii) the development of a detailed plan of action for the collection of entomological baseline data, (iii) a parasitological and serological survey of livestock in the target area, and (iv) entomological baseline data collection (scheduled to be finalised in December 2008/January 2009).

A workshop was organized in Dakar by the DIREL in October 2008 to review programme progress. All entomological data were collected using a representative grid-based sampling approach (FAO/IAEA Guidelines outlining this approach are being published under the FAO Animal Production and Health series), that aims at the collection of data from carefully selected habitats within a given grid cell that are considered to be representative of other similar habitat areas in other grid cells. The sampling process was fine tuned and improved using modern tools of spatial analysis (GIS/RS/GPS) and mathematical modelling and taking into account the ecological affinities of the target species *G. p. gambiensis*. The data so far collected indicate that the total infested area in the Niayes/ La Petite Côte is 525 km^2 and the total control area, taking into consideration an assumed dispersal capacity of 5 km of the flies, is currently estimated at 975 km^2. The target area will be adjusted after the completion of the entomological baseline data collection.

During the veterinary survey of 2007, 1 329 cattle were screened for trypanosome infection using the buffy coat technique. Positive animals were found in 13 of the 38 sites where animals were screened. Interestingly, infections were found in Mboro (north of Dakar) and in Joal (in La Petite Côte, south east of Dakar) where so far no flies have been trapped. The infestation rate in livestock varied between 2.5 percent and 13.3 percent, which can be considered as high for a vertical parasitological survey with a moderate sensitivity (microscopic observation of the buffy coat). All the samples were also screened serologically using a specific Ab-ELISA for each *Trypanosoma* species at the CIRDES, which is also a partner in this project. The parasitological data were confirmed, with 28 percent of the animals being positive for *Trypanosoma vivax* and 4 percent for *T. congolense*. The geographical distribution of the serological prevalence was very heterogeneous, with up to 96 percent of the animals being positive in some herds located inside the tsetse-infested area. In 2008, 394 small ruminants (44 ovines and 350 goats) and 155 horses (55 of local breed and 97 of exotic breeds) were also screened using the buffy coat technique, with prevalence rates of 0.34 percent and 0 percent respectively. Positive animals were found in two of the 12 sites sampled.

Preliminary analysis of the gene frequencies of the *G. p. gambiensis* flies sampled in the various areas of the Niayes /La Petite Côte and in Missira (population in the south eastern part of the country and part of the larger tsetse fly belt of West Africa) indicate complete isolation of the two main *G. p. gambiensis* populations of the Niayes (Sebikotane, Pout, Diacksao Peul and Hann) from those of the main belt in the south eastern part of the country. The fly population in the Parc de Hann (in Dakar) seems likewise strongly isolated from the remaining fly pockets in the Niayes. Gene flow however, seems to exist between the different fly populations in Pout, Diacksao Peul, and Sebikotane, which probably occurs during the rainy season when the fly populations spread from their dry season foci. These data are extremely important as they confirm the isolated character of the fly pockets in the Niayes.

Although a complete analysis of the entomological and parasitological/serological data still has to be completed, all stakeholders in the project are of the opinion that a sustainable tsetse-free zone can be created in the Niayes/La Petite Côte using an area-wide integrated pest management approach that would include the release of sterile males. Initial trial releases using sterile males from the *G. p. gambiensis* colony that is maintained at CIRDES are scheduled to be initiated in 2009 to develop transport and release procedures and to assess the performance of the flies in the field.

The Director of the DIREL has requested increased support from the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) to reinforce the expertise within the project. As such, CIRAD has agreed to transfer a tsetse ecologist from CIRDES in Burkina Faso to Senegal in December 2008 for an initial period of two years.

SECTION B - ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

14539. **Alphey, L., Nimmo, D., O'Connell, S. & Alphey, N., 2008**. Insect population suppression using engineered insects. *Advances in Experimental Medicine and Biology,* **627**: 93-103.

Department of Zoology, Oxford University, Oxford, UK.
[luke.alphey@zoo.ox.ac.uk].

Suppression or elimination of vector populations is a tried and tested method for reducing vector-borne disease, and a key component of integrated control programmes. Genetic methods have the potential to provide new and improved methods for vector control. The required genetic technology is simpler than that required for strategies based on population replacement and is likely to be available earlier. In particular, genetic methods that enhance the sterile insect technique (e.g. RIDL) are already available for some species.

14540. **Antoine-Moussiaux, N., Magez, S. & Desmecht, D., 2008**. Contributions of experimental mouse models to the understanding of African trypanosomiasis. *Trends in Parasitology,* **24** (9): 411-418.

Department of Animal Pathology, Veterinary College, University of Liege, 20 Boulevard de Colonster, B43, 4000 Liege, Belgium. [nantoine@ulg.ac.be].

African trypanosomiasis is the collective name for a wide variety of trypanosome infections that affect humans and livestock. In recent years, experimental mice infection models have provided new insights into both human and animal trypanosomiasis. Mouse models seem to be a valuable and versatile tool in trypanosomiasis-associated pathology and immunology research and highlight the variety shown by African trypanosomiases. Indeed, inbred mouse strains have enabled the study of genetic determinants of susceptibility and of the roles of anti-parasite antibodies, inflammatory mediators and anti-inflammatory mediators for each trypanosome species. Remarkable advances relating to the encephalitic stage of sleeping sickness have also been achieved thanks to murine models. The different contributions of murine models to the African trypanosomiases knowledge are presented here. Future search directions are finally proposed, with respect to mouse model opportunities and limitations.

14541. **Balasegaram, M., Balasegaram, S., Malvy, D. & Millet, P., 2008**. Neglected diseases in the news: a content analysis of recent international media coverage focussing on leishmaniasis and trypanosomiasis. *PLoS Neglected Tropical Diseases,* **2** (5): e234.

Centre René Labusquière EA 3677, Bases Thérapeutiques des Inflammations et des Infections, Université Bordeaux 2, Bordeaux, France.

Although the pharmaceutical industry's "neglect" of neglected tropical diseases (NTDs) has been investigated, no study evaluating media coverage of NTDs has been published. Poor media coverage exacerbates the neglect. This study aimed to investigate, describe, and analyse international media coverage of "neglected diseases" in general and three specific NTDs-African trypanosomiasis, leishmaniasis, and Chagas disease-from 1 January 2003 to 1 June 2007. Archives of 11 leading international, English-language media were searched. A content analysis was done, coding for media organisation, date, author, type of report, slant, and themes. Semi-structured interviews with journalists and key informants were conducted for further insight. Only 113 articles in a 53-month time period met the inclusion criteria, with no strong trends or increases in coverage. Overall, the BBC had the highest coverage with 20 results, followed by the Financial Times and Agence France Presse. CNN had the least coverage with one result. The term "neglected diseases" had good media currency and "sleeping sickness" was far more widely used than trypanosomiasis. The disease most covered was leishmaniasis and the least covered was Chagas. Academic researchers were most commonly quoted as a main source, while the World Health Organization (WHO) and pharmaceutical industry were the least quoted. Journalists generally agreed NTDs had not been adequately covered, but said a lack of newsworthy development and the need to cater to domestic audiences were major obstacles for NTD reporting. All journalists said health agencies, particularly WHO, were not communicating adequately about the burden of NTDs. It is concluded that public health agencies need to raise the priority for NTD advocacy. Innovative strategies, such as reporting grants or creating a network of voices, may be needed.

14542. **Bern, C., Montgomery, S. P., Katz, L., Caglioti, S. & Stramer, S. L., 2008**. Chagas disease and the US blood supply. *Current Opinion in Infectious Diseases,* **21** (5): 476-482.

Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30341, USA. [CBern@cdc.gov].

This review describes new developments in blood-bank screening and management of patients with chronic *Trypanosoma cruzi* infection in the United States. The first US Food and Drug Administration licensed serological test for *T. cruzi* blood screening went into widespread usage in January 2007 and more than 500 confirmed *T. cruzi*-infected donations were detected by mid-June 2008. Until recently, drug therapy was recommended for acute and congenital infections, but seldom for chronic infections, which were believed to respond poorly. However, in the 1990s, efficacy was demonstrated in two placebo-controlled trials of benznidazole in children with chronic *T. cruzi* infection. In 2006, a non-randomized, non-blinded trial demonstrated that benznidazole treatment may slow progression of cardiomyopathy and decrease mortality risk in infected adults. Blood-bank screening will continue to detect *T. cruzi*-infected donors. Based on recent data, antitrypanosomal treatment is recommended for all acute and congenital *T. cruzi* infections, reactivated infection, and chronically infected children. In adults aged 19-50 years without advanced heart disease, treatment should generally be offered; management should be individualized for older adults. Less toxic, more effective drugs, a sensitive, specific assay for response to treatment, and improved healthcare access would promote more effective management.

14543. **Boraschi, D., Abebe Alemayehu, M., Aseffa, A., Chiodi, F., Chisi, J., Del Prete, G., Doherty, T. M., Elhassan, I., Engers, H., Gyan, B., Harandi, A. M., Kariuki, T., Kironde, F., Kouriba, B., Langhorne, J., Laskay, T., Medaglini, D., Olesen, O., Onyebujoh, P., Palma, C., Sauerwein, R., Sibanda, E., Steinhoff, U., Tagliabue, A., Thiel, A., Vahedi, M. & Troye-Blomberg, M., 2008**. Immunity against HIV/AIDS, malaria, and tuberculosis during co-infections with neglected infectious diseases: recommendations for the European Union research priorities. *PLoS Neglected Tropical Diseases,* **2** (6): e255.

CNR, Pisa, Italy.

Infectious diseases remain a major health and socioeconomic problem in many low-income countries, particularly in sub-Saharan Africa. For many years, the three most devastating diseases, HIV/AIDS, malaria, and tuberculosis (TB) have received most of the world's attention. However, in rural and impoverished urban areas, a number of infectious diseases remain neglected and cause massive suffering. It has been calculated that a group of 13 neglected infectious diseases affects over one billion people, corresponding to a sixth of the world's population. These diseases include infections with different types of worms and parasites, cholera, and sleeping sickness, and can cause significant mortality and severe disabilities in low-income countries. For most of these diseases, vaccines are either not available, ineffective, or too expensive. Moreover, these neglected diseases often occur in individuals who are also affected by HIV/AIDS, malaria, or TB, making the problem even more serious and indicating that co-infections are the rule rather than the exception in many

geographical areas. To address the importance of combating co-infections, scientists from 14 different countries in Africa and Europe met in Addis Ababa, Ethiopia, on September 9-11, 2007. The message coming from these scientists is that the only possibility for winning the fight against infections in low-income countries is by studying, in the most global way possible, the complex interaction between different infections and conditions of malnourishment. The new scientific and technical tools of the post-genomic era can allow us to reach this goal. However, a concomitant effort in improving education and social conditions will be needed to make the scientific findings effective.

14544. **Cecchi, G., Mattioli, R. C., Slingenbergh, J. & de la Rocque, S., 2008**. Land cover and tsetse fly distributions in sub-Saharan Africa. *Medical and Veterinary Entomology.* **Published online 8 September 2008.**

Food and Agriculture Organization of the United Nations (FAO), Animal Production and Health Division, Rome, Italy. [raffaele.mattioli@fao.org].

This study aims to provide trypanosomiasis-affected countries with standardized datasets and methodologies for mapping the habitat of the tsetse fly (*Glossina* spp., the disease vector) by customizing and integrating state-of-the-art land cover maps on different spatial scales. Using a combination of inductive and deductive approaches, land cover and fly distribution maps are analysed in a geographic information system (GIS) to estimate the suitability of different land cover units for the three groups (subgenera) of *Glossina*. All land cover datasets used for and produced by the study comply with the land cover classification system (LCCS). At the continental scale, a strong correlation between land cover and tsetse habitat is found for both the *fusca* and *palpalis* groups, whereas the weaker correlation found for the *morsitans* group may be indicative of less restrictive ecological requirements. At the regional and national levels, thematic aggregation of the multi-purpose Africover datasets yielded high-resolution, standardized land cover maps tailored for tsetse habitat for eight East African countries. The national maps provide remarkable spatial resolution, thematic detail and geographical coverage. They may be applied in subsequent phases of tsetse and trypanosomiasis control projects, including the planning of entomological surveys, actual tsetse control operations and planning for land use in reclaimed areas. The methodology and datasets discussed in the paper may have applications beyond the tsetse and trypanosomiasis issue and may be used with reference to other arthropod vectors, vector-borne and parasitic diseases.

14545. **Chung, M. C., Ferreira, E. I., Santos, J. L., Giarolla, J., Rando, D. G., Almeida, A. E., Bosquesi, P. L., Menegon, R. F. & Blau, L., 2008**. Prodrugs for the treatment of neglected diseases. *Molecules,* **13** (3): 616-677.

Laboratório de Desenvolvimento de Fármacos, Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmaceuticas - UNESP Rodovia Araraquara-Jau Km 1, 14801-902, Brazil. [chungmc@fcfar.unesp.br].

Recently, the World Health Organization (WHO) and Médecins sans Frontières (MSF) proposed a classification of diseases as global, neglected and extremely neglected. Global diseases, such as cancer, cardiovascular and mental (CNS) diseases represent the targets of the majority of the R&D efforts of pharmaceutical companies. Neglected diseases affect

millions of people in the world yet existing drug therapy is limited and often inappropriate. Furthermore, extremely neglected diseases affect people living under miserable conditions who barely have access to the bare necessities for survival. Most of these diseases are excluded from the goals of the R&D programmes in the pharmaceutical industry and therefore fall outside the pharmaceutical market. About 14 million people, mainly in developing countries, die each year from infectious diseases. From 1975 to 1999, 1 393 new drugs were approved yet only 1 percent were for the treatment of neglected diseases. These numbers have not changed until now, so in those countries there is an urgent need for the design and synthesis of new drugs and in this area the prodrug approach is a very interesting field. It provides, among other effects, activity improvements and toxicity decreases for current and new drugs, improving market availability. It is worth noting that it is essential in drug design to save time and money, and prodrug approaches can be considered of high interest in this respect. The present review covers 20 years of research on the design of prodrugs for the treatment of neglected and extremely neglected diseases such as Chagas disease (American trypanosomiasis), sleeping sickness (African trypanosomiasis), malaria, sickle cell disease, tuberculosis, leishmaniasis and schistosomiasis.

14546. **Click Lambert, R., Kolivras, K. N., Resler, L. M., Brewster, C. C. & Paulson, S. L., 2008**. The potential for emergence of Chagas disease in the United States. *Geospatial Health,* **2** (2): 227-239.

Department of Geography, Virginia Polytechnic Institute and State University (Virginia Tech), 115 Major Williams Hall, Blacksburg, VA 24061, USA.

To determine the risk for Chagas disease (American trypanosomiasis) in the United States, the characteristics that make the triatomine vector effective and the areas most at risk for transmission were delineated. In addition, the status of Chagas disease awareness among physicians in areas with a potential risk for the disease was determined. A geographical information system (GIS) was used to analyze three triatomine species within the United States known to harbour *Trypanosoma cruzi* and that exhibit qualities of domesticity. An analysis of the minimum temperature threshold for increased triatomine activity delineates the current population at increased risk, and by incorporating temperature predictions for 2030, the population at risk under a future climate scenario was also delineated. Considering both environmental and social factors, a vignette-based physician survey, based on the results of the GIS analysis, was used to gauge the level of awareness of Chagas disease within the delineated higher risk range. The current area at increased risk for Chagas disease includes much of the southern United States, and the higher risk range is expected to expand into the central United States based upon the 1 ^{0}C (1.8 ^{0}F) increase in temperature predicted by the Intergovernmental Panel on Climate Change (IPCC) by the year 2030. Survey results indicate a limited consideration of Chagas disease during differential diagnosis, illustrating that the low number of Chagas disease cases discovered in the United States may be attributable to a lack of disease awareness as opposed to a lack of disease threat. This study combines GIS and survey analyses to evaluate the role that temperature variability and disease awareness among physicians play in the potential emergence of Chagas disease in the United States. This approach indicates that there is a potential for Chagas disease to emerge in the United States.

14547. **Cook, P. E., McMeniman, C. J. & O'Neill, S. L., 2008**. Modifying insect population age structure to control vector-borne disease. *Advances in Experimental Medicine and Biology,* **627**: 126-140.

School of Integrative Biology, The University of Queensland, Brisbane, Queensland, Australia.

Age is a critical determinant of the ability of most arthropod vectors to transmit a range of human pathogens. This is due to the fact that most pathogens require a period of extrinsic incubation in the arthropod host before pathogen transmission can occur. This developmental period for the pathogen often comprises a significant proportion of the expected lifespan of the vector. As such, only a small proportion of the population that is oldest contributes to pathogen transmission. Given this, strategies that target vector age would be expected to obtain the most significant reductions in the capacity of a vector population to transmit disease. The recent identification of biological agents that shorten vector lifespan, such as *Wolbachia*, entomopathogenic fungi and densoviruses, offer new tools for the control of vector-borne diseases. Evaluation of the efficacy of these strategies under field conditions will be possible due to recent advances in insect age-grading techniques. Implementation of all of these strategies will require extensive field evaluation and consideration of the selective pressures that reductions in vector longevity may induce on both vector and pathogen.

14548. **Courtin, F., Jamonneau, V., Duvallet, G., Camara, M., Kaba, D. & Solano, P., 2008**. One century of "sleeping sickness" in West Africa. *Bulletin de la Société pathologie exotique,* **101** (3): 287-289.

Centre International de recherche développement sur l'élevage en zone subhumide (CIRDES)/Institut de recherche pour le développement (IRD), UMR 177, BP 454, Bobo-Dioulasso, Burkina Faso. [courtinfabrice@yahoo.fr].

This paper summarizes the geography of sleeping sickness disease (or Human African Trypanosomiasis, HAT) over the last 100 years in West Africa, with the objective of identifying today's priority areas for the sleeping sickness surveillance. The history and geography of the disease are based on a bibliographic review of old reports and recent publications on recent results obtained from medical surveys conducted in West Africa up to 2007. This allowed us to situate the historical geography of HAT from the beginning of the 20th century to nowadays. For instance, active HAT foci seem to have moved from the North (savannah area) to the South (forest area) in the last century. Taking into account the limited nature of the information available, endemic HAT presently appears to be limited to areas where annual rainfall is higher than 1 200 mm, although the reasons for this remain unknown. During this period of time 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, but many parts of West Africa still lack information on HAT and remain to be investigated. These observations are put in the current context of demographic growth and global climatic change responsible for landscape evolution, political instability and population movements.

14549. **Croft, S. L., 2008**. Kinetoplastida: new therapeutic strategies. *Parasite,* **15** (3): 522-527.

Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK. [simon.croft@lshtm.ac.uk].

New formulations and therapeutic switching of the established drugs, amphotericin B and paromomycin, together with the discovery of miltefosine, have significantly improved the opportunities for treatment of visceral leishmaniasis. However, for human African trypanosomiasis (HAT), Chagas disease and cutaneous leishmaniases there has been limited progress. For HAT, a novel diamidine, parfuramidine, is in phase III clinical trial for early-stage disease, but for the treatment of late-stage disease there are no new drugs and combinations of eflornithine with melarsoprol or nifurtimox have been the focus of clinical studies. For Chagas disease, different classes of compounds that have validated biochemical targets, sterol biosynthesis methylases and cysteine proteases, are in various stages of development. The genome sequences that are now available for the pathogens that cause the leishmaniases and trypanosomiases, and new methods for rapid validation of targets, are part of the solution to discover new drugs. The integration of medicinal chemistry, pharmacokinetics, project planning and interaction with the pharma/biotech sector are essential if progress is to be made. Although there are financial constraints, the appearance of new funding sources and not-for-profit product development partnerships offers hope for drug development.

14550. **de Koning, H. P., 2008**. Ever-increasing complexities of diamidine and arsenical cross-resistance in African trypanosomes. *Trends in Parasitology,* **24** (8): 345-349.

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

The treatment of both human and veterinary African trypanosomiasis is still, to a large extent, dependent on diamidines and melaminophenyl arsenicals. Sixty years after the introduction of pentamidine, a large effort is being made to develop a new generation of diamidines for the treatment of sleeping sickness. However, given the reports of resistance to both diamidines and melaminophenyl arsenicals from the field, including cross-resistance to both classes in single isolates, researchers should proceed with some caution before introducing new diamidines, and a thorough understanding of the causes of resistance and cross-resistance will be essential.

14551. **Doyle, P. S., Sajid, M., O'Brien, T., Dubois, K., Engel, J. C., Mackey, Z. B. & Reed, S., 2008.** Drugs targeting parasite lysosomes. *Current Pharmaceutical Design,* **14** (9): 889-900.

Tropical Diseases Research Unit and Sandler Center, Department of Pathology, University of California, San Francisco, USA. [Patricia.Doyle-Engel@ucsf.edu].

Lysosomes were first described as vacuolar structures containing various hydrolytic enzymes at acidic pH. Subsequent studies revealed that the lysosome/vacuolar system is complex and composed of distinct membrane-enclosed vesicles including endosomes, primary and mature lysosomes, autophagic vesicles, residual bodies, multivesicular bodies, and digestive lysosomes. Lysosomes express a battery of hydrolytic enzymes including proteases, acid phosphatases, glycosidases, and lipases. Parasitic protozoa also possess complex intracellular lysosomes/endosomes/vesicles involved in digestion, transport and recycling of molecules similar to those of mammalian cells. Unique characteristics are ascribed to lysosomes of different parasites and may even differ between parasite stages. Transport of hydrolases and proteins to parasite lysosomes is directed either from the Golgi complex via endosomal vesicles or from endocytic vesicles originated in the cell surface. Inhibition of lysosomal proteases demonstrated that different proteolytic machineries catabolize distinct classes of proteins, and this selectivity may be exploited for the development of effective antiparasitic drugs. This review describes lysosomal molecules that are either validated or potential drug targets for Chagas disease, sleeping sickness, leishmaniasis, toxoplasmosis, malaria, amebiasis, and giardiasis.

14552. **Ebi, K. L., Helmer, M. & Vainio, J., 2008**. The health impacts of climate change: getting started on a new theme. *Prehospital and Disaster Medicine,* **23** (4): s60-64.

Red Cross/Red Crescent Climate Centre, The Hague, the Netherlands.

Climate change is widely acknowledged as a key global challenge for the 21st century, and is projected to significantly affect population health and human well-being. All of the climate change-related changes in weather patterns will affect human health, from boosting mental well-being to mortality from large-scale disasters. Human health can be affected both directly and indirectly. For various reasons, the health sector has been slow in responding to the projected health impacts of climate change. To effectively prepare for and cope with climate change impacts, public health must move from a focus on surveillance and response to a greater emphasis on prediction and prevention. The targeted agenda programme dialogue identified three priorities for climate change related health actions: heat waves, vector-borne diseases; and malnutrition.

14553. **Eisen, R. J. & Eisen, L., 2008**. Spatial modelling of human risk of exposure to vector-borne pathogens based on epidemiological versus arthropod vector data. *Journal of Medical Entomology,* **45** (2): 181-192.

Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, 3150 Rampart Rd., Fort Collins, CO 80522, USA. [dyn2@cdc.gov].

Understanding spatial patterns of human risk of exposure to arthropod vectors and their associated pathogens is critical for targeting limited prevention, surveillance, and control resources (e.g., spatial targeting of vaccination, drug administration, or education campaigns; use of sentinel sites to monitor vector abundance; and identifying areas for most effective use of pesticides). Vector-borne disease risk can, in many cases, be modeled with high predictive accuracy by using geographic information system approaches because abundances of vectors and pathogen reservoirs often are associated with environmental factors. Spatial risk models for human exposure to vector-borne pathogens, which ideally should have high accuracy for

predicting areas of elevated risk without overestimating risk coverage, can be constructed based on epidemiological data or abundance of vectors or infected vectors. We use five bacterial or viral vector-borne diseases occurring in the United States and with pathogen transmission by fleas (plague), ticks (Lyme disease and tularaemia), or mosquitoes (dengue and West Nile virus disease) to i) examine how spatial risk of human exposure to vector-borne pathogens typically is presented to the public health community and public, and ii) evaluate the utility of basing spatial risk models on epidemiological data relative to data for arthropod vectors or infected vectors. Recommended future directions for vector-borne disease risk modeling include development of subcounty level spatial risk models combining epidemiological and vector data and the use of simulation or analytical models to assess critical vector abundance thresholds required for enzootic pathogen maintenance.

14554. **Forman, S., Hungerford, N., Yamakawa, M., Yanase, T., Tsai, H. J., Joo, Y. S., Yang, D. K. & Nha, J. J., 2008**. Climate change impacts and risks for animal health in Asia. *Revue scientifique et technique,* **27** (2): 581-597.

World Bank, Washington, DC 20433, USA.

The threat of climate change and global warming is now recognised worldwide and some alarming manifestations of change have occurred. The Asian continent, because of its size and diversity, may be affected significantly by the consequences of climate change, and its new status as a “hub” of livestock production gives it an important role in mitigating possible impacts of climate variability on animal health. Animal health may be affected by climate change in four ways: heat-related diseases and stress, extreme weather events, adaptation of animal production systems to new environments, and emergence or re-emergence of infectious diseases, especially vector-borne diseases critically dependent on environmental and climatic conditions. To face these new menaces, the need for strong and efficient veterinary services is irrefutable, combined with good coordination of public health services, as many emerging human diseases are zoonoses. Asian developing countries have acute weaknesses in their veterinary services, which jeopardises the global surveillance network essential for early detection of hazards. Indeed, international cooperation within and outside Asia is vital to mitigating the risks of climate change to animal health in Asia.

14555. **Gage, K. L., Burkot, T. R., Eisen, R. J. & Hayes, E. B., 2008**. Climate and vector-borne diseases. *American Journal of Preventive Medicine,* **35** (5): 436-450.

Division of Vector-Borne Infectious Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, CDC, Atlanta, Georgia, USA. [kgage@cdc.gov].

Climate change could significantly affect vector borne disease in humans. Temperature, precipitation, humidity, and other climatic factors are known to affect the reproduction, development, behaviour, and population dynamics of the arthropod vectors of these diseases. Climate also can affect the development of pathogens in vectors, as well as the population dynamics and ranges of the non-human vertebrate reservoirs of many vector-borne diseases. Whether climate changes increase or decrease the incidence of vector-borne diseases in humans will depend not only on the actual climatic conditions but also on local non-climatic epidemiologic and ecologic factors. Predicting the relative impact of sustained climate

change on vector-borne diseases is difficult and will require long-term studies that look not only at the effects of climate change but also at the contributions of other agents of global change such as increased trade and travel, demographic shifts, civil unrest, changes in land use, water availability, and other issues. Adapting to the effects of climate change will require the development of adequate response plans, enhancement of surveillance systems, and development of effective and locally appropriate strategies to control and prevent vector-borne diseases.

14556. **Gould, M. K., Vu, X. L., Seebeck, T. & de Koning, H. P., 2008**. Propidium iodide-based methods for monitoring drug action in the kinetoplastidae: comparison with the Alamar Blue assay. *Analytical Biochemistry,* **382** (2): 87-93.

Institute of Biomedical and Life Sciences, Division of Infection and Immunity, University of Glasgow, 120 University Place, Glasgow G128TA, UK.

The urgent need for new drug development for African trypanosomiasis is widely recognized. This requires reliable and informative high-throughput assays. Currently, drug action is determined with a fluorimetric/colorimetric assay based on the metabolism of the dye Alamar Blue (resazurin) by live cells. However, this assay does not easily distinguish between cell death and growth arrest, nor supply information about the rate at which test compounds affect these parameters. We report here an alternative fluorimetric assay, based on the interaction of propidium iodide with DNA, that allows either real-time monitoring of cell viability or the generation of EC(50) values at a predetermined time-point. The assay is highly sensitive and fluorescence readings easily correlate to numbers of parasites or DNA content. The EC(50) values were highly similar to those obtained with the standard Alamar Blue assay. The procedure lends itself readily to applications in drug development or resistance monitoring.

14557. **Grant, K. M., 2008**. Targeting the cell cycle in the pursuit of novel chemotherapies against parasitic protozoa. *Current Pharmaceutical Design,* **14** (9): 917-924.

Department of Medicine, Lancaster University, Lancaster, UK. [k.grant1@lancaster.ac.uk].

Protozoan parasites, such as those responsible for malaria and African sleeping sickness, represent a huge burden to the developing world. Current chemotherapy to combat these diseases is inadequate: antiquated, toxic and increasingly ineffective due to drug resistance. In this article, the potential usefulness of targeting key regulators of the parasite cell cycle is discussed, paying particular attention to three families of protein kinases: cyclin-dependent kinases, glycogen synthase kinases and Aurora kinases. This review outlines their identification, which has been greatly accelerated by the availability of parasite genome data, their validation as *bona fide* regulators of the parasite cell cycle and current data on the availability and anti-parasite activity of inhibitors.

14558. **Hosack, G. R., Rossignol, P. A. & van den Driessche, P., 2008**. The control of vector-borne disease epidemics. *Journal of Theoretical Biology,* **255** (1): 16-25.

Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, OR 97331-3803, USA. [Geoff.Hosack@oregonstate.edu].

The theoretical underpinning of our struggle with vector-borne disease, and still our strongest tool, remains the basic reproduction number, R (0), the measure of long term endemicity. Despite its widespread application, R (0) does not address the dynamics of epidemics in a model that has an endemic equilibrium. We use the concept of reactivity to derive a threshold index for epidemicity, E (0), which gives the maximum number of new infections produced by an infective individual at a disease-free equilibrium. This index describes the transitory behaviour of disease following a temporary perturbation in prevalence. We demonstrate that if the threshold for epidemicity is surpassed, then an epidemic peak can occur, that is, prevalence can increase further, even when the disease is not endemic and so dies out. The relative influence of parameters on E (0) and R (0) may differ and lead to different strategies for control. We apply this new threshold index for epidemicity to models of vector-borne disease because these models have a long history of mathematical analysis and application. We find that both the transmission efficiency from hosts to vectors and the vector-host ratio may have a stronger effect on epidemicity than endemicity. The duration of the extrinsic incubation period required by the pathogen to transform an infected vector to an infectious vector, however, may have a stronger effect on endemicity than epidemicity. We use the index E (0) to examine how vector behaviour affects epidemicity. We find that parasite modified behaviour, feeding bias by vectors for infected hosts, and heterogeneous host attractiveness contribute significantly to transitory epidemics. We anticipate that the epidemicity index will lead to a re-evaluation of control strategies for vector-borne disease and be applicable to other disease transmission models.

14559. **Kennedy, P. G., 2008**. The continuing problem of human African trypanosomiasis (sleeping sickness). *Annals of Neurology,* **64** (2): 116-126.

Department of Neurology, Division of Clinical Neurosciences, Faculty of Medicine, University of Glasgow Institute of Neurological Sciences, Southern General Hospital, Glasgow, Scotland, UK. [p.g.kennedy@clinmed.gla.ac.uk].

Human African trypanosomiasis, also known as sleeping sickness, is a neglected disease, and it continues to pose a major threat to 60 million people in 36 countries in sub-Saharan Africa. Transmitted by the bite of the tsetse fly, the disease is caused by protozoan parasites of the genus *Trypanosoma* and comes in two types: East African human African trypanosomiasis caused by *Trypanosoma brucei rhodesiense* and the West African form caused by *Trypanosoma brucei gambiense*. There is an early or haemolymphatic stage and a late or encephalitic stage, when the parasites cross the blood-brain barrier to invade the central nervous system. Two critical current issues are disease staging and drug therapy, especially for late-stage disease. Lumbar puncture to analyze cerebrospinal fluid will remain the only method of disease staging until reliable non-invasive methods are developed, but there is no widespread consensus as to what exactly defines biologically central nervous system disease or what specific cerebrospinal fluid findings should justify drug therapy for late-stage involvement. All four main drugs used for human African trypanosomiasis are

toxic, and melarsoprol, the only drug that is effective for both types of central nervous system disease, is so toxic that it kills 5 percent of patients who receive it. Eflornithine, alone or combined with nifurtimox, is being used increasingly as first-line therapy for *gambiense* disease. There is a pressing need for an effective, safe oral drug for both stages of the disease, but this will require a significant increase in investment for new drug discovery from Western governments and the pharmaceutical industry.

14560. **Lozano-Fuentes, S., Elizondo-Quiroga, D., Farfan-Ale, J. A., Lorono-Pino, M. A., Garcia-Rejon, J., Gomez-Carro, S., Lira-Zumbardo, V., Najera-Vazquez, R., Fernandez-Salas, I., Calderon-Martinez, J., Dominguez-Galera, M., Mis-Avila, P., Morris, N., Coleman, M., Moore, C. G., Beaty, B. J. & Eisen, L., 2008.** Use of Google Earth to strengthen public health capacity and facilitate management of vector-borne diseases in resource-poor environments. *Bulletin of the World Health Organization,* **86** (9): 718-725.

Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO., USA.

Novel, inexpensive solutions are needed for improved management of vector-borne and other diseases in resource-poor environments. Emerging free software providing access to satellite imagery and simple editing tools (e.g. Google Earth) complement existing geographic information system (GIS) software and provide new opportunities for: (i) strengthening overall public health capacity through development of information for city infrastructures; and (ii) display of public health data directly on an image of the physical environment. We used freely accessible satellite imagery and a set of feature-making tools included in the software (allowing for production of polygons, lines and points) to generate information for city infrastructure and to display disease data in a dengue decision support system (DDSS) framework. Two cities in Mexico (Chetumal and Merida) were used to demonstrate that a basic representation of city infrastructure useful as a spatial backbone in a DDSS can be rapidly developed at minimal cost. Data layers generated included labelled polygons representing city blocks, lines representing streets, and points showing the locations of schools and health clinics. City blocks were colour-coded to show presence of dengue cases. The data layers were successfully imported in a format known as shapefile into a GIS software. It is concluded that the combination of Google Earth and free GIS software (e.g. HealthMapper, developed by WHO, and SIGEpi, developed by PAHO) has tremendous potential to strengthen overall public health capacity and facilitate decision support system approaches to prevention and control of vector-borne diseases in resource-poor environments.

14561 **Martinez-Girón, R., Esteban, J. G., Ribas, A. & Doganci, L., 2008**. Protozoa in respiratory pathology: A review. *European Respiratory Journal,* **32** (5): 1354-1370.

Protozoal Respiratory Pathology Research Unit, Fundacion INCLINICA, Anatomical Pathology Service, Hospital Universitario Central de Asturias, Oviedo, Spain. [rmartinezgiron@hotmail.com].

Among the microorganisms that may affect the respiratory apparatus are the protozoa. The diseases they may give rise to constitute a relatively uncommon group of respiratory

ailments with, in the majority of cases, an underlying clinical situation corresponding to states of suppressed immunity (AIDS, transplants, malign haemopathies, corticotherapy, etc.). Other factors, such as visits to endemic areas and immigration, also have to be taken into account. In view of the probable increase in the number of cases and the appearance of new emerging diseases, it is the intention of the present work to review the publications available, in different fields of medicine, that refer to the principal kinds of protozoa (*Entamoeba, Acanthamoeba, Balamuthia, Leishmania, Trypanosoma, Trichomonas, Lophomonas, Cryptosporidium, Cyclospora, Toxoplasma, Plasmodium, Babesia, Encephalitozoon, Enterocytozoon* and *Balantidium*) and, at the same time, detail and comment on the latest findings on this subject.

14562 **McCoy, K. D., 2008**. The population genetic structure of vectors and our understanding of disease epidemiology. *Parasite,* **15** (3): 444-448.

Génétique et Evolution des Maladies Infectieuses, UMR 2724 CNRS-IRD, Centre IRD, 911, avenue Agropolis, BP 64501, F-34394 Montpellier, France. [mccoy@mpl.ird.fr].

Understanding and predicting disease epidemiology relies on clear knowledge about the basic biology of the organisms involved. Despite the key role that arthropod vectors play in disease dynamics and detailed mechanistic work on the vector-pathogen interface, little information is often available about how these populations function under natural conditions. Population genetic studies can help fill this void by providing information about the taxonomic status of species, the spatial limits of populations, and the nature of gene flow among populations. Here, the different types of population genetic structure and some recent examples of where this information has provided key elements for understanding pathogen transmission in tick-borne systems are reviewed.

14563. **Monzote, L., 2008**. A review of anti-parasitic patents (1988-2008). *Recent Patents on Anti-Infective Drug Discovery,* **3** (3): 177-191.

Parasitology Department, Institute of Tropical Medicine "Pedro Kouri", Havana City, Cuba. [monzote@ipk.sld.cu.].

New anti-parasitic drugs are urgently needed to treat and control diseases such as malaria, leishmaniasis, sleeping sickness, filariasis and schistosomiasis, which affect millions of people each year. In this review, we are focusing on patents of anti-parasitic agents that have been published during the last 20 years. The data collected demonstrate that the number of patents concerning this issue has been increasing. In addition, the reports for antiprotozoan compounds are more numerous when compared with anthelminths drugs. The synthetic products were the most patented, followed by natural ones and combinations of existing drugs. The discovery of new antiparasitic drugs to obtain solutions for millions of people who suffer and die due to parasitic diseases is an urgent need.

14564. **Muskavitch, M. A., Barteneva, N. & Gubbels, M. J., 2008**. Chemogenomics and parasitology: small molecules and cell-based assays to study infectious processes. *Combinatorial Chemistry and High Throughput Screening,* **11** (8): 624-646.

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

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

14565. **Pepin, J. & Labbe, A. C., 2008**. Noble goals, unforeseen consequences: control of tropical diseases in colonial Central Africa and the iatrogenic transmission of blood-borne viruses. *Tropical Medicine and International Health,* **13** (6): 744-753.

Department of Microbiology and Infectious Diseases, University of Sherbrooke, Canada. [jacques.pepin@usherbrooke.ca].

In southern Cameroon, 40 percent-50 percent of individuals born before 1945 have antibodies against hepatitis C virus (HCV), suggesting massive iatrogenic transmission of at least one blood-borne virus in the region of the world where SIV(cpz) emerged into HIV-1. To estimate the potential role of disease control programmes that used intravenous (IV) drugs in the transmission of blood-borne viruses, especially HCV, we reviewed the records of health services in Cameroun, Oubangui-Chari, Gabon and Moyen-Congo between 1921 and 1959. We calculated the incidence of diseases whose treatment required the administration of IV drugs, and compared these with previously published data on HCV prevalence. The results showed that several IV drugs were used against African trypanosomiasis, leprosy, yaws and syphilis. However, yaws was the only disease whose incidence was high enough so that up to half of some birth cohorts could have acquired HCV. Yaws incidence varied dramatically between regions, and was often >200 per 1 000 per year in southern Cameroon, where extremely high HCV prevalence was found. Yaws incidence peaked between 1935 and

1955, a period which coincided with the emergence of HCV and HIV. In conclusion, age, geographical and temporal distributions of yaws suggest that the HCV epidemic in Cameroon was driven by campaigns against yaws (and, secondarily, syphilis) using arsenicals and other metallic drugs. The same interventions may have exponentially amplified other blood-borne viruses, including SIV (cpz)/HIV-1.

14566. **Pinto, J., Bonacic, C., Hamilton-West, C., Romero, J. & Lubroth, J., 2008**. Climate change and animal diseases in South America. *Revue scientifique et technique,* **27** (2): 599-613.

Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases/ Global Early Warning and Response System (EMPRES/GLEWS), Food and Agriculture Organization of the United Nations, Rome, Italy.

Climate strongly affects agriculture and livestock production and influences animal diseases, vectors and pathogens, and their habitat. Global warming trends predicted in the 2007 Intergovernmental Panel on Climatic Change (IPCC) report for South America are likely to change the temporal and geographical distribution of infectious diseases, including those that are vector-borne such as bluetongue, West Nile fever, vesicular stomatitis and New World screwworm. Changes in distribution will be partially modulated by El Niño Southern Oscillation events, which will become more frequent and lead to a greater frequency of droughts and floods. Active disease surveillance for animal diseases in South America, particularly for vector-borne diseases, is very poor. Disease reporting is often lacking, which affects knowledge of disease distribution and impact, and preparedness for early response. Improved reporting for animal diseases that may be affected by climate change is needed for better prevention and intervention measures in susceptible livestock, wildlife and vectors in South America. This requires contributions from multidisciplinary experts, including meteorologists, epidemiologists, biologists and ecologists, and from local communities.

14567. **Rasgon, J. L., 2008**. Using predictive models to optimize *Wolbachia*-based strategies for vector-borne disease control. *Advances in Experimental Medicine and Biology,* **627**: 114-125.

W. Harry Feinstone Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland 21205, USA. [jrasgon@jhsph.edu].

The development of resistance to insecticides by vector arthropods, the evolution of resistance to chemotherapeutic agents by parasites and the lack of clinical cures or vaccines for many diseases have stimulated a high-profile effort to develop vector-borne disease control strategies based on release of genetically modified mosquitoes. Because transgenic insects are likely to be less fit than their wild-type counterparts, transgenic traits must be actively driven into the population in spite of fitness costs (population replacement). *Wolbachia* are maternally-inherited symbionts that are associated with numerous alterations in host reproductive biology. By a variety of mechanisms, *Wolbachia*-infected females have a reproductive advantage relative to uninfected females, allowing infection to spread rapidly through host populations to high frequency in spite of fitness costs. In theory, *Wolbachia* can be exploited to drive costly transgenes into vector populations for disease control. Before

conducting an actual release, it is important to be able to predict how released *Wolbachia* infected females are expected to behave. While inferences can be made by observing the dynamics of naturally-occurring infections, there is no ideal way to empirically test the efficacy of a *Wolbachi*a gene driver under field conditions prior to the first actual release. Mathematical models are a powerful way to predict the outcomes of transgenic insect releases and allow one to identify knowledge gaps, identify parameters that are critical to the success of releases, conduct risk-assessment analysis and investigate worst-case scenarios, and ultimately identify the most effective, most logistically feasible control method or methods. In this chapter, current and historical advances in applied models of *Wolbachia* spread are reviewed, specifically within the context of applied population replacement strategies for vector-borne disease control.

14568. **Rasgon, J. L., 2008**. Stable isotope analysis can potentially identify completely-digested blood meals in mosquitoes. *PLoS ONE,* **3** (5): e2198.

The W. Harry Feinstone Department of Molecular Microbiology and Immunology, and the Johns Hopkins Malaria Research Institute, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA. [jrasgon@jhsph.edu].

Vertebrate blood feeding is a critical component of a mosquito's ability to transmit pathogens that cause diseases such as malaria, dengue fever and viral encephalitis. Due to degradation by the digestive process, current methods to identify mosquito blood meal sources are only useful for approximately 36 hours post-feeding. A critical need exists for technologies to extend this window and gain a more complete picture of mosquito feeding behaviour for epidemiological studies. Stable isotopes are useful for investigating organism feeding behaviour because the isotopic ratio of an organism's tissues reflects that of the material it ingests. Proof-of-principle data indicates that after blood feeding, *Aedes albopictus* mosquitoes acquire diagnostic Carbon and Nitrogen stable isotope profiles from their vertebrate hosts that can be accurately identified one week post-feeding, approximately 4 days after the entire blood meal has been digested. Total C/N ratio served as a biomarker marker for blood feeding ($P<0.02$), while delta N was the most informative variable which could distinguish between unfed, chicken-fed and human-fed mosquitoes ($P<0.01$). By plotting C/N vs. delta N, all feeding treatments could be identified in a double-blind analysis. These proof-of-principle experiments indicate that analysis of stable isotopes can be used to distinguish blood-fed from un-fed mosquitoes, and also distinguish between different vertebrate blood meal sources even after all blood has been digested. The development of stable isotope-based assays for mosquito blood meal identification may be a powerful tool to investigate mosquito feeding ecology and the dynamics of vector-borne pathogens.

14569. **Roche, B., Guegan, J. F. & Bousquet, F., 2008**. Multi-agent systems in epidemiology: a first step for computational biology in the study of vector-borne disease transmission. *BMC Bioinformatics,* **9** (1): 435.

UMR 2724 Génétique et Evolution des Maladies Infectieuses, IRD-CNRS-Université de Montpellier I, Montpellier, France, French Center on Globalization and Infectious Diseases, French School of Public Health,

Montpellier, France, and Centre International de Recherche en Agronomie pour le Développement, Montpellier, France [roche@mpl.ird.fr].

Computational biology is often associated with genetic or genomic studies only. However, thanks to the increase in computational resources, computational models are appreciated as useful tools in many other scientific fields. Such modelling systems are particularly relevant for the study of complex systems, like the epidemiology of emerging infectious diseases. So far, mathematical models remain the main tool for the epidemiological and ecological analysis of infectious diseases, with SIR models could be seen as an implicit standard in epidemiology. Unfortunately, these models are based on differential equations and, therefore, can become very rapidly unmanageable due to the too many parameters which need to be taken into consideration. For instance, in the case of zoonotic and vector-borne diseases in wildlife many different potential host species could be involved in the life-cycle of disease transmission, and SIR models might not be the most suitable tool to truly capture the overall disease circulation within that environment. This limitation underlines the necessity to develop a standard spatial model that can cope with the transmission of disease in realistic ecosystems. Computational biology may prove to be flexible enough to take into account the natural complexity observed in both natural and man-made ecosystems. In this paper, we propose a new computational model to study the transmission of infectious diseases in a spatially explicit context. We developed a multi-agent system model for vector-borne disease transmission in a realistic spatial environment. Here we describe in detail the general behaviour of this model that hopefully will become a standard reference for the study of vector-borne disease transmission in wildlife. We show how this simple model could be easily adapted and modified for use as a common framework for further research developments in this field.

14570. **Van den Bossche, P. & Coetzer, J. A., 2008**. Climate change and animal health in Africa. *Revue scientifique et technique,* **27** (2): 551-562.

Institute of Tropical Medicine, Animal Health Department, Antwerp, Belgium.

Climate change is expected to have direct and indirect impacts on African livestock. Direct impacts include increased ambient temperature, floods and droughts. Indirect impacts are the result of reduced availability of water and forage and changes in the environment that promote the spread of contagious diseases through increased contact between animals, or increased survival or availability of the agent or its intermediate host. The distribution and prevalence of vector-borne diseases may be the most significant effect of climate change. The potential vulnerability of the livestock industry will depend on its ability to adapt to such changes. Enhancing this adaptive capacity presents a practical way of coping with climate change. Adaptive capacity could be increased by enabling the African livestock owner to cope better with animal health problems through appropriate policy measures and institutional support. Developing an effective and sustainable animal health service, associated surveillance and emergency preparedness systems and sustainable disease control and prevention programmes is perhaps the most important strategy for dealing with climate change in many African countries.

14571 **Volfova, V., Hostomska, J., Cerny, M., Votypka, J. & Volf, P., 2008**. Hyaluronidase of bloodsucking insects and its enhancing effect on *Leishmania* infection in mice. *PLoS Neglected Tropical Diseases,* **2** (9): e294.

Department of Parasitology, Faculty of Science, Charles University in Prague, Czech Republic. [volf@cesnet.cz].

Salivary hyaluronidases have been described in a few bloodsucking arthropods. However, very little is known about the presence of this enzyme in various bloodsucking insects and no data are available on its effect on transmitted microorganisms. Here, we studied hyaluronidase activity in thirteen bloodsucking insects belonging to four different orders. In addition, we assessed the effect of hyaluronidase co-inoculation on the outcome of *Leishmania major* infection in BALB/c mice. High hyaluronidase activity was detected in several Diptera tested, namely deer fly *Chrysops viduatus*, blackflies *Odagmia ornata* and *Eusimilium latipes*, mosquito *Culex quinquefasciatus*, biting midge *Culicoides kibunensis* and sand fly *Phlebotomus papatasi*. Lower activity was detected in cat flea *Ctenocephalides felis*. No activity was found in kissing bug *Rhodnius prolixus*, mosquitoes *Anopheles stephensi* and *Aedes aegypti*, tsetse fly *Glossina fuscipes*, stable fly *Stomoxys calcitrans* and human louse *Pediculus humanus*. Hyaluronidases of different insects vary substantially in their molecular weight, the structure of the molecule and the sensitivity to reducing conditions or sodium dodecyl sulphate. Hyaluronidase exacerbates skin lesions caused by *Leishmania major*; more severe lesions developed in mice where *L. major* promastigotes were co-injected with hyaluronidase. It appears that high hyaluronidase activities seem to be essential for insects with pool-feeding mode, where they facilitate the enlargement of the feeding lesion and serve as a spreading factor for other pharmacologically active compounds present in saliva. As this enzyme is present in all *Phlebotomus* and *Lutzomyia* species studied to date, it seems to be one of the factors responsible for enhancing activity present in sand fly saliva. We propose that salivary hyaluronidase may facilitate the spread of other vector-borne microorganisms, especially those transmitted by insects with high hyaluronidase activity, namely blackflies (Simuliidae), biting midges (Ceratopogonidae) and horse flies (Tabanidae).

14572. **Wang, X., Jobe, M., Tyler, K. M. & Steverding, D., 2008.** Efficacy of common laboratory disinfectants and heat on killing trypanosomatid parasites. *Parasite Vectors*, **1** (1): 35.

BioMedical Research Centre, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, NR4 7TJ, UK. [dsteverding@hotmail.com].

The disinfectants TriGene, bleach, ethanol and liquid hand soap, and water and temperature were tested for their ability to kill bloodstream forms of *Trypanosoma brucei*, epimastigotes of *Trypanosoma rangeli* and promastigotes of *Leishmania major*. A five minute exposure to 0.2 percent TriGene, 0.1 percent liquid hand soap and 0.05 percent bleach (0.05 percent NaOCl) killed all three trypanosomatids. Ethanol and water destroyed the parasites within 5 min at concentrations of 15 percent-17.5 percent and 80 percent-90 percent, respectively. All three organisms were also killed when treated for five minutes at 50 ^{o}C. The results indicate that the disinfectants, water and temperature treatment (i.e. autoclaving) are suitable laboratory hygiene measures against trypanosomatid parasites.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

14573. **Attardo, G. M., Lohs, C., Heddi, A., Alam, U. H., Yildirim, S. & Aksoy, S., 2008**. Analysis of milk gland structure and function in *Glossina morsitans*: Milk protein production, symbiont populations and fecundity. *Journal of Insect Physiology,* **54** (8): 1236-1242.

Yale School of Public Health, Department of Epidemiology and Microbial Diseases, 60 College Street, 606 LEPH New Haven, CT 06510, USA. [serap.aksoy@yale.edu].

A key process in the tsetse reproductive cycle is the transfer of essential nutrients and bacterial symbionts from mother to intrauterine offspring. The tissue mediating this transfer is the milk gland. This work focuses upon the localization and function of two milk proteins (milk gland protein (*Gmm*MGP) and transferrin (*Gmm*Tsf)) and the tsetse endosymbionts (*Sodalis* and *Wigglesworthia*), in the context of milk gland physiology. Fluorescent *in situ* hybridization (FISH) and immunohistochemical analysis confirm that the milk gland secretory cells synthesize and secrete milk gland protein and transferrin. Knockdown of *Gmm*MGP by double stranded RNA (dsRNA) mediated RNA interference results in reduction of tsetse fecundity, demonstrating its functional importance in larval nutrition and development. Bacterial species-specific *in situ* hybridizations of milk gland sections reveal large numbers of *Sodalis* and *Wigglesworthia* within the lumen of the milk gland. *Sodalis* is also localized within the cytoplasm of the secretory cells. Within the lumen, *Wigglesworthia* localize close to the channels leading to the milk storage reservoir of the milk gland secretory cells. We discuss the significance of the milk gland in larval nutrition and in transmission of symbiotic bacteria to developing offspring.

14574. **Krafsur, E. S., 2008**. Tsetse flies: Genetics, evolution, and role as vectors. *Infection, Genetics and Evolution.* **Available online 17 October 2008**

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

Tsetse flies (Diptera: Glossinidae) are an ancient taxon of one genus, *Glossina*, and limited species diversity. All are exclusively haematophagous and confined to sub-Saharan Africa. The *Glossina* are the principal vectors of African trypanosomes *Trypanosoma* sp. (Kinetoplastida: Trypanosomatidae) and as such, are of great medical and economic importance. Clearly tsetse flies and trypanosomes are co-adapted and evolutionary interactions between them are manifest. Numerous clonally reproducing strains of *Trypanosoma* sp. exist and their genetic diversities and spatial distributions are inadequately known. Here the breeding structures of the principal trypanosome vectors, *G. morsitans s.l., G. pallidipes, G. palpalis s.l.* and *G. fuscipes fuscipes* are reviewed. All show highly structured populations among which there is surprisingly little detectable gene flow. Rather less is known of the breeding structure of *T. brucei* vis-a-vis their vector tsetse flies but many

genetically differentiated strains exist in nature. Genetic recombination in *Trypanosoma* via meiosis has recently been demonstrated in the laboratory thereby furnishing a mechanism of strain differentiation in addition to that of simple mutation. Conducting spatially and genetically representative sampling of both trypanosome species and strains and their *Glossina* vectors is a major barrier to a comprehensive understanding of their mutual relationships.

14575. **Pais, R., Lohs, C., Wu, Y., Wang, J. & Aksoy, S., 2008**. The obligate mutualist *Wigglesworthia glossinidia* influences reproduction, digestion, and immunity processes of its host, the tsetse fly. *Applied and Environmental Microbiology,* **74** (19): 5965-5974.

Yale School of Public Heath, Department of Epidemiology of Microbial Diseases, New Haven, CT 06520, USA. [serap.aksoy@yale.edu].

Tsetse flies (Diptera: Glossinidae) are vectors for trypanosome parasites, the agents of the deadly sleeping sickness disease in Africa. Tsetse flies also harbour two maternally transmitted enteric mutualist endosymbionts: the primary intracellular obligate *Wigglesworthia glossinidia* and the secondary commensal *Sodalis glossinidius*. Both endosymbionts are transmitted to the intrauterine progeny through the milk gland secretions of the viviparous female. We administered various antibiotics either continuously by *per os* supplementation of the host blood meal diet or discretely by haemocoelic injections into fertile females in an effort to selectively eliminate the symbionts to study their individual functions. A symbiont-specific PCR amplification assay and fluorescence *in situ* hybridization analysis were used to evaluate symbiont infection outcomes. Tetracycline and rifampin treatments eliminated all tsetse symbionts but reduced the fecundity of the treated females. Ampicillin treatments did not affect the intracellular *Wigglesworthia* localized in the bacteriome organ and retained female fecundity. The resulting progeny of ampicillin-treated females, however, lacked *Wigglesworthia* but still harboured the commensal *Sodalis*. Our results confirm the presence of two physiologically distinct *Wigglesworthia* populations: the bacteriome-localized *Wigglesworthia* involved with nutritional symbiosis and free-living *Wigglesworthia* in the milk gland organ responsible for maternal transmission to the progeny. We evaluated the reproductive fitness, longevity, digestion, and vectorial competence of flies that were devoid of *Wigglesworthia*. The absence of *Wigglesworthia* completely abolished the fertility of females but not that of males. Both the male and female *Wigglesworthia*-free adult progeny displayed longevity costs and were significantly compromised in their blood meal digestion ability. Finally, while the vectorial competence of the young newly hatched adults without *Wigglesworthia* was comparable to that of their wild-type counterparts, older flies displayed higher susceptibility to trypanosome infections, indicating a role for the mutualistic symbiosis in host immunobiology. The ability to rear adult tsetse that lack the obligate *Wigglesworthia* endosymbionts will now enable functional investigations into this ancient symbiosis.

14576. **Pontes, M. H., Babst, M., Lochhead, R., Oakeson, K., Smith, K. & Dale, C., 2008**. Quorum sensing primes the oxidative stress response in the insect endosymbiont, *Sodalis glossinidius*. *PLoS ONE,* **3** (10): e3541.

Department of Biology, University of Utah, Salt Lake City, Utah, USA. [pontes.biology@utah.edu].

Sodalis glossinidius, a maternally transmitted bacterial endosymbiont of tsetse flies (*Glossina* spp.), uses an acylated homoserine lactone (AHL)-based quorum sensing system to modulate gene expression in accordance with bacterial cell density. The *S. glossinidius* quorum sensing system relies on the function of two regulatory proteins; SogI (a LuxI homologue) synthesizes a signalling molecule, characterized as N-(3-oxohexanoyl) homoserine lactone (OHHL), and SogR1 (a LuxR homologue) interacts with OHHL to modulate transcription of specific target genes. We used a tiling microarray to analyze the *S. glossinidius* transcriptome in the presence and absence of exogenous OHHL. The major finding is that OHHL increases transcription of a large number of genes that are known to be involved in the oxidative stress response. We also show that the obligate symbiont of the rice weevil, *Sitophilus oryzae* (SOPE), maintains copies of the quorum sensing regulatory genes that are found in *S. glossinidius*. Molecular evolutionary analyses indicate that these sequences are evolving under stabilizing selection, consistent with the maintenance of their functions in the SOPE symbiosis. Finally, the expression studies in *S. glossinidius* also reveal that quorum sensing regulates the expression of a cryptic, degenerate gene (carA) that arose from an ancient deletion in the last common ancestor of *S. glossinidius* and SOPE. This oxidative stress response is likely mandated under conditions of dense intracellular symbiont infection, when intense metabolic activity is expected to generate a heavy oxidative burden. Such conditions are known to arise in the bacteriocytes of grain weevils, which harbour dense intracellular infections of symbiotic bacteria that are closely related to *S. glossinidius*. The presence of a degenerate carA sequence in *S. glossinidius* and SOPE indicates the potential for neofunctionalization to occur during the process of genome degeneration.

14577. **Tuck, E. J., Windmill, J. F. & Robert, D., 2008**. Hearing in tsetse flies? Morphology and mechanics of a putative auditory organ. *Bulletin of Entomological Research.* **Published online 28 October 2008.**

School of Biological Sciences, Woodland Road, University of Bristol, Bristol, UK. [D.Robert@bristol.ac.uk].

Tympanal hearing organs are widely used by insects to detect sound pressure. Such ears are relatively uncommon in the order Diptera, having only been reported in two families thus far. This study describes the general anatomical organization and experimentally examines the mechanical resonant properties of an unusual membranous structure situated on the ventral prothorax of the tsetse fly, *Glossina morsitans* (Diptera: Glossinidae). Anatomically, the prosternal membrane is backed by an air-filled chamber and attaches to a pair of sensory chordotonal organs. Mechanically, the membrane shows a broad resonance around 5.3-7.2 kHz. Unlike previously reported dipteran tympana, a directional response to sound was not found in *G. morsitans*. Collectively, the morphology, the resonant properties and acoustic sensitivity of the tsetse prothorax are consistent with those of the tympanal hearing organs in *Ormia* sp. and *Emblemasoma* sp. (Tachinidae and Sarcophagidae). The production of sound by several species of tsetse flies has been repeatedly documented. Yet, clear behavioural evidence for acoustic behaviour is sparse and inconclusive. Together with sound production, the presence of an ear-like structure raises the enticing possibility of auditory communication in tsetse flies and renews interest in the sensory biology of these medically important insects.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

14578. **Abila, P. P., Slotman, M. A., Parmakelis, A., Dion, K. B., Robinson, A. S., Muwanika, V. B., Enyaru, J. C., Lokedi, L. M., Aksoy, S. & Caccone, A., 2008.** High levels of genetic differentiation between Ugandan *Glossina fuscipes fuscipes* populations separated by Lake Kyoga. *PLoS Neglected Tropical Diseases,* **2** (5): e242.

National Livestock Resources Research Institute, Tororo, Uganda.

Glossina fuscipes fuscipes is the major vector of human African trypanosomiasis, commonly referred to as sleeping sickness, in Uganda. In western and eastern Africa, the disease has distinct clinical manifestations and is caused by two different parasites: *Trypanosoma brucei rhodesiense* and *T. b. gambiense*. Uganda is exceptional in that it harbours both parasites, which are separated by a narrow 160-km belt. This separation is puzzling considering there are no restrictions on the movement of people and animals across this region. We investigated whether genetic heterogeneity of *G. f. fuscipes* vector populations can provide an explanation for this disjunct distribution of the *Trypanosoma* parasites. Therefore, we examined genetic structuring of *G. f. fuscipes* populations across Uganda using newly developed microsatellite markers, as well as mtDNA. Our data show that *G. f. fuscipes* populations are highly structured, with two clearly defined clusters that are separated by Lake Kyoga, located in central Uganda. Interestingly, we did not find a correlation between genetic heterogeneity and the type of *Trypanosoma* parasite transmitted. The lack of a correlation between genetic structuring of *G. f. fuscipes* populations and the distribution of *T. b. gambiense* and *T. b. rhodesiense* indicates that it is unlikely that genetic heterogeneity of *G. f. fuscipes* populations explains the disjunct distribution of the parasites. These results have important epidemiological implications, suggesting that a fusion of the two disease distributions is unlikely to be prevented by an incompatibility between vector populations and parasite.

14579. **Dyer, N. A., Lawton, S. P., Ravel, S., Choi, K. S., Lehane, M. J., Robinson, A. S., Okedi, L. M., Hall, M. J., Solano, P. & Donnelly, M. J., 2008.** Molecular phylogenetics of tsetse flies (Diptera: *Glossinidae*) based on mitochondrial (COI, 16S, ND2) and nuclear ribosomal DNA sequences, with an emphasis on the *palpalis* group. *Molecular Phylogenetics and Evolution,* **49** (1): 227-239.

Vector Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, Merseyside, L3 5QA, UK. [Naomi.Dyer@liv.ac.uk].

Relationships of 13 species of the genus *Glossina* (tsetse flies) were inferred from mitochondrial (cytochrome oxidase 1, NADH dehydrogenase 2 and 16S) and nuclear (internal transcribed spacer 1 of rDNA) sequences. The resulting phylogeny confirms the monophyly of the morphologically defined *fusca, morsitans* and *palpalis* subgenera. Genetic distances between *palpalis* and *morsitans* subspecies suggest that their status needs revision. In particular, cytochrome oxidase 1 sequences showed large geographical differences within *G. palpalis palpalis*, suggesting the existence of cryptic species within this subspecies. The

morphology of *palpalis* group female genital plates was examined, and individuals were found varying outside the ranges specified by the standard identification keys, making definitive morphological classification impossible. A diagnostic PCR to distinguish *G. palpalis palpalis*, *G. tachinoides* and *G. palpalis gambiensis* based on length differences of internal transcribed spacer 1 sequences is presented.

14580. **Krafsur, E. S., Marquez, J. G. & Ouma, J. O., 2008**. Structure of some East African *Glossina fuscipes fuscipes* populations. *Medical and Veterinary Entomology,* **22** (3): 222-227.

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

Glossina fuscipes fuscipes Newstead 1910 (Diptera: Glossinidae) is the primary vector of human sleeping sickness in Kenya and Uganda. This is the first report on its population structure. A total of 688 nucleotides of mitochondrial ribosomal 16S2 and cytochrome oxidase I genes were sequenced. Twenty-one variants were scored in 79 flies from three geographically diverse natural populations. Four haplotypes were shared among populations, eight were private and nine were singletons. The mean haplotype and nucleotide diversities were 0.84 and 0.009, respectively. All populations were genetically differentiated and were at demographic equilibrium. In addition, a longstanding laboratory culture originating from the Central African Republic (CAR-lab) in 1986 (or before) was examined. Haplotype and nucleotide diversities in this culture were 0.95 and 0.012, respectively. None of its 27 haplotypes were shared with the East African populations. A first approximation of relative effective population sizes was Uganda > CAR-lab > Kenya. It was concluded that the structure of *G. f. fuscipes* populations in East Africa is localized.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also: **31:** 14539, 14544, 14547, 14557, 14560, 14567]

14581. **Aksoy, S., Weiss, B. & Attardo, G., 2008**. Paratransgenesis applied for control of tsetse transmitted sleeping sickness. *Advances in Experimental Medicine and Biology,* **627**: 35-48.

Yale University School of Medicine, Department of Epidemiology and Public Health, New Haven, CT 06520, USA. [serap.aksoy@yale.edu].

African trypanosomiasis (sleeping sickness) is a major cause of morbidity and mortality in sub Saharan Africa for human and animal health. In the absence of effective vaccines and efficacious drugs, vector control is an alternative intervention tool to break the disease cycle. This paper describes the vectorial and symbiotic biology of tsetse with emphasis on the current knowledge on tsetse symbiont genomics and functional biology, and tsetse's trypanosome transmission capability. The ability to culture one of tsetse's commensal symbiotic microbes, *Sodalis in vitro* has allowed for the development of a genetic transformation system for this organism. Tsetse can be repopulated with the modified *Sodalis* symbiont, which can express foreign gene products (an approach we refer to as

paratransgenic expression system). Expanding knowledge on tsetse immunity effectors, on genomics of tsetse symbionts and on tsetse's parasite transmission biology stands to enhance the development and potential application of paratransgenesis as a new vector control strategy. We describe the hallmarks of the paratransgenic transformation technology where the modified symbionts expressing trypanocidal compounds can be used to manipulate host functions and lead to the control of trypanosomiasis by blocking trypanosome transmission in the tsetse vector.

14582. **Bouyer, J., 2008.** Does isometamidium chloride treatment protect tsetse flies from trypanosome infections during SIT campaigns? *Medical and Veterinary Entomology*, **22** (2): 140-143.

Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Département Systèmes Biologiques, Montpellier, France. [bouyer@cirad.fr]

African animal trypanosomosis is a major pathological constraint to cattle breeding across 10 million km^2 of sub-Saharan West African countries infested by tsetse flies, their cyclic vectors. The release of sterile males (sterile insect technique, SIT) is a potentially important control technique aimed at eliminating the vectors. Prior to release, tsetse are generally treated with isometamidium chloride, a trypanocide, to prevent them from transmitting parasites. The present study investigated the preventive action of isometamidium chloride (0.5 mg/L) on the subsequent susceptibility of tsetse released into the wild. A total of 1 755 *Glossina palpalis gambiensis* Vanderplank and 744 *Glossina tachinoides* Westwood were released, of which 50 and 48, respectively, were recaptured 22-43 days after release. Their probosces were analysed by polymerase chain reaction to identify mature infections with three trypanosome species (*Trypanosoma vivax*, *Trypanosoma brucei* and *Trypanosoma congolense* savannah type). Two mature infections with *T. vivax* and four with *T. congolense* were detected, indicating that the use of this treatment regimen in an SIT campaign would not totally prevent sterile males from transmitting trypanosomes.

14583. **Mugisha, A., McLeod, A., Percy, R. & Kyewalabye, E., 2008**. Socio-economic factors influencing control of vector-borne diseases in the pastoralist system of south western Uganda. *Tropical Animal Health and Production,* **40** (4): 287-297.

Faculty of Veterinary Medicine, Makerere University, Box 7062, Kampala, Uganda. [amugisha@vetmed.mak.ac.ug].

Research in control of tick-borne diseases and trypanosomosis, and their vectors, namely, ticks and tsetse flies respectively, has been on-going for decades. However, very little attention has been paid to the socio-economic factors that are likely to influence the outcome of the interventions to control these diseases. Thus, this study was designed to investigate these factors, mainly the intra-household factors influencing decision-making in the control of vector-borne diseases in the pastoralist areas of Uganda. These factors included: indigenous technical knowledge, household economic factors, and gender. Both qualitative and quantitative methods were used in the collection and analysis of data. The tools used for data collection included among others, participatory learning and action (PLA), and case studies. The findings included the following: In pastoralist households, a big

proportion of the household budget was allocated to vector-borne diseases control. In the male-headed households, men dominated decision-making on vector-borne diseases control, although the goals and priorities of men and women in these households were not the same. Also, vector-borne disease control was predominantly by use of modern veterinary drugs, and pastoralists treated sick cattle by themselves even in situations where there were veterinary personnel.

14584. **Schofield, C. J. & Kabayo, J. P., 2008**. Trypanosomiasis vector control in Africa and Latin America. *Parasite Vectors,* **1** (1): 24.

ECLAT Coordinator, LSHTM, London ,WC1E7HT, UK.
[cj.schofield@lshtm.ac.uk].

Vectors of trypanosomiasis - tsetse (Glossinidae) in Africa, kissing-bugs (Triatominae) in Latin America - are very different insects but share demographic characteristics that render them highly vulnerable to available control methods. For both, the main operational problems relate to re-invasion of treated areas, and the solution seems to be in very large-scale interventions covering biologically-relevant areas rather than adhering to administrative boundaries. In this review we present the underlying rationale, operational background and progress of the various trypanosomiasis vector control initiatives active in both continents.

14585. **Sindato, C., Kimbita, E. N. & Kibona, S. N., 2008**. Factors influencing individual and community participation in the control of tsetse flies and human African trypanosomiasis in Urambo District, Tanzania. *Tanzanian Journal of Health Research,* **10** (1): 20-27.

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

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

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

[See also **31**: 14544, 14553, 14555, 14562, 14565, 14568, 14569, 14701]

14586. **Akoda, K., Harouna, S., Marcotty, T., De Deken, R. & Van den Bossche, P., 2008**. Investigations on the transmissibility of *Trypanosoma congolense* by the tsetse fly *Glossina morsitans morsitans* during its development in a mammalian host. *Acta Tropica,* **107** (1): 17-19.

Department of Animal Health, Institute of Tropical Medicine, Antwerp, Belgium.

Experiments were conducted to investigate the effect of the developmental stage of a monomorphic *T. congolense* IL1180 strain, in a vertebrate host, on its transmissibility by the tsetse fly *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). Batches of 160 male teneral tsetse flies were given a single bloodmeal on mice infected with this *T. congolense* strain 4, 5, 6, 7 or 10 days post-infection. The proportion of infected flies in each of those batches showed that the stage of development of the trypanosome does affect the proportion of flies that develop a mature or immature infection with immature and mature infection rates of flies infected on days 5 or 10 significantly higher. The proportion of infected flies was not affected by the parasitaemia at the moment of infection. Results show that tsetse flies can become infected at any phase of the development of the *T. congolense* IL 1180 strain but the ease with which trypanosomes develop in the fly depends on the phase in the parasite's development in the host. Those observations suggest that in analogy with the pleomorphic *T. brucei* s.l. adaptation of the monomorphic *T. congolense* to development in the fly may also determine the parasite's transmissibility. Moreover, the findings stress the importance of standardising experiments in which the vectorial capacity of tsetse flies is determined and compared.

14587. **Balmer, O. & Caccone, A., 2008**. Multiple-strain infections of *Trypanosoma brucei* across Africa. *Acta Tropica,* **107** (3): 275-279.

Department of Ecology and Evolutionary Biology, Yale University, 165 Prospect Street, New Haven, CT 06511, USA. [oliver.balmer@aya.yale.edu].

It is becoming increasingly clear that parasitic infections frequently contain multiple strains of the same parasite species. This may have important consequences for the parasite dynamics in the host and thus alter disease and transmission dynamics. In *Trypanosoma brucei*, the causal agent of human African trypanosomiasis (sleeping sickness), multiple-strain infections have previously been demonstrated to occur. Here, we analyzed field isolates of *T. b. gambiense*, *T. b. rhodesiense*, and *T. b. brucei*, isolated throughout Africa to assess the commonness of multiple-strain infections across the natural range of this parasite. Using eight highly variable microsatellite loci, we found multiple strains in 8.8 percent of our isolates. Due to the technical challenges of detecting multiple infections this number represents a minimum estimate and the true frequency of multiple-strain infections is likely to

be higher. Multiple-strain infections occurred across the entire East-West range of the parasite. Together with previous results, these findings strongly suggest that multiple-strain infections are common for this parasite and that their consequences for epidemiology and parasite evolution should be investigated in detail.

14588. **Bett, B., Irungu, P., Nyamwaro, S. O., Murilla, G., Kitala, P., Gathuma, J., Randolph, T. F. & McDermott, J., 2008**. Estimation of tsetse challenge and its relationship with trypanosomosis incidence in cattle kept under pastoral production systems in Kenya. *Veterinary Parasitology,* **155** (3-4): 287-298.

Trypanosomiasis Research Centre, Kenya Agricultural Research Institute, P.O. Box 362-00902, Kikuyu, Kenya. [b.bett@cgiar.org].

In an on-farm trial conducted amongst the Maasai pastoralists in Nkuruman and Nkineji areas of Kenya between April 2004 and August 2005 designed to evaluate the effectiveness of a synthetic tsetse repellent technology, we assessed the relationship between tsetse challenge and trypanosomosis incidence in cattle. Six villages were used in each area. Each of these villages had a sentinel cattle herd that was screened for trypanosomosis on monthly basis using the buffy coat technique. Animals found infected at each sampling were treated with diminazene aceturate at 7 mg kg^{-1} body weight. Treatments administered by the owners over the sampling intervals were recorded as well. Tsetse flies were trapped at the time of sampling using baited stationary traps and apparent tsetse density estimated as flies per trap per day (FTD). A fixed proportion (10 percent) of the flies was dissected and their infection status determined through microscopy. Blood meals were also collected from some of the flies and their sources identified using enzyme-linked immunosorbent assay (ELISA). Tsetse challenge was obtained as a product of tsetse density, trypanosome prevalence and the proportion of blood meals obtained from cattle. This variable was transformed using logarithmic function and fitted as an independent factor in a Poisson model that had trypanosomosis incidence in the sentinel cattle as the outcome of interest. The mean trypanosomosis incidence in the sentinel group of cattle was 7.2 percent and 10.2 percent in Nkuruman and Nkineji, respectively. *Glossina pallidipes* was the most prevalent tsetse species in Nkuruman while *G. swynnertoni* was prevalent in Nkineji. The proportions of tsetse that had mature infections in the respective areas were 0.6 percent and 4.2 percent. Most tsetse (28 percent) sampled in Nkuruman had blood meals from warthogs while most of those sampled in Nkineji (30 percent) had blood meals from cattle. A statistically significant association between tsetse challenge and trypanosomosis incidence was obtained only in Nkuruman when data was pooled and analyzed at the area but not at the village level. In the latter scenario, correlating trypanosomosis incidence with tsetse challenge 1 month later improved the strength but not the significance of the association. These findings show that when the spatial unit of analysis in observational studies or on-farm trials is small, for instance a village, it may not be possible to demonstrate a statistically significant association between tsetse challenge and trypanosomosis incidence in livestock so as to effectively control for tsetse challenge.

14589. **Herrera, H. M., Abreu, U. G., Keuroghlian, A., Freitas, T. P. & Jansen, A. M., 2008**. The role played by sympatric collared peccary (*Tayassu tajacu*), white-lipped peccary (*Tayassu pecari*), and feral pig (*Sus scrofa*) as maintenance hosts for *Trypanosoma evansi* and *Trypanosoma cruzi* in a sylvatic area of Brazil. *Parasitology Research,* **103** (3): 619-624.

Instituto Oswaldo Cruz/FIOCRUZ, Rio de Janeiro, RJ, Brazil. [herrera@ioc.fiocruz.br].

The Brazilian Pantanal has been considered one of the richest and most diverse wetland ecosystems in the world. It is occupied by cattle ranching, and a variety of wildlife species share the same habitats with domestic livestock. We investigated infections of *Trypanosoma evansi* and *Trypanosoma cruzi* in the sympatric suiformes-collared peccary (*Tayassu tajacu*), white-lipped peccary (*Tayassu pecari*), and feral pig (*Sus scrofa*) by parasitological, serological, and molecular tests. Additionally, we evaluated the health status of both positive and negative suiformes by haematological and biochemical parameters. The results show that peccaries and feral pigs play an important role on the maintenance of both *T. evansi* and *T. cruzi* in the Brazilian Pantanal. Health impairment was observed only in the white-lipped peccary infected with *T. evansi*. Despite presenting low *T. evansi* parasitaemia, all infected white-lipped peccaries displayed low haematocrit values and marked leucopaenia. The haematological values showed that the *T. evansi* infection is more severe in young white-lipped peccaries. The data presented show that feral pigs and peccaries are involved in the transmission of both trypanosome species in the Pantanal region.

14590 **Jensen, R. E., Simpson, L. & Englund, P. T., 2008**. What happens when *Trypanosoma brucei* leaves Africa. *Trends in Parasitology,* **24** (10): 428-431.

Department of Cell Biology, Johns Hopkins Medical School, Baltimore, MD 21205, USA.

Julius Lukes and co-workers evaluated the evolutionary origin of *Trypanosoma equiperdum* and *Trypanosoma evansi*, parasites that cause horse and camel diseases. Although similar to *T. brucei*, the sleeping-sickness parasite, these trypanosomes do not cycle through the tsetse fly and have been able to spread beyond Africa. Transmission occurs sexually, or via blood-sucking flies or vampire bats. They concluded that these parasites, which resemble yeast petite mutants, are *T. brucei* sub-species, which have evolved recently through changes in mitochondrial DNA.

14591. **Martins, J. R., Leite, R. C. & Doyle, R. L., 2008**. Trypanosomatides like *Trypanosoma theileri* in the cattle tick *Boophilus microplus*. *Revista brasileira de parasitologia veterinario,* **17** (2): 113-114.

Instituto de Pesquisas Veterinárias Desidério Finamor/Fundaçáo Estadual de Pesquisa Agropecuária, Estrada do Conde 6000, Eldorado do Sul, RS, Brazil. [joaorsm@terra.com.br]

Epimastigotes forms of a trypanosomatide are reported in the haemolymph of the cattle tick *Boophilus microplus* in the state of Rio Grande do Sul, southern Brazil. Morphological evidence suggests they are similar to *Trypanosoma theileri*, a species described as non pathogenic to cattle, and usually transmitted by tabanids.

14592. **Mekata, H., Konnai, S., Simuunza, M., Chembensofu, M., Kano, R., Witola, W. H., Tembo, M. E., Chitambo, H., Inoue, N., Onuma, M. & Ohashi, K., 2008**. Prevalence and source of trypanosome infections in field-captured vector flies (*Glossina pallidipes*) in south eastern Zambia. *Journal of Veterinary Medical Science,* **70** (9): 923-928.

Department of Disease Control, School of Veterinary Medicine, Hokkaido University, Sapporo, Japan. [okazu@vetmed.hokudai.ac.jp].

The prevalence of trypanosome infections in tsetse flies, *Glossina pallidipes*, collected from Chiawa and Chakwenga in Zambia which have endemic trypanosomosis was assessed by the polymerase chain reaction (PCR). Out of the 550 *G. pallidipes* sampled, 58 (10.5 percent) flies were found to harbour trypanosome DNA. Infection rates of tsetse with *T. vivax*, *T. congolense* savannah, *T. congolense* forest and *T. congolense* Kilifi were 4.2 percent (23/550), 4.7 percent (26/550), 1.1 percent (6/550) and 1.6 percent (9/550), respectively. To determine the mammalian hosts of *T. congolense* and *T. vivax* infections from the tsetse flies, mammalian mitochondrion DNA of the blood meals in these flies was analyzed by PCR, followed by gene sequence analysis of the amplicons. Sequence analysis showed the presence of the cytochrome b gene (cyt b) of seven different mammalian species such as human, elephant, buffalo, goat, warthog, greater kudu and cattle. Goats which were the main livestock in these areas were further examined to determine the extent of its contribution to spreading the infection. We examined the prevalence of trypanosome infections in the domestic goat population in 6 settlements in Chiawa. Of the 86 goats sampled, four (4.6 percent), five (5.8 percent), four (4.6 percent) and four (4.6 percent) were positive for *T. vivax*, *T. congolense* savannah, forest and Kilifi, respectively. These findings showed that the host source of trypanosome infections in the vector flies provide vital information about the spread of infection. The result of this study will certainly contribute in elucidating the epidemiology of trypanosomosis in more detail.

14593. **Motta, M. C., 2008**. Kinetoplast as a potential chemotherapeutic target of trypanosomatids. *Current Pharmaceutical Design,* **14** (9): 847-854.

Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, 21941-590, Rio de Janeiro, RJ, Brazil. [motta@biof.ufrj.br].

Many trypanosomatid protozoa, such as those belonging to the *Trypanosoma* and *Leishmania* genera cause serious diseases to man. Such parasites present an unusual feature, a mitochondrial DNA arranged in catenated circles, known as kinetoplast DNA (kDNA). The replication of kDNA network is a complex process, which involves many proteins. Some of them are classified as topoisomerases and play essential biological roles, not only on kDNA synthesis, but also in the dynamics of the network topology, constituting the main target for drugs in kinetoplast. DNA binding drugs are also reported as chemotherapeutic agents against

trypanosomatid infections. This review summarizes what is known about kinetoplast as a potential chemotherapeutic target for trypanosomatid protozoa.

14594. **Peacock, L., Ferris, V., Bailey, M. & Gibson, W., 2008**. Fly transmission and mating of *Trypanosoma brucei brucei* strain 427. *Molecular and Biochemical Parasitology,* **160** (2): 100-106.

School of Biological Sciences, University of Bristol, Woodland Road, Bristol, UK. [w.gibson@bristol.ac.uk].

Like yeast, *Trypanosoma brucei* is a model organism and has a published genome sequence. Although *T. b. brucei* strain 427 is used in many laboratories to study trypanosome molecular biology, particularly antigenic variation, this strain was not selected for the genome sequencing project as it is monomorphic and unable to complete development in the insect vector. Instead, the fly transmissible, mating competent strain TREU 927 was used for the genome project, but this is not as easily grown or genetically manipulable as strain 427; furthermore, recent findings have raised concerns about the potential human infectivity of TREU 927. Here we show that a 40-year-old cryopreserved line of strain 427, Variant 3, is fly-transmissible and also able to undergo genetic exchange with another strain of *T. b. brucei.* Comparison of Variant 3 with laboratory isolates of 427 shows that all have variant surface glycoprotein genes 117, 121 and 221, and identical alleles for three microsatellite loci. Therefore, despite some differences in molecular karyotype, there is no doubt that Variant 3 is an ancestral line of present day 427 isolates. Since Variant 3 grows fast both as bloodstream forms and procyclics and is readily genetically manipulable, it may prove useful where a fly transmissible version of 427 is required.

14595. **Poinsignon, A., Remoue, F., Rossignol, M., Cornelie, S., Courtin, D., Grebaut, P., Garcia, A. & Simondon, F., 2008**. Human IgG antibody response to *Glossina* saliva: an epidemiologic marker of exposure to *Glossina* bites. *American Journal of Tropical Medicine and Hygiene,* **78** (5): 750-753.

Epidémiologie et Prévention, Institut de Recherche pour le Développement, Dakar, Sénégal. [anne.poinsignon@ird.fr].

The evaluation of human antibody response specific to arthropod saliva may be a useful marker of exposure to vector-borne disease. Such an immunologic tool, applied to the evaluation of the exposure to *Glossina* bites, could be integrated in the control of human African trypanosomiasis (HAT). The antibody (IgG) response specific to uninfected *Glossina fuscipes fuscipes* saliva was evaluated according to the vector exposure and trypanic status in individuals residing in an HAT-endemic area. A high level of anti-saliva IgG antibodies was only detected in exposed individuals, whether infected or not by *Trypanosoma brucei gambiense*. In addition, the evaluation of specific IgG response represented spatial heterogeneity according to the studied sites. These results suggest that the evaluation of anti-saliva IgG could be an indicator of *Glossina* exposure and thus could be integrated with other available tools to identify populations presenting risks of HAT transmission.

14596. **Roditi, I. & Lehane, M. J., 2008**. Interactions between trypanosomes and tsetse flies. *Current Opinion in Microbiology,* **11** (4): 345-351.

Institut fur Zellbiologie, Universitat Bern, Baltzerstrasse 4, Bern, Switzerland. [isabel.roditi@izb.unibe.ch]

African trypanosomes are insect-borne parasites that cause sleeping sickness in humans and nagana in domesticated animals. Successful transmission is the outcome of crosstalk between the trypanosome and its insect vector, the tsetse fly. This enables the parasite to undergo successive rounds of differentiation, proliferation and migration, culminating in the infection of a new mammalian host. Several stage- and species-specific parasite surface molecules have been identified and there are new insights into their regulation in the fly. Tsetse flies are often refractory to infection with trypanosomes. While many environmental and physiological factors are known to influence infection, our detailed understanding of tsetse-trypanosome relationships is still in its infancy. Recent studies have identified a number of tsetse genes that show altered expression patterns in response to microbial infections, some of which have also been implicated in modulating trypanosome transmission.

14597. **Wang, J., Hu, C., Wu, Y., Stuart, A., Amemiya, C., Berriman, M., Toyoda, A., Hattori, M. & Aksoy, S., 2008**. Characterization of the antimicrobial peptide attacin loci from *Glossina morsitans*. *Insect Molecular Biology,* **17** (3): 293-302.

Yale University School of Medicine, Department of Epidemiology and Public Health, 60 College Street, New Haven, CT 06510, USA. [serap.aksoy@yale.edu].

The antimicrobial peptide attacin is an immune effector molecule that can inhibit the growth of gram-negative bacteria. In *Glossina morsitans morsitans*, which serves as a vector of African trypanosomes, attacins also play a role in trypanosome resistance, and in maintaining parasite numbers at homeostatic levels in infected individuals. We characterized the attacin encoding loci from a bacterial artificial chromosome (BAC) library. The attacin genes are organized into three clusters. Cluster 1 contains two attacin (attA) genes located in head-to-head orientation, cluster 2 contains two closely related genes (attA and attB) located in a similar transcriptional orientation, and cluster 3 contains a single attacin gene (attD). Coding and transcription regulatory sequences of attA and attB are nearly identical, but differ significantly from attD. Putative AttA and AttB have signal peptide sequences, but lack the pro domain typically present in insect attacins. Putative AttD lacks both domains. Analysis of attacin cDNA sequences shows polymorphisms that could arise either from allelic variations or from the presence of additional attacin genomic loci. Real time-PCR analysis reveals that attA and attB expression is induced in the fat body of flies challenged *per os* with *Escherichia coli* and parasitized with trypanosomes. In the midgut, expression of these attacins is similarly induced following microbial challenge, but reduced in response to parasite infections. Transcription of AttD is significantly less relative to the other two genes, and is preferentially induced in the fat body of parasitized flies. These results indicate that the different attacin genes may be differentially regulated.

14598. **Weiss, B. L., Wu, Y., Schwank, J. J., Tolwinski, N. S. & Aksoy, S., 2008**. An insect symbiosis is influenced by bacterium-specific polymorphisms in outer-membrane protein A. *Proceedings of the National Academy of Sciences USA,* **105** (39): 15088-15093.

Department of Epidemiology and Public Health, Section of Vector Biology, Yale University School of Medicine, LEPH 606, 60 College Street, New Haven, CT 06520, USA. [serap.aksoy@yale.edu].

Beneficial bacterial symbioses are ubiquitous in nature. However, the functional and molecular basis of host tolerance to resident symbiotic microbes, in contrast to resistance to closely related bacteria that are recognized as foreign, remain largely unknown. We used the tsetse fly (*Glossina morsitans*), which depends on symbiotic flora for fecundity and has limited exposure to foreign microbes, to investigate the tolerance phenomenon exhibited during symbiosis. We examined the potential role of bacterium-specific polymorphisms present in the major bacterial surface protein, outer-membrane protein A (OmpA), on host infection outcomes. Tsetse flies were successfully superinfected with their mutualistic facultative symbiont, *Sodalis glossinidius*, whereas infections with *Escherichia coli* K12 were lethal. In contrast, tsetse flies were resistant to an *E. coli* OmpA mutant strain, whereas recombinant *Sodalis* expressing *E. coli* OmpA became pathogenic. Profiling of tsetse immunity-related gene expression incriminated peptidoglycan recognition protein (pgrp)-lb as a determinant of the infection outcomes we observed. RNAi-induced knockdown of tsetse pgrp-lb significantly reduced host mortality after infection with otherwise lethal *E. coli* K12. Our results show that polymorphisms in the exposed loop domains of OmpA represent a microbial adaptation that mediates host tolerance of endogenous symbiotic bacteria.

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE

[See also **31**: 14702]

14599. **Kohagne Tongue, L., Guengai, M., Dologuele, N. F., Louis, F. J. & Moka, J. J., 2008**. Status of human African trypanosomiasis in the Nola-Salo-Bilolo in the Central African Republic in 2005. *Médicine Tropicale (Mars),* **68** (3): 247-250.

Programme sous-régional de lutte contre la trypanosomiase humaine africaine, Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale, BP 15665 Yaoundé, Cameroon. [kohagne_oceac@yahoo.fr]

In November-December 2005, the technical staff of the Organisation for Coordination of the Control of Endemic Diseases in Central Africa (OCEAC) and the National Programme for Control of Human African Trypanosomiasis (PNLTHA) undertook screening for human African trypanosomiasis in the historical focus of Nola-Salo-Biolo in Central Africa. A total of 31 new cases of trypanosomiasis were detected for a prevalence of 0.44 percent. This

study also provided insight into the limits of this old focus and showed that circulation of the parasite is still heavy. The endemic of sleeping sickness has now been contained in Nola-Salo-Bilolo but maintaining control measures is essential to preventing a potentially major recrudescence.

14600. **Konstantinov, O. K., Camara, S. K., Balde, M. S., Lienko, B. P. & Danilkin, B. K., 2008.** African trypanosomiasis in the Republic of Guinea. *Meditsinskaia parazitologiia i parazitarnyen bolezni* (Moscow), **3**: 36-40.

Address not available.

The information on the Gambian form of human African trypanosomiasis (HAT), collected in Guinea, is analyzed. The fauna of tsetse flies currently numbers at least 8 species. Two species are the vectors of HAT. These include *G. palpalis* and *G. tachinoides*, the latter of which is the vector of animal trypanosomiasis ("nagana" cattle disease) as well. In the period of 1991 to 1997, the country's incidence of HAT was 9.6 per 100 000 inhabitants. The highest morbidity was established in the natural region of Lower Guinea (23.4 per 100 000, with mortality rates of 1.1 percent to 18.5 percent). A clinical study of the population of a few villages in this region revealed 6 patients with HAT. Its clinical diagnosis was parasitologically verified. Preliminary studies suggest the circulation of the pathogen of HAT in Guinea, the most active foci of which are in Lower Guinea. The epidemiological features of HAT and its epidemic significance for Guinea are yet to be studied.

14601. **Louis, F. J., Kohagne Tongue, L., Ebo, O. E. V. & Simarro, P. P., 2008**. Organizing an active screening campaign for human African trypanosomiasis due to *Trypanosoma brucei gambiense*. *Médecine Tropicale (Mars),* **68** (1): 11-16.

O.C.E.A.C., Yaoundé, Cameroon. [louis_oceac@yahoo.fr]

Organization of an active screening programme for human African trypanosomiasis in an outbreak area is subject to strict guidelines that must take into account the size of the population, the specificity and sensitivity of the diagnostic techniques used, and the cost of screening. Numerous parameters can affect the outcome including accessibility of the outbreak area (road conditions, rainy season); awareness of village populations and of local administrative, traditional, and religious personalities; quality of local health-care facilities and personnel; possibility of referring patients to a health care institution able to provide treatment, etc. For these reasons the cost of screening programmes can be high in terms of human, physical, and financial resources. Careful planning and preparation is necessary to ensure worthwhile results. The model described in this article allows screening of 300 to 600 persons a day in areas in which the endemic disease prevalence is higher than 1 percent. A variant for areas with lower endemicity allows screening of up to 1 500 persons a day.

14602. **Sosa-Estani, S., Gamboa-León, M. R., Del Cid-Lemus, J., Althabe, F., Alger, J., Almendares, O., Cafferata, M. L., Chippaux, J. P., Dumonteil, E., Gibbons, L., Padilla-Raygoza, N., Schneider, D., Belizán, J. M. & Buekens, P., 2008.** Use of a rapid test on umbilical cord blood to screen for *Trypanosoma cruzi* infection in pregnant women in Argentina, Bolivia, Honduras, and Mexico. *American Journal of Tropical Medicine and Hygiene*, **79** (5): 755-759.

Instituto de Efectividad Clínica y Sanitaria, Centro Nacional de Diagnóstico e Investigación de Endemoepidemias (CeNDIE) ANLIS Dr. Carlos G. Malbrán, Ministerio de Salud, Buenos Aires, Argentina. [ssosa@msal.gov.ar].

We conducted a cross-sectional study of Chagas disease in five endemic areas in Argentina, Bolivia, Honduras, and Mexico to estimate the prevalence of *Trypanosoma cruzi*-specific antibodies in pregnant women, and to assess the use of a rapid test (Chagas Stat-Pak) to screen for *T. cruzi* infection at the time of delivery. The prevalence of antibodies to *T. cruzi* measured by enzyme-linked immunosorbent assay (ELISA) in maternal blood was 5.5 percent (a range of 0.8 percent-28.8 percent among the countries) in 2, 495 women enrolled. Compared with ELISA in maternal blood samples, the Chagas Stat-Pak rapid test sensitivity and specificity in umbilical cord blood were 94.6 percent and 99.0 percent, respectively. These results show the ability for a rapid determination of the presence of *T. cruzi*-specific antibodies in umbilical cord blood as a pragmatic strategy to screen for infection in pregnant women.

14603. **Wilson, L. S., Ramsey, J. M., Koplowicz, Y. B., Valiente-Banuet, L., Motter, C., Bertozzi, S. M. & Tobler, L. H., 2008**. Cost-effectiveness of implementation methods for ELISA serology testing of *Trypanosoma cruzi* in California blood banks. *American Journal of Tropical Medicine and Hygiene,* **79** (1): 53-68.

University of California, San Francisco, California 94010-0613, USA. [wilsonL@pharmacy.ucsf.edu].

The first ELISA test for *T. cruzi* antibodies was licensed by the Food and Drug Administration (FDA) on December 13 2006. Blood banks have begun screening in absence of FDA recommendations to determine best implementation methods. We surveyed 2 029 blood donors at five Californian sites with three risk-based Chagas risk-screening questions. Semi-Markov models compared the cost-effectiveness of three testing strategies. 30 percent of donors screened positively. Screening all dominated doing nothing, being less costly, and saving more lives. The choice to "screen and test" compared with "testing all" varied by Chagas prevalence, "screening and testing" being cost-effective for high (0.004) and low (0.00004) prevalences, and "testing all" cost-effective for moderate risk (0.0004). It is cost-effective to screen by ELISA rather than do nothing. The best strategy depends on site-specific risk. Census estimates of Hispanics do not predict donor risk well. We suggest using our screening questions to determine risk level and the most cost-effective testing strategy.

14604. **Zoller, T., Fevre, E. M., Welburn, S. C., Odiit, M. & Coleman, P. G., 2008**. Analysis of risk factors for *T. brucei rhodesiense* sleeping sickness within villages in south-east Uganda. *BMC Infectious Diseases,* **8**: 88.

Medizinische Klinik mit Schwerpunkt Infektiologie und Pneumologie, Charite - Universitätsmedizin Berlin, Chariteplatz 1, 10117 Berlin, Germany. [thomas.zoller@charite.de].

Sleeping sickness (HAT) caused by *T. b. rhodesiense* is a major veterinary and human public health problem in Uganda. Previous studies have investigated spatial risk factors for *T. b. rhodesiense* at large geographic scales, but none have properly investigated such risk factors at small scales, i.e. within affected villages. In the present work, we use a case-control methodology to analyse both behavioural and spatial risk factors for HAT in an endemic area. In the present study, behavioural and occupational risk factors for infection with HAT within villages were investigated using a questionnaire-based case-control study conducted in 17 villages endemic for HAT in SE Uganda, and spatial risk factors in four high risk villages. For the spatial analysis, the location of homesteads with one or more cases of HAT up to three years prior to the beginning of the study was compared with all non-case homesteads. Analysing spatial associations with respect to irregularly shaped geographical objects required the development of a new approach to geographical analysis in combination with a logistic regression model. The study was able to identify, among other behavioural risk factors, having a family member with a history of HAT ($p = 0.001$) as well as proximity of a homestead to a nearby wetland area ($p < 0.001$) as strong risk factors for infection. The novel method of analysing complex spatial interactions used in the study can be applied to a range of other diseases. It is concluded that spatial risk factors for HAT are maintained across geographical scales, and this consistency is useful in the design of decision support tools for intervention and prevention of the disease. Familial aggregation of cases was confirmed for *T. b. rhodesiense* HAT in the study and probably results from shared behavioural and spatial risk factors among members of a household.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **31**: 14540, 14548, 14559, 14638, 14641, 14645, 14652, 14659, 14665]

14605. **Blum, J. A., Zellweger, M. J., Burri, C. & Hatz, C., 2008**. Cardiac involvement in African and American trypanosomiasis. *Lancet Infectious Diseases,* **8** (10): 631-641.

Swiss Tropical Institute, Basel, Switzerland. [johannes.blum@unibas.ch].

American trypanosomiasis (Chagas disease) and human African trypanosomiasis (HAT; sleeping sickness) are both caused by single-celled flagellates that are transmitted by arthropods. Cardiac problems are the main cause of morbidity in chronic Chagas disease, but neurological problems dominate in HAT. Physicians need to be aware of Chagas disease and HAT in patients living in or returning from endemic regions, even if they left those regions long ago. Chagas heart disease has to be taken into account in the differential diagnosis of cardiomyopathy, primarily in patients with pathological electrocardiographic (ECG) findings,

such as right bundle branch block or left anterior hemiblock, with segmental wall motion abnormalities or aneurysms on echocardiography, and in young patients with stroke in the absence of arterial hypertension. In HAT patients, cardiac involvement as seen by ECG alterations, such as repolarisation changes and low voltage, is frequent. HAT cardiopathy in general is benign and does not cause relevant congestive heart failure and subsides with treatment. We review the differences between the American and African trypanosomiases with the main focus on the heart.

14606. **Courtioux, B., Pervieux, L., Bisser, S. & Bouteille, B., 2008**. Criteria for diagnosis of the neurological stage of human African trypanosomiasis: update and perspectives. *Médecine Tropicale (Mars),* **68** (1): 17-23.

UPRES EA 3174 Neuroparasitologie et neuroépidémiologie tropicale, Faculté de Médecine, Université de Limoges, France. [bertrand.courtioux@unilim.fr].

Sleeping sickness or human African trypanosomiasis (HAT) is due to parasite infection by a sanguicolous flagellate protozoan of the *Trypanosoma brucei* genus. The disease is classically divided into two stages, i.e., the haemolymphatic stage and the CNS stage. Disease staging is currently a major challenge for therapeutic decision-making. In the field, diagnosis is based solely on white blood cell (WBC) count and detection of the parasite in the patient's cerebrospinal fluid (CSF). This technique is unreliable and invasive. Numerous studies are now under way to adapt staging to field conditions and to develop a reliable, low-cost, non-invasive test. This article describes the mechanisms underlying CNS involvement during HAT and reviews the different techniques now being studied to simplify and improve diagnosis of the CNS stage.

14607. **Cuellar, A., Rojas, F., Bolaños, N., Diez, H., Del Carmen Thomas, M., Rosas, F., Velasco, V., López, M. C., González, J. M. & Puerta, C., 2008**. Natural CD 4(+) T-cell responses against *Trypanosoma cruzi* KMP-11 protein in chronic chagasic patients. *Immunoly and Cell Biology*. **Published online 28 October 2008**

Grupo de Inmunobiología y Biología Celular, Departamento de Microbiología, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, Colombia. [cpuerta@javeriana.edu.co].

Trypanosoma cruzi kinetoplastid membrane protein-11 (KMP-11) is able to induce protective immunity in mice. In humans, T-cell immunity during Chagas disease has been documented using parasite antigens allowing the identification of specific CD8(+) T cells. However, little is known about the CD4(+) T-cell response during the evolution of the disease. In this paper, the induction of a natural CD4(+) T-cell response against the KMP-11 protein in *T. cruzi* infected humans was studied to assess whether this parasite-derived protein could be processed, presented and detected as a major histocompatibility complex class II restricted epitope. The results show that helper T cells from 5 out of 13 chagasic patients specifically produced interferon-gamma after exposure to the KMP-11 antigen, whereas healthy donors and non-chagasic cardiopathic patients did not respond. This is the first description of *T. cruzi* KMP-11 protein recognition by CD4(+) T cells in chronic chagasic patients.

14608. **Deborggraeve, S., Koffi, M., Jamonneau, V., Bonsu, F. A., Queyson, R., Simarro, P. P., Herdewijn, P. & Buscher, P., 2008**. Molecular analysis of archived blood slides reveals an atypical human *Trypanosoma* infection. *Diagnostic Microbiology and Infectious Disease,* **61** (4): 428-433.

Department of Parasitology, Institute of Tropical Medicine, 2000 Antwerp, Belgium. [sdeborggraeve@itg.be]

In 2003, a ten-month-old Ghanaian boy recovered from a *Trypanosoma brucei* infection, although the patient was not treated with antitrypanosomal drugs. Only *T. brucei gambiense* and *T. brucei rhodesiense* are able to infect humans, causing human African trypanosomiasis. The disease is considered 100 percent fatal if left untreated. The identity of the trypanosome was determined by DNA extraction from the archived stained blood slides followed by sequential application of polymerase chain reactions (PCRs) that are specific for the order, subgenus, species and subspecies, followed by genotyping with microsatellite PCR. Molecular analysis indicated that the parasites observed in the patient's blood in 2003 belong to the *T. brucei* subspecies *brucei*, which is normally not infectious to humans. Next to the clinical message, this article provides technical information to extract successfully DNA from archived blood slides for subsequent molecular analysis and to identify a trypanosome by taxon-specific PCRs and microsatellite genotyping.

14609. **Kennedy, P. G., 2008**. Cytokines in central nervous system trypanosomiasis: cause, effect or both? *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **e-Publication ahead of print September 22 2008.**

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

The late, or encephalitic, stage of human African trypanosomiasis (HAT), or sleeping sickness, is typified by a diffuse meningoencephalitis characterised neuropathologically by perivascular infiltration of inflammatory cells. While the cause of this neuroinflammatory reaction is not understood, there is evidence for the roles of pro-inflammatory cytokines such as IFN-gamma and TNF-alpha and counter-inflammatory cytokines such as IL-10, with the balance of these influencing disease outcome. Because of the practical difficulties of obtaining serial measurements in patients, it has proved difficult to assign either cause or effect properties to measured cytokines, but mechanistic animal modelling studies are proving helpful.

14610. **Pays, E. & Vanhollebeke, B., 2008**. Mutual self-defence: the trypanolytic factor story. *Microbes and Infection,* **10** (9): 985-989.

Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles, 12 rue des Professeurs Jeener et Brachet, B-6041 Gosselies, Belgium. [epays@ulb.ac.be].

Around 1900 Laveran and Mesnil discovered that African trypanosomes (prototype: *Trypanosoma brucei brucei*) do not survive in the blood of some primates and humans. The nature of the trypanolytic factor present in these sera has been the focus of a long-standing debate between different groups, but recent developments have allowed the proposal of a coherent model incorporating most seemingly divergent views and providing an interesting example of the complex interplay that continuously occurs between hosts and parasites. Possibly as an adaptation to their natural environment, great African apes and humans have acquired a new member of the apolipoprotein-L family, termed apoL1. This protein is the only one of the family to be secreted in the blood, where it binds to a subset of HDL particles that also contain another human-specific protein, haptoglobin-related protein or Hpr. *T. b. brucei* possesses a specific surface receptor for the haptoglobin-hemoglobin (Hp-Hb) complex, as a way to capture haeme into haemoproteins that contribute to cell growth and resistance to the oxidative stress of the host. As this receptor does not discriminate between Hp and Hpr, Hpr-containing HDL particles of human serum are efficiently taken up by the parasite, leading to the simultaneous internalization of apoL1, Hpr and Hb-derived heme. Once in the lysosome, apoL1 is targeted to the lysosomal membrane, where its colicin-like anionic pore-forming activity triggers an influx of chloride ions from the cytoplasm. Osmotic effect linked to this ionic flux leads to uncontrolled swelling of the lysosome, ultimately causing the death of the parasite. Two *T. brucei* clones, termed *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*, have managed to resist this lysis mechanism and, therefore, cause sleeping sickness in humans. While the mechanism of this resistance is still not known in the case of *T. b. gambiense*, the dominant factor responsible for resistance of *T. b. rhodesiense* has been identified. This protein, named SRA for serum resistance-associated, is a truncated version of the major and variable surface antigen of the parasite, the variant surface glycoprotein or VSG. Presumably due to its defective nature, SRA is not targeted to the plasma membrane as do regular VSGs, but ends up in the late endosomal compartment. In this location SRA is thought to neutralize apoL1 through coiled-coil interactions between alpha-helices. We discuss the potential of these discoveries in terms of fight against the disease.

14611. **Vanhollebeke, B., De Muylder, G., Nielsen, M. J., Pays, A., Tebabi, P., Dieu, M., Raes, M., Moestrup, S. K. & Pays, E., 2008.** A haptoglobin-haemoglobin receptor conveys innate immunity to *Trypanosoma brucei* in humans. *Science,* **320** (5876): 677-681.

Laboratory of Molecular Parasitology, Institute for Molecular Biology and Medicine, Université Libre de Bruxelles, 12 rue des Profs Jeener et Brachet, B6041 Gosselies, Belgium. [epays@ulb.ac.be].

The protozoan parasite *Trypanosoma brucei* is lysed by apolipoprotein L-I, a component of human high-density lipoprotein (HDL) particles that are also characterized by the presence of haptoglobin-related protein. We report that this process is mediated by a parasite glycoprotein receptor, which binds the haptoglobin-haemoglobin complex with high affinity for the uptake and incorporation of haeme into intracellular haemoproteins. In mice, this receptor was required for optimal parasite growth and the resistance of parasites to the oxidative burst by host macrophages. In humans, the trypanosome receptor also recognized the complex between haemoglobin and haptoglobin-related protein, which explains its ability

to capture trypanolytic HDLs. Thus, in humans the presence of haptoglobin-related protein has diverted the function of the trypanosome haptoglobin-haemoglobin receptor to elicit innate host immunity against the parasite.

(c) TREATMENT

[See also **31**: 14545, 14549, 14550, 14551, 14556, 14561, 14563, 14646, 14682, 14690, 14695]

14612. **Balasegaram, M., Young, H., Chappuis, F., Priotto, G., Raguenaud, M. E. & Checchi, F., 2008**. Effectiveness of melarsoprol and eflornithine as first-line regimens for *gambiense* sleeping sickness in nine Médecins Sans Frontières programmes. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* **e - Publication ahead of print 22 October 2008.**

Médecins Sans Frontières-United Kingdom, 67-74 Saffron Hill, London EC1N 8QX, UK.

This paper describes the effectiveness of first-line regimens for stage 2 human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* infection in nine Médecins Sans Frontieres HAT treatment programmes in Angola, Republic of Congo, Sudan and Uganda. Regimens included eflornithine and standard- and short-course melarsoprol. Outcomes for 1 0461 naive stage 2 patients fitting a standardised case definition and allocated to one of the above regimens were analysed by intention-to-treat analysis. Effectiveness was quantified by the case fatality rate (CFR) during treatment, the proportion probably and definitely cured and the Kaplan-Meier probability of relapse-free survival at 12 months and 24 months post admission. The CFR was similar for the standard- and short-course melarsoprol regimens (4.9 percent and 4.2 percent, respectively). The CFR for eflornithine was 1.2 percent. Kaplan-Meier survival probabilities varied from 71.4 percent-91.8 percent at one year and 56.5-87.9 percent at 2 years for standard-course melarsoprol, to 73.0 percent-91.1 percent at one year for short-course melarsoprol, and 79.9 percent-97.4 percent at 1 year and 68.6 percent-93.7 percent at two years for eflornithine. With the exception of one programme, survival at 12 months was >90 percent for eflornithine, whilst for melarsoprol it was <90 percent except in two sites. Eflornithine is recommended where feasible, especially in areas with low melarsoprol effectiveness.

14613. **Dorlo, T. P. & Kager, P. A., 2008**. Pentamidine dosage: a base/salt confusion. *PLoS Neglected Tropical Diseases,* **2** (5): e225.

Center for Infection and Immunity Amsterdam (CINIMA), Division of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands. [thomasdorlo@gmail.com].

Pentamidine has a long history in the treatment of human African trypanosomiasis (HAT) and leishmaniasis. Early guidelines on the dosage of pentamidine were based on the base-moiety of the two different formulations available. Confusion on the dosage of pentamidine arose from a different labelling of the two available products, either based on the

salt or base moiety available in the preparation. We provide an overview of the various guidelines concerning HAT and leishmaniasis over the past decades and show the confusion in the calculation of the dosage of pentamidine in these guidelines and the subsequent published reports on clinical trials and reviews. At present, only pentamidine isethionate is available, but the advised dosage for HAT and leishmaniasis is (historically) based on the amount of pentamidine base. In the treatment of leishmaniasis this is probably resulting in a subtherapeutic treatment. There is thus a need for a new, more transparent and concise guideline concerning the dosage of pentamidine, at least in the treatment of HAT and leishmaniasis.

14614. **Gehrig, S. & Efferth, T., 2008**. Development of drug resistance in *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense:* Treatment of human African trypanosomiasis with natural products (review). *International Journal of Molecular Medicine,* **22** (4): 411-419.

Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany.

Human African trypanosomiasis is an infectious disease which has resulted in the deaths of thousands of people in sub-Saharan Africa. Two subspecies of the protozoan parasite *Trypanosoma brucei* are the causative agents of the infection, whereby *T. b. gambiense* leads to chronic development of the disease and *T. b. rhodesiense* establishes an acute form which is fatal within months or even weeks. Current chemotherapy treatment is complex, since special drugs have to be used for the different development stages of the disease, as well as for the parasite concerned. Melarsoprol is the only approved drug for effectively treating both subspecies of human African trypanosomiasis in its advanced stage, however, the drug's potency is constrained due to an unacceptable side effect: encephalopathy, which develops in one out of every 20 patients who are treated with the drug. In addition to the deleterious treatment with melarsoprol, the number of drug-resistant strains of *T. brucei* spp. increases. Mechanisms of drug resistance have been elucidated and involve decreased drug import through the loss of the purine transporter P2 as well as enhanced drug export, mediated by a multidrug resistance-associated protein called *Tb*MRPA. Thereby, the medical treatment with the available chemotherapeutics becomes exceedingly difficult. A promising strategy for research into new drugs and moreover, to overcome drug resistance, are compounds derived from natural sources. This study provides an overview of the recently discovered small molecules with trypanocidal activity against *T. b. gambiense* and *T. b. rhodesiense*. In addition, former promising compounds are touched upon.

14615. **Kinoshita, T., 2008**. Designing sleeping sickness control. *ACS Chemical Biology,* **3** (10): 601-603.

Laboratory of Immunoglycobiology, WPI Immunology Frontier Research Center, and Department of Immunoregulation, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan. [tkinoshi@biken.osaka-u.ac.jp]

Control of African trypanosomiasis caused by the protozoan parasite *Trypanosoma brucei* is an important issue in medicine, veterinary medicine, and agricultural economy. Because vaccine development is unlikely, development of safer and more effective chemotherapeutics is critical. The biosynthetic pathway of glycosylphosphatidylinositol (GPI), which acts as membrane anchors of coat proteins, variant surface glycoproteins and transferrin receptors, is a validated target of drug development. An article in this issue reports the first chemically synthesized inhibitor of the third mannosyltransferase from the GPI pathway, stimulating further investigation toward practical and useful compounds.

14616. **Lejon, V., Roger, I., Mumba Ngoyi, D., Menten, J., Robays, J., N'Siesi F, X., Bisser, S., Boelaert, M. & Buscher, P., 2008**. Novel markers for treatment outcome in late-stage *Trypanosoma brucei gambie*nse trypanosomiasis. *Clinical Infectious Diseases,* **47** (1): 15-22.

Department of Parasitology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium. [vlejon@itg.be]

To date, no biological marker for treatment outcome in human African trypanosomiasis (HAT) has been described. The accuracy of biological markers for prediction of treatment outcome of HAT caused by *Trypanosoma brucei gambiense* was assessed. Cerebrospinal fluid (CSF) white blood cell (WBC) count and immunoglobulin M (IgM), trypanosome-specific antibody, total protein, and interleukin-10 levels were determined before and up to 24 months after treatment of late-stage HAT. Treatment failure was experienced by 48 of 260 patients. Pretreatment CSF WBC counts > or = 102 cells/µL, IL-10 concentrations > or = 37 pg/mL, LATEX/IgM end titres > or = 1:32, LATEX/*T. b. gambiense* end titres > or = 1:2, and protein concentrations > or = 674 mg/L were associated with treatment failure. Six months after treatment, patients with CSF WBC counts < or = 5 cells/µL were at low risk of HAT recurrence (negative predictive value, >0.93). After 12 months, the combination of CSF WBC count > or = 8 cells/µL and LATEX/IgM end titre > or = 1:4 predicted treatment failure with 97 percent specificity and 79 percent sensitivity. Eighteen months after treatment, each marker accurately predicted treatment outcome. The combination of CSF WBC count > or = 8 cells/µL and LATEX/IgM end titre > or = 1:4 was 100 percent specific for treatment failure after 18 and 24 months. It was concluded that HAT-affected patients with elevated pretreatment CSF levels of WBC, interleukin-10, IgM, trypanosome-specific antibody, and total protein are at risk of treatment failure. Six months after treatment, patients with CSF WBC counts < or = 5 cells/µL can be considered to be cured. The assessment of a combination of CSF WBC count and LATEX/IgM level allowed accurate prediction of outcome beginning at 12 months after treatment, as did each individual marker at 18 months after treatment.

14617. **McGeary, R. P., Bennett, A. J., Tran, Q. B., Cosgrove, K. L. & Ross, B. P., 2008**. Suramin: clinical uses and structure-activity relationships. *Mini Reviews in Medicinal Chemistry,* **8** (13): 1384-1394.

School of Molecular & Microbial Sciences, The University of Queensland, Brisbane, Qld 4072, Australia. [r.mcgeary@uq.edu.au].

Suramin is a polysulfonated polyaromatic symmetrical urea. It is currently used to treat African river blindness and African sleeping sickness. Suramin has also been extensively trialed recently to treat a number of other diseases, including many cancers. Here, we examine its modes of action and discuss its structure-activity relationships.

14618. **Robays, J., Nyamowala, G., Sese, C., Betu Ku Mesu Kande, V., Lutumba, P., Van der Veken, W. & Boelaert, M., 2008**. High failure rates of melarsoprol for sleeping sickness, Democratic Republic of Congo. *Emerging Infectious Diseases,* **14** (6): 966-967.

Institute of Tropical Medicine, Antwerp, Belgium. [jrobays@itg.be]

A retrospective medical chart review of 4 925 human African trypanosomiasis patients treated with melarsoprol in 2001-2003 in Equateur Nord Province of the Democratic Republic of Congo showed a treatment failure rate of 19.5 percent. This rate increased over the 3 years. Relapse rates were highest in the central part of the province.

14619. **Salerno, M. & Garnier-Suillerot, A., 2003**. Resistance to arsenic- and antimony-based drugs. *Bioinorganic Chemistry and Applications* **1** (2): 189-198.

Laboratoire de Physicochimie Biomoléculaire et Cellulaire (LPBC/CSSB UMR 7033) Université Paris Nord, 74 rue Marcel Cachin, Bobigny 93017, France.

Organic arsenicals were the first antimicrobial agents specifically synthesized for the treatment of infectious diseases such as syphilis and sleeping sickness. For the treatment of diseases caused by trypanosomatid parasites, organic derivatives of arsenic and the related metalloid antimony are still the drugs of choice. Arsenic trioxide has been used for a long time in traditional Chinese medicines for treatment of various diseases, and it has recently been shown to be clinically active in acute promyelocytic leukemias. Resistance to metalloid salts is found in bacteria, fungi, parasites and animals. In some organisms, resistance involves overproduction of intracellular thiols. In many cases, resistance to arsenic salts is the result of removal of the metalloid from the cytosol usually by extrusion from the cell. In eukaryotes resistance to arsenic and antimony is conferred by membrane transport proteins of the MRP family. The human MRP1, a member of this family, is frequently amplified in cancer cells and it is well-documented that MRPl-overexpressing cells poorly accumulate arsenic and antimony because of enhanced cellular efflux which depends on the presence of GSH.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

[See also **31**: 14554, 14566, 14570, 14703]

14620. **Guedes, D. S., Jr., Araújo, F. R., Silva, F. J., Rangel, C. P., Barbosa Neto, J. D. & Fonseca, A. H., 2008**. Frequency of antibodies to *Babesia bigemina, B. bovis, Anaplasma marginale, Trypanosoma vivax* and *Borrelia burgdorferi* in cattle from

the north eastern region of the State of Para, Brazil. *Revista brasileira de parasitologia veterinaria,* **17** (2): 105-109.

Departamento de Epidemiologia e Saúde Pública, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil. RJ. [guedesjr@ufrrj.br]

Babesiosis, anaplasmosis, and trypanosomosis are relevant diseases, potentially causing morbidity in cattle, leading to economic losses. Borreliosis is important as a potential zoonosis. The objective of this study was to determine, by indirect enzyme-linked immunosorbent assay (ELISA), the frequency of seropositive cattle to *Babesia bigemina, B. bovis, Anaplasma marginale, Trypanosoma vivax* and *Borrelia burgdorferi* in cattle from the north eastern region of Para, Brazil. Serum samples from 246 female adult cattle from municipalities of Castanhal and Sao Miguel do Guama were used. Crude antigen ELISAs were used to detect antibodies to all agents, except to *A. marginale*, for which an indirect ELISA with recombinant major surface 1a protein (MSP1a) antigen was used. Overall frequencies of seropositive animals were: *B. bigemina* -99.2 percent; *B. bovis* -98.8 percent; *A. marginale* -68.3 percent; *T. vivax* -93.1 percent and *B. burgdorferi* -54.9 percent. The frequencies of seropositive cattle to *B. bovis* and *B. bigemina* suggest a high rate of transmission of these organisms by tick in the studied region, which can be classified as enzootically stable to these haemoprotozoans. The low frequency of seropositive cattle to *A. marginale* may be attributed to a lower sensitivity of the recombinant antigen ELISA utilized or a distinct rate of inoculation of this rickettsia by ticks, as compared with *Babesia* sp. transmission. The high frequency of seropositive cattle to *T. vivax* indicates that this hemoprotozoan is prevalent in herds from the north eastern region of Para. The rate of animal that showed homologous antibodies to *B. burgdorferi* indicates the presence of the tick-borne spirochaetal agent in the cattle population in the studied region.

14621. **Laha, R. & Sasmal, N. K., 2008**. Endemic status of *Trypanosoma evansi* infection in a horse stable of eastern region of India: A field investigation. *Tropical Animal Health and Production,* **40** (5): 357-361.

Department of Veterinary Parasitology, West Bengal University of Animal and Fishery Sciences, 37, Belgachia Road, Kolkata, 700 037 West Bengal, India. [dnksasmal@rediffmail.com].

Diagnosis of *Trypanosoma evansi* infection in a horse stable in the eastern region of India was done on the basis of examination of Giemsa stained blood smears. A high percentage (12.74 percent) of horses of this stable was found to be suffering from *T evansi* infection. The high prevalence of *T. evansi* in horses in this area could be considered as an alarming situation which has never been explored previously. After a period of two months and 18 days of treatment with quinapyramine sulphate and quinapyramine chloride, reinfection with *T. evansi* in treated horses of this stable was noticed. Clinical signs in affected horses and possible causes of reinfection are discussed.

14622. **Mandal, M., Laha, R. & Sasmal, N. K., 2008**. First report of establishment of *Trypanosoma evansi* infection in pigeon nestlings (*Columba livia*). *Journal of Parasitology*. **e - Publication ahead of print 16 May 2008.**

West Bengal University of Animal and Fishery Sciences Indian Veterinary Research Institute, Department of Parasitology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal & Fishery Sciences, 37, K.B. Sarani, Kolkata, West Bengal, India 700 037. [dnksasmal@rediffmail.com].

Trypanosomosis (surra) caused by *Trypanosoma evansi* is quite common among horses where the parasite is endemic. In the present study, *T. evansi* was isolated from an infected horse and maintained by sub-inoculation in Swiss albino mice. At the peak of parasitaemia (5 x 10^6 parasites per ml of blood), 0.25 ml of the tail blood from infected mice was inoculated intraperitoneally and subcutaneously to two groups of adult pigeons and two groups of pigeon nestlings. Four days following inoculation, the trypanosomes appeared in the peripheral circulation of pigeon nestlings, but no parasitaemia was observed in adult pigeons. The body temperatures of infected nestlings increased to 104 ^{o}F, while uninfected controls remained steady at 102 ^{o}F; thus, elevated temperatures coincided with parasite presence in the peripheral circulation. A decrease in haemoglobin concentration of blood was also observed in infected nestlings. On microscopic examination, increases in length and breadth of trypomastigotes and vigorous flagellar movement of the parasites were observed. The virulence and pathogenicity of the parasites after adaptation to nestlings remained unchanged for albino mice as proved by the death of all sub-inoculated mice. Further, polymerase chain reaction (PCR) studies confirmed that the genomic DNA of trypanosomes in pigeon blood was same as *T. evansi*. This is the first report of the establishment of *T. evansi* infection in pigeon nestlings.

14623. **Matjila, P. T., Leisewitz, A. L., Jongejan, F. & Penzhorn, B. L., 2008**. Molecular detection of tick-borne protozoal and *Ehrlichial* infections in domestic dogs in South Africa. *Veterinary Parasitology,* **155** (1-2): 152-157.

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag 04, 0110 Onderstepoort, South Africa. [tshepo.matjila@up.ac.za]

A total of 1 138 blood specimens were collected over a six-year period (2000-2006) from domestic dogs in South Africa. Specimens from domestic dogs were obtained from the Onderstepoort Veterinary Academic Hospital in Pretoria, the Society for the Prevention of Cruelty to Animals (Johannesburg, Durban, East London and Bloemfontein) and private practices from four provinces (Gauteng, Mpumalanga, KwaZulu-Natal and Western Cape). All specimens were screened for *Babesia, Theileria, Hepatozoon* and *Ehrlichia/Anaplasma* species using PCR and reverse line blot (RLB) assays. On RLB, 560/1, 137 domestic dog-specimens were positive for one or more parasites. Of the positive domestic dog specimens, 420 (75 percent) were infected with *Babesia rossi*; 82 (15 percent) dogs were infected with *Theileria* sp. (dog); 18 (3 percent) dogs were infected with *Babesia vogeli*; 14 (3 percent) specimens were infected with *Ehrlichia canis*. Mixed infections were also found: *B. rossi* and *E. canis* were detected in 12 (2 percent) specimens; *B. vogeli* and *E. canis* occurred in seven (1 percent) specimens; *Theileria* sp. (dog) and *E. canis* in three (0.5 percent) specimens; *B. rossi* and *B. vogeli* in one specimen. *B. rossi*, *B. vogeli* and *E. canis* occurred simultaneously in one dog. There was also one incidental finding of a dog positive for *Trypanosoma*

congolense. The results indicate that a wide range of tick-borne pathogens are circulating in the canine populations in South Africa.

14624. **Mingala, C. N. & Gundran, R. S., 2008**. Assessment of water buffalo health and productivity in a communal management system in the Philippines. *Tropical Animal Health and Production,* **40** (1): 61-68.

Department of Veterinary Studies, Institute of Graduate Studies, Central Luzon State University, Science City of Munoz, 3120 Nueva Ecija, Philippines. [cnmingala@hotmail.com].

This study aimed to generate a profile of the health and productivity of water buffaloes in a communal setting. Using the Epi-Info version 6.04 for data management, a coded information system was used to accommodate data coming from the reference population. Calves and cows that were born and milked, respectively, were enrolled and monitored for six months. The key outcomes of interest monitored in this study included mortality, morbidity and productivity. Results of the study showed a 93.7 percent probability of the calves surviving up to six months with a calculated mortality true rate of 0.7 deaths per 1 000 calf-days at risk. Three calves died during the six month observation period with a mean age at death of three days. Analysis of variance on productivity showed that the parasitic load, specifically *Coccidia*, liver fluke and *Trypanosoma* affected the growth rate of the calves. The productivity of cows in the study in terms of milk production was also highly affected by the endoparasitic load and disease condition of the animal. Univariate analysis revealed a significant association between calf scouring and cow's mastitis ($p=0.066$). Meanwhile, for the cows, the parasitic load particularly fasciolosis ($p=0.000$), coccidiosis ($p=0.002$) and trypanosomosis ($p=0.094$) ($p<0.10$) also significantly affected the milk production. The results give a clearer view of the relationship between the health and productivity profiles of these animals.

14625. **Nelder, M. P., Reeves, W. K., Adler, P. H., Wozniak, A. & Wills, W., 2008**. Ectoparasites and associated pathogens of free-roaming and captive animals in zoos of South Carolina. *Vector Borne and Zoonotic Diseases*. **In press, corrected proof.**

Department of Entomology, Soils & Plant Sciences, Clemson University, Clemson, South Carolina, USA.

A survey of ectoparasites and their associated pathogens was conducted in two South Carolina zoos, from 2004 to 2007. Dead, wild birds and mammals, as well as captive animals examined during routine veterinary checks constituted the study populations. Ectoparasites were tested for species of *Anaplasma, Bartonella, Coxiella burnetii, Ehrlichia, Rickettsia*, and *Trypanosoma*. Forty-six species of ectoparasites were collected from 133 free-roaming and captive hosts and their associated nesting and bedding materials. Six vector-borne pathogens were detected molecularly in the ectoparasites, including *Anaplasma phagocytophilum* in the tick *Ixodes dentatus* Marx from an eastern cottontail rabbit,

Bartonella clarridgeiae in the cat flea *Ctenocephalides felis* (Bouche) from a Virginia opossum, *Bartonella* sp. Oh6 in the squirrel flea *Orchopeas howardi* (Baker) from an eastern grey squirrel, *Bartonella* sp. T7498 in the sucking louse *Neohaematopinus sciuri* Jancke from a squirrel, *Rickettsia* sp. Rf2125 in *C. felis* from a zookeeper and a grizzly bear, and *Rickettsiales* sp. Ib 2006 in *Ixodes brunneus* Koch from an American crow. While the pathology of some of these pathogens is poorly known, *Anaplasma phagocytophilum* (causative agent of human granulocytic anaplasmosis) and *Bartonella clarridgeiae* (causative agent of a disease similar to cat-scratch disease) can infect humans. Ectoparasites and their pathogens, especially those originating from free-roaming animals, present a potential threat to captive animals and humans.

14626. **Smith, A., Clark, P., Averis, S., Lymbery, A. J., Wayne, A. F., Morris, K. D. & Thompson, R. C., 2008**. Trypanosomes in a declining species of threatened Australian marsupial, the brush-tailed bettong *Bettongia penicillata* (Marsupialia: *Potoroidae*). *Parasitology,* **135** (11): 1329-1335.

WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, State Agricultural Biotechnology Centre, School of Veterinary and Biomedical Sciences, Murdoch University, Western Australia 6150, Australia. [andrew.smith@murdoch.edu.au].

The brush-tailed bettong (*Bettongia penicillata*), or woylie, is a medium-sized macropod marsupial that has undergone a rapid and substantial decline throughout its home range in the Upper Warren region of Western Australia over a period of approximately 5 years. As part of an investigation into possible causes of the decline a morphologically distinct *Trypanosoma* sp. was discovered by light microscopy in the declining population but was absent in a stable population within the Karakamia Wildlife Sanctuary. Further investigations employing molecular methods targeting variations in the 18s rRNA gene determined that the trypanosome was novel and was also present within the Karakamia population albeit at a much lower overall prevalence and individual parasitaemia levels. Phylogenetic analysis suggests the novel *Trypanosoma* sp. to be closely related to other trypanosomes isolated from native Australian wildlife species. Although it appears unlikely that the parasite is solely responsible for the decline in woylie population size, it may (singularly or in conjunction with other infectious agents) predispose woylies to increased mortality.

14627. **Specht, E. J., 2008**. Prevalence of bovine trypanosomosis in Central Mozambique from 2002 to 2005. *Onderstepoort Journal of Veterinary Research,* **75** (1): 73-81.

Laboratorio Regional de Veterinaria, P.O. Box 42, Chimoio, Mozambique. [vetlabman@teledata.mz]

The study is the result of analyzing 16 895 blood smears of cattle collected at 180 sites in the provinces of Manica, Sofala, Zambezia and Tete in Mozambique. Of the blood smears 73.9 percent were from Manica, 11.8 percent from Tete, 8.5 percent from Sofala and 5.8 percent from Zambezia; 75.6 percent of these were collected from smallholder cattle. Infections with trypanosomes were highest in smallholder cattle from Sofala Province with 36.8 percent of the 872 blood smears examined positive for trypanosomes, and lowest in

cattle of commercial farmers in Manica Province with only 6.2 percent of 2 252 blood smears being positive. *Trypanosoma congolense* was the predominant species, followed by *Trypanosoma vivax* and *Trypanosoma brucei*. *Trypanosoma brucei*, which also infects humans, was more frequent in the districts of Buzi, Mutarara and Morrumbala with 15.1 percent, 10.5 percent and 9.8 percent of all examined cattle in 2005 being infected with it, respectively. The results show a significant increase in the infection rate with trypanosomes compared with results obtained in previous years by the Regional Veterinary Laboratory in Manica Province and by the Regional Tsetse and Trypanosomiasis Control Programme in Zambezia, Tete and Sofala provinces.

14628. **Yeshitila, A. Y., Getachew, A., Hagos, A., & Basu A. K., 2006**: Prevalence of bovine trypanosomosis in Sokoru District, Jimma Zone, Oromiya Region, southwest of Ethiopia. *Bulletin of Animal Health and Production in Africa*. **54** (4): 242-258.

Faculty of Veterinary Medicine, Addis Ababa University, PO.Box 34, Debre Zeit, Ethiopia. [asokebasu@gmail.com].

The study was conducted to investigate the prevalence and magnitude of bovine trypanosomosis in selected villages of Sokoru District, Jimma Zone, Oromiya Region, Ethiopia, to assess and analyze the efficacy of trypanocidals in use, and the sustainable and effective control options of tsetse transmitted trypanosomosis. The questionnaire survey revealed that trypanosomosis was the most important and major disease in the study area. Entomological survey revealed that *Glossina m. submorsitans* was the highly prevalent tsetse fly species followed by *Glossina pallidipes*. The apparent fly density tsetse was relatively higher in late rainy season at Abelty compared to Tiroshashama (0.194 and 0.028 fly/ trap /day respectively). In Tiroshashama 0.017 fly /trap/day of *G. m. submorsitans* and 0 .017 *G. pallidipes* was caught in dry season whereas no fly was caught in Abelty. The prevalence of trypanosomosis was higher in dry season 8.36 percent, compared to 7.22 percent in late rainy season. A significant difference ($p< 0.05$) was noticed between the mean PCV values in parasitaemic (95 percent CI 21.24, 23.59) and aparasitaemic (95 percent CI 23.93, 24.73) cattle. The result also indicated a better protection by isometamidium chloride for prophylaxis of animal trypanosomosis in the study area.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **31**: 14540, 14640, 14643, 14647, 14648, 14653, 14655].

14629. **Auty, H., Mundy, A., Fyumagwa, R. D., Picozzi, K., Welburn, S. & Hoare, R., 2008**. Health management of horses under high challenge from trypanosomes: a case study from Serengeti, Tanzania. *Veterinary Parasitology,* **154** (3-4): 233-241.

Centre for Tropical Veterinary Medicine, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, Midlothian EH25 9RG, UK. [h.k.auty@sms.ed.ac.uk]

Horses kept for recreational riding purposes by a wildlife tourism company in a heavily tsetse fly-infested region of north-western Tanzania were systematically monitored to

investigate the occurrence, presentation and management of tsetse-transmitted trypanosomosis. During a 23-month period, 18 clinical cases were diagnosed (*Trypanosoma brucei* or *Trypanosoma congolense* were identified) and treated and trypanosomes were implicated of involvement in four deaths. Pyrexia consistently aided early detection (17 cases). Ataxia, weight loss and anaemia were seen in chronic cases and conferred a poor prognosis. Delaying treatment by more than two days from the onset of clinical signs led to prolonged disease course and more severe anaemia. Early detection, prompt treatment, thorough post-treatment health monitoring and rigorous prophylactic measures helped keep clinical cases to manageable levels, but re-infection remained a constant, insidious threat.

14630. **Desquesnes, M., Bossard, G., Patrel, D., Herder, S., Patout, O., Lepetitcolin, E., Thevenon, S., Berthier, D., Pavlovic, D., Brugidou, R., Jacquiet, P., Schelcher, F., Faye, B., Touratier, L. & Cuny, G., 2008.** First outbreak of *Trypanosoma evansi* in camels in metropolitan France. *Veterinary Record,* **162** (23): 750-752.

Cirad & IRD, UR Trypanosomes, Montpellier, F-34000, France. [m.desquesnes@fasonet.bf].

The first outbreak of trypanosomosis caused by *Trypanosoma evansi* in camels in France was reported on a farm in the Aveyron Department. Five camels were imported from the Canary Islands to the farm in early July 2006, and trypanosomes were observed on a stained blood smear from one of them, which died in October. On further investigations, trypanosomes were observed in the blood of five camels, three of them indigenous to the farm and two that had been imported. On the basis of microscopical examination (morphological criteria and measurements) and serological results based on the card agglutination *T evansi* test and PCR typing, the parasites were identified as *T. evansi*. After treatment with melarsomine, the infected camels rapidly became negative by parasitological tests and were negative two to four months later by serological tests. The parasite was probably transmitted by tabanids and *Stomoxys calcitrans*, which were abundant in July to September 2006. No parasites were observed in other animals on the farm or on neighbouring farms, but some of the sheep on these farms were positive by PCR or serology.

14631. **Magona, J. W., Walubengo, J. & Odimin, J. T., 2008**. Acute haemorrhagic syndrome of bovine trypanosomosis in Uganda. *Acta Tropica,* **107** (2): 186-191.

National Livestock Resources Research Institute, Tororo, Uganda. [magonaw@live.com]

A study was undertaken in July 2005 to investigate an acute haemorrhagic syndrome that caused cattle mortality starting March 2005 in Mifumi, Senda, Kainja and Nyagoke villages of Tororo district in Uganda; areas dominantly infested with *Glossina fuscipes fuscipes* with scanty *G. pallidipes*. Four hundred and one (401) cattle belonging to 158 farmers were randomly sampled from a population of 549 and screened using a combination of haematocrit centrifugation technique (HCT) and buffy coat technique (BCT) for trypanosomosis. Of these animals 49 (12.2 percent) had trypanosome infections. Clinical cases manifested bleeding through the ears, severe weight loss, anaemia, weakness and enlarged lymph nodes prior to death. Out of an original population of 844 cattle 295 (35 percent) had died. The prevalence of bovine trypanosomosis in herds experiencing mortality

(21.5 percent) was significantly higher than in those without mortality (2.6 percent) ($p<0.001$). Herd size, number of draught oxen and lactating cows in a given herd significantly influenced the risk of mortality ($p<0.001$). Males had a significantly higher prevalence of trypanosomosis (17.8 percent) than females (9.5 percent) ($p <0.05$) and significantly lower mean packed cell volumes (PCV) (23.7 percent) than females (25.4 percent) ($p<0.05$). Older calves (7-12 months), yearlings (13-24 months) and adults (>24 months) with prevalences of 11.1 percent, 15.4 percent and 11.8 percent, respectively, were the most affected age categories. Trypanosome-infected cattle had a significantly lower mean PCV (17.9 percent) than non-infected ones (25.8 percent) ($p<0.001$), and a significantly higher proportion of anaemic animals (81.6 percent) than non-infected ones (37.2 percent) ($p<0.001$). *Trypanosoma vivax* was the dominant trypanosome species, constituting 82 percent of trypanosome infections. This work has provided further evidence on the importance of *T. vivax*-induced acute haemorrhagic syndrome in livestock trypanosomosis.

(c) TRYPANOTOLERANCE

[See also **31**: 14657]

14632. **Rennie, C., Hulme, H., Fisher, P., Halp, L., Agaba, M., Noyes, H. A., Kemp, S. J. & Brass, A., 2008**. A systematic, data-driven approach to the combined analysis of microarray and QTL data. *Developments in Biologicals (Basel),* **132**: 293-299.

School of Biological Sciences, Biosciences Building, Liverpool, UK. [catriona.rennie@postgrad.manchester.ac.uk].

High-throughput technologies inevitably produce vast quantities of data. This presents challenges in terms of developing effective analysis methods, particularly where the analysis involves combining data derived from different experimental technologies. In this investigation, a systematic approach was applied to combine microarray gene expression data, quantitative trait loci (QTL) data and pathway analysis resources in order to identify functional candidate genes underlying tolerance to *Trypanosoma congolense* infection in cattle. We automated much of the analysis using Taverna workflows previously developed for the study of trypanotolerance in the mouse model. Pathways represented by genes within the QTL regions were identified, and this list was subsequently ranked according to which pathways were over-represented in the set of genes that were differentially expressed (over time or between tolerant N'dama and susceptible Boran breeds) at various timepoints after *T. congolense* infection. The genes within the QTL that played a role in the highest ranked pathways were flagged as good targets for further investigation and experimental confirmation.

(d) TREATMENT

[See also **31**: 14550, 14556, 14557, 14675, 14691]

14633. **Delespaux, V., Geysen, D., Van den Bossche, P. & Geerts, S., 2008**. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. *Trends in Parasitology,* **24** (5): 236-242.

Animal Health Department, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. [vdelespaux@itg.be]

There are currently 17 African countries in which animal trypanocidal drug resistance has been reported. Large-scale surveys were carried out in only ten of them. The lack of baseline information is mainly due to the fact that the methods currently available for the detection of drug resistance are laborious, expensive and time consuming. In this review the mechanisms involved in resistance to isometamidium and diminazene will be discussed, together with some new molecular detection tools that have been developed recently enabling faster diagnosis of drug resistance than conventional laboratory or field tests.

14634. **Delespaux, V., Dinka, H., Masumu, J., Van den Bossche, P. & Geerts, S., 2008**. Five-fold increase in *Trypanosoma congolense* isolates resistant to diminazene aceturate over a seven-year period in Eastern Zambia. *Drug Resistance Updates,* **11** (6): 205-209.

Animal Health Department, Institute of Tropical Medicine (Antwerp), Nationalestraat 155, B-2000, Belgium. [vdelespaux@itg.be].

Two groups of *Trypanosoma congolense* isolates collected from cattle in 1996 (n=39) and 2003 (n=38) in the Eastern Province of Zambia were analyzed by BclI-PCR-RFLP to assess the evolution of diminazene aceturate (DA) resistance over a period of seven years. The results show a significant increase of DA resistance in this relatively short period of time. In 1996, among the 39 isolates, 61.5 percent were found sensitive, 12.8 percent resistant and 25.7 percent had a mixed BclI-PCR-RFLP profile. In 2004, among the 38 isolates, 10.5 percent were found sensitive, 63.2 percent were resistant and 26.3 percent showed a mixed BclI-PCR-RFLP profile. *In vivo* tests in mice showed that isolates with a sensitive or mixed RFLP profile were sensitive to DA whereas isolates with a resistant RFLP profile were resistant. Since there are no indications that the drug pressure has increased between 1996 and 2003, it is suggested that genetic exchange of resistance genes might explain the increased frequency of resistance to DA.

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

14635. **Fernández, D., González-Baradat, B., Eleizalde, M., González-Marcano, E., Perrone, T. & Mendoza, M., 2008**. *Trypanosoma evansi*: A comparison of PCR and parasitological diagnostic tests in experimentally infected mice. *Experimental Parasitology.* **e - Publication ahead of print 26 September 2008.**

Universidad Nacional Experimental Simón Rodríguez, Instituto de Estudios Cientificos y Tecnológicos (IDECYT), Centro de Estudios Biomédicos y Veterinarios, Apartado Postal 47925, Caracas 1041A, Venezuela.

Trypanosoma evansi is the causative agent of equine trypanosomosis, disease that affects the productivity and health of horses. Parasitological and molecular methods are mostly used to detect the infection. The aim of this work was to evaluate the sensitivity of

PCR to detect *T. evansi* using the primers 21/22-mer, ITS1, ESAG 6/7 and TBR 1/2 designed from repetitive (multicopies) genomic sequences. The results were compared with two parasitological tests in mice, the micro-haematocrit centrifugation technique and direct microscopic examination. The results showed (i) that the minimum amount of DNA from blood of highly parasitaemic mice that was detectable by PCR was 0.001 ng, using the ESAG6/7 and TBR1/2 primers, (ii) using TBR1/2 primer for purified parasites could detect 0.000001 ng and (iii) in the prepatent period, PCR detected the presence of parasites earlier than parasitological techniques. Nevertheless, the percentage of detection for PCR varied depending on the primer employed with 60 percent and 66 percent for ITS1 and 21/22-mer, and 80 percent for ESAG6/7 and TBR1/2 respectively. Consequently, TBR1/2 and ESAG6/7 were the best primers to monitor *T. evansi* infections in mice. For epidemiological application, such a comparative evaluation should be made for detection of *T. evansi* in livestock such as horses.

14636. **Fitzwater, S., Calderon, M., Lafuente, C., Galdos-Cardenas, G., Ferrufino, L., Verastegui, M., Gilman, R. H. & Bern, C., 2008**. Polymerase chain reaction for chronic *Trypanosoma cruzi* infection yields higher sensitivity in blood clot than buffy coat or whole blood specimens. *American Journal of Tropical Medicine and Hygiene,* **79** (5): 768-770.

Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA. [CBern@cdc.gov].

Trypanosoma cruzi polymerase chain reaction (PCR) is widely used, but sensitivity varies widely. We compared PCR using 121/122 primers targeting kinetoplast minicircle DNA in whole blood, buffy coat, and clot from Bolivian women. Sensitivity was significantly higher in clot (60.1 percent) than buffy coat (46.5 percent) or whole blood (40 percent). The use of clot could simplify specimen collection while improving sensitivity.

14637. **Mugasa, C. M., Schoone, G. J., Ekangu, R. A., Lubega, G. W., Kager, P. A. & Schallig, H. D., 2008**. Detection of *Trypanosoma brucei* parasites in blood samples using real-time nucleic acid sequence-based amplification. *Diagnostic Microbiology and Infectious Disease,* **61** (4): 440-445.

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

Currently, the conventional diagnosis of human African trypanosomiasis (HAT) is by microscopic demonstration of trypomastigotes in blood, lymph, and/or cerebrospinal fluid. However, microscopic diagnosis of HAT is not sensitive enough and may give false-negative results, thus, denying the patient the necessary treatment of the otherwise fatal disease. For this reason, a highly sensitive technique needs to be developed to enhance case findings. In this study, the real-time nucleic acid sequence-based amplification assay described is based on amplification and concurrent detection of small subunit rRNA (18S rRNA) of *Trypanosoma brucei*. The sensitivity of the assay was evaluated on nucleic acid from *in vitro* cultured parasites and blood spiked with various parasites quantities. The assay detected 10 parasites/mL using cultured parasites as well as spiked blood. A sensitive assay such as the

one developed in this study may become an alternative tool to confirm diagnosis of human African trypanosomiasis.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **31**: 14706, 14716, 14741, 14744, 14749, 14755, 14783, 14789, 14798]

14638. **Abdulla, M. H., O'Brien, T., Mackey, Z. B., Sajid, M., Grab, D. J. & McKerrow, J. H., 2008**. RNA interference of *Trypanosoma brucei* cathepsin B and L affects disease progression in a mouse model. *PLoS Neglected Tropical Diseases,* **2** (9): e298.

Sandler Center for Basic Research in Parasitic Diseases, California Institute for Quantitative Biomedical Research, University of California San Francisco, San Francisco, California, USA. [maha.abdulla@ucsf.edu].

We investigated the roles played by the cysteine proteases cathepsin B and cathepsin L (brucipain) in the pathogenesis of *Trypanosoma brucei brucei* in both an *in vivo* mouse model and an *in vitro* model of the blood-brain barrier. Doxycycline induction of RNAi targeting cathepsin B led to parasite clearance from the bloodstream and prevented a lethal infection in the mice. In contrast, all mice infected with *T. brucei* containing the uninduced *Trypanosoma brucei* cathepsin B (*Tb*CatB) RNA construct died by day 13. Induction of RNAi against brucipain did not cure mice from infection; however, 50 percent of these mice survived 60 days longer than uninduced controls. The ability of *T. b. brucei* to cross an *in vitro* model of the human blood-brain barrier was also reduced by brucipain RNAi induction. Taken together, the data suggest that while *Tb*CatB is the more likely target for the development of new chemotherapy, a possible role for brucipain is in facilitating parasite entry into the brain.

14639. **Antoine-Moussiaux, N., Saerens, D. & Desmecht, D., 2008**. Flow cytometric enumeration of parasitaemia and haematologic changes in *Trypanosoma*-infected mice. *Acta Tropica,* **107** (2): 139-144.

Department of Animal Pathology, Faculty of Veterinary Medicine, Sart Tilman B43, University of Liege (ULg), Liege, Belgium.[nantoine@ulg.ac.be].

African trypanosomiasis is a severe parasitic disease affecting both man and livestock. It is crucial to expand our fundamental knowledge of the intimate interactions between trypanosomes and their vertebrate hosts in order to develop new and efficient control strategies. The mouse model of trypanosomiasis is the most popular for research purposes because of all the logistic advantages of using this species. Studies of any aspect of trypanosomiases in the mouse systematically require the quantification of some phenotypic traits which translate its degree of resistance/susceptibility to the disease, as blood cell counts. The present study presents a methodological approach combining everyday micro sampling of tail blood and its analysis by flow cytometry. The technical options and conditions permitting a fast, reliable and reproducible daily quantification of erythrocyte, reticulocyte, leucocyte and trypanosome counts in the inoculated mouse were established. The protocol proposed allows the multiplication of blood samplings without being exposed to

the time-consuming constraint of visual countings, without causing iatrogenic blood cell alterations in the mouse and without requiring specific anti-trypanosome antibodies.

14640. **Barkhuizen, M., Magez, S., Ryffel, B. & Brombacher, F., 2008**. Interleukin-12p70 deficiency increases survival and diminishes pathology in *Trypanosoma congolense* infection. *Journal of Infectious Diseases,* **198** (9): 1284-1291.

Division of Immunology, Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa; Laboratory of Cellular and Molecular Immunology, Department of Molecular and Cellular Recognition, Flanders Interuniversity for Biotechnology, Vrije Universiteit Brussels, Brussels, Belgium; Université d'Orleans, Centre National de la Recherche Scientifique, Molecular Immunology and Embryology, Orléans, France. [fbrombac@mweb.co.za].

To determine the immunological role played by interleukin (IL)-12 family members in *Trypanosoma congolense* infection, IL-12p35(-/-), IL-12p40(-/-), and IL-12p35(-/-)/p40(-/-) mice were used. While the latter two strains lack all IL-12 homologues, IL-12p35(-/-) mice still produce IL-12p80 homodimers and IL-23. Compared with wild-type mice, all infected IL-12-deficient mouse strains showed prolonged survival, whereas parasitaemia levels were unaltered. Interferon (IFN)-gamma production in IL-12-deficient mice was strikingly reduced during the acute and chronic stages of infection, coinciding with significantly reduced chronic-stage hepatocellular damage, as demonstrated by histological analysis and plasma aspartate transaminase measurements. In contrast, IL-10 production was not affected by the absence of IL-12. Taken together, these results show that, during *T. congolense* infection, the absence of IL-12, but not the IL-12p80 homodimer or IL-23, leads to a reduction in IFN-gamma production, which reduces hepatic pathology and improves host survival in conjunction with IL-10 without negatively affecting parasitaemia control.

14641. **de Abreu Vieira, P. M., Francisco, A. F., de Souza, S. M., Malaquias, L. C., Reis, A. B., Giunchetti, R. C., Veloso, V. M., de Lana, M., Tafuri, W. L. & Carneiro, C. M., 2008**. *Trypanosoma cruzi*: serum levels of nitric oxide and expression of inducible nitric oxide synthase in myocardium and spleen of dogs in the acute stage of infection with metacyclic or blood trypomastigotes. *Experimental Parasitology*. **e - Publication ahead of print 18 October 2008**.

Núcleo de Pesquisas em Ciências Biológicas (NUPEB), Instituto de Ciências Exatas e Biológicas (ICEB), Universidade Federal de Ouro Preto (UFOP), Campus Universitário Morro do Cruzeiro, 35 400-000 Ouro Preto, MG, Brazil.

The participation of nitric oxide (NO) in the control of blood parasitaemia and parasitism during the acute phase of infection in dogs inoculated with blood trypomastigotes (BT) or metacyclic trypomastigotes (MT group) of Berenice-78 *Trypanosoma cruzi* strain was evaluated. Animals of the MT group (n=4) presented increased levels of serum NO throughout the infection when compared with the BT (n=4) or control (n=4) groups, and a delay in parasitaemia peak compared with the BT group. In spleen fragments, tissue parasitism was not observed but the MT group presented larger areas associated with

inducible NO synthase (iNOS) in relation to BT and control groups. Heart fragments of MT-infected animals exhibited comparatively low tissue parasitism and high iNOS expression, while animals of the BT group presented high inflammatory infiltrate, high tissue parasitism and low iNOS expression. These results indicate that the source of inoculum can interfere with the development of the acute phase of Chagas disease, and may also trigger a distinct parasite-host interaction during this phase.

14642. **Dagenais, T. R., Demick, K. P., Bangs, J. D., Forest, K., Paulnock, D. M. & Mansfield, J. M., 2008**. T cell responses to the trypanosome variant surface glycoprotein are not limited to hypervariable subregions. *Infection and Immunity*. **Online Publication ahead of print 20 October 2008**

Departments of Bacteriology, and Medical Microbiology and Immunology, Microbial Sciences Building, 1550 Linden Drive, University of Wisconsin-Madison, Madison, WI 53706 USA. [mansfield@bact.wisc.edu].

Variable subregions within the variant surface glycoprotein (VSG) coat displayed by African trypanosomes are predicted sites for T and B cell recognition. Hypervariable subregion 1 (HV-1) is localized to an internal amphipathic alpha helix in VSG monomers and may have evolved due to selective pressure by host T cell responses to epitopes within this subregion. The prediction of TCR-reactive sites and MHC-II binding motifs within the HV-1 subregion, coupled with the conservation of amino acid residues in other regions of the molecule sufficient to maintain secondary and tertiary VSG structure, prompted us to test the hypothesis that Th cells may preferentially recognize HV-1 subregion peptides. Thus, we examined the fine specificity of VSG-specific T cell lines, T cell hybridomas and Th cells activated during infection. Our results demonstrate that T cell epitopes are distributed throughout the N-terminal domain of VSG but are not clustered exclusively within HV-1 or other hypervariable subregions. In contrast, T cell reactive sites were not detected within the relatively conserved C-terminal domain of VSG. Overall, this study is the first to dissect the fine specificity of T cell responses to the trypanosome VSG and suggests that evolution of a conserved HV-1 region may be unrelated to selective pressures exerted by host T cell responses. This study also demonstrates that T cells do not recognize the relatively invariant C-terminal region of the VSG molecule during infection, suggesting that it could serve as a potential subunit vaccine to provide variant cross-specific immunity for African trypanosomiasis.

14643. **Fatihu, M. Y., Adamu, S., Umar, I. A., Ibrahim, N. D., Eduvie, L. O. & Esievo, K. A., 2008**. Studies on effects of lactose on experimental *Trypanosoma vivax* infection in Zebu cattle. 1. Plasma kinetics of intravenously administered lactose at onset of infection and pathology. *Onderstepoort Journal of Veterinary Research,* **75** (2): 163-172.

Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, Nigeria. [myfatihuy@yahoo.com]

Lactose in normal saline was administered intravenously to a group of Zebu cattle infected with *Trypanosoma vivax* to determine the blood plasma kinetics at onset of an experimental infection and its ability to protect tissues against damage as part of preliminary

studies to determine its suitability for use in the treatment of trypanosomosis. Significantly ($p < 0.01$) higher lactose concentrations were observed in the *T. vivax*-infected bulls at 30 min and one h ($p< 0.05$) post-infection (p.i.) and by four h p.i. the plasma lactose remained above the level prior to infusion, after which it fell slightly below the pre-infusion level in the uninfected group. Calculated pharmacokinetic parameters revealed delayed excretion of lactose in the *T. vivax*-infected group soon after infection. The total body clearance (Cl (B)) was significantly ($p< 0.05$) reduced. The biological half-life ($t_{1/2}$), elimination rate constant (k(el)) and apparent volume of distribution (V(d)) were relatively decreased ($p> 0.05$) as a result of the *T. vivax* infection. Retention of lactose in the plasma was attributed to decreased plasma clearance. It is suggested that the presence of trypanosomes in circulation rather than organic lesions could have been responsible for the delay observed in the excretion of lactose. At 12 weeks p.i., when the experiment was terminated, the group infected and given lactose infusion (despite higher parasitaemia) had no gross or histopathological lesions in the brain, spleen, lymph nodes, heart, kidneys, liver and testes. However, the group infected but not infused with lactose were emaciated, had pale mucosae, watery blood, general muscular atrophy, serous atrophy of coronary fat and other adipose tissue, hepatomegaly, splenomegaly, swollen and oedematous lymph nodes, all of which are suggestive of trypanosomosis. Histopathological lesions included narrowing of Bowman's space and hypercellularity of glomerular tufts in the kidneys with the mean glomerular tuft nuclear indices (GTNs) in the group significantly higher ($p< 0.01$) than the mean GTNs of the lactose-infused and control bulls. Degenerative changes occurred in the myocardium, spleen, testes and epididymides. The testicular and epididymal lesions are indicative of male reproductive dysfunction.

14644. **Forlenza, M., Scharsack, J. P., Kachamakova, N. M., Taverne-Thiele, A. J., Rombout, J. H. & Wiegertjes, G. F., 2008**. Differential contribution of neutrophilic granulocytes and macrophages to nitrosative stress in a host-parasite animal model. *Molecular Immunology,* **45** (11): 3178-3189.

Department of Animal Sciences, Cell Biology and Immunology Group, Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

Tyrosine nitration is a hallmark for nitrosative stress caused by the release of reactive oxygen and nitrogen species by activated macrophages and neutrophilic granulocytes at sites of inflammation and infection. In the first part of the study, we used an informative host-parasite animal model to describe the differential contribution of macrophages and neutrophilic granulocytes to *in vivo* tissue nitration. With this aim common carp (*Cyprinus carpio*) were infected with the extracellular blood parasite *Trypanoplasma borreli* (Kinetoplastida). After infection, serum nitrite levels significantly increased concurrently to the upregulation of inducible nitric oxide synthase (iNOS) gene expression. Tyrosine nitration, as measured by immunohistochemistry using an anti-nitrotyrosine antibody, dramatically increased in tissues from parasite-infected fish, demonstrating that elevated NO production during *T. borreli* infection coincides with nitrosative stress in immunologically active tissues. The combined use of an anti-nitrotyrosine antibody with a panel of monoclonal antibodies specific for several carp leucocytes, revealed that fish neutrophilic granulocytes strongly contribute to *in vivo* tissue nitration most likely through both, a peroxynitrite- and an MPO-mediated mechanism. Conversely, fish macrophages, by restricting the presence of

radicals and enzymes to their intraphagosomal compartment, contribute to a much lesser extent to *in vivo* tissue nitration. In the second part of the study, we examined the effects of nitrosative stress on the parasite itself. Peroxynitrite, but not NO donor substances, exerted strong cytotoxicity on the parasite *in vitro*. *In vivo*, however, nitration of *T. borreli* was limited if not absent despite the presence of parasites in highly nitrated tissue areas. Further, we investigated parasite susceptibility to the human anti-trypanosome drug Melarsoprol (Arsobal), which directly interferes with the parasite-specific trypanothione anti-oxidant system. Arsobal treatment strongly decreased *T. borreli* viability both *in vitro* and *in vivo*. All together, our data suggest an evolutionary conservation in modern bony fish of the function of neutrophilic granulocytes and macrophages in the nitration process and support the common carp as a suitable animal model for investigations on nitrosative stress in host-parasite interactions. The potential of *T. borreli* to serve as an alternative tool for pharmacological studies on human anti-trypanosome drugs is discussed.

14645. **Guilliams, M., Bosschaerts, T., Herin, M., Hunig, T., Loi, P., Flamand, V., De Baetselier, P. & Beschin, A., 2008**. Experimental expansion of the regulatory T cell population increases resistance to African trypanosomiasis. *Journal of Infectious Diseases,* **198** (5): 781-791.

Department of Molecular and Cellular Interactions, Vlaams Instituut voor Biotechnologie, Vrije Universiteit Brussel, Brussels, Belgium. [abeschin@vub.ac.be].

Inflammatory responses mounted to eliminate parasites can be lethal if not counterbalanced by regulatory responses protecting the host from collateral tissue damage. Here, we show that the maintained inflammation associated with tissue damage, anaemia, and reduced survival of *Trypanosoma brucei*-infected mice correlates with the absence of the expansion of the regulatory T (T (reg)) cell population. Induction of T (reg) cell expansion via CD28 superagonist antibody treatment in these mice down-regulated interferon-gamma production by T cells and tumour necrosis factor-alpha and reactive oxygen species production by classically activated macrophages, triggered the development of alternatively activated macrophages, delayed the onset of liver injury, diminished the anaemia burden, and prolonged the survival of infected animals. Thus, triggering the expansion of the T (reg) cell population coupled with the induction of alternatively activated macrophages can restore the balance between pro- and anti-inflammatory signals and thereby limit the pathogenicity of African trypanosomiasis.

14646. **Karori, S. M., Ngure, R. M., Wachira, F. N., Wanyoko, J. K. & Mwangi, J. N., 2008**. Different types of tea products attenuate inflammation induced in *Trypanosoma brucei* infected mice. *Parasitology International,* **57** (3): 325-333.

Department of Biochemistry and Molecular Biology, Egerton University, PO Box 536, Egerton, Kenya. [fwachira@yahoo.com].

An *in vivo* study was carried out to determine the effect of different types of Kenyan tea extracts on male Swiss albino mice infected with *Trypanosoma brucei brucei* isolate KETRI 2710. The isolate produced a similar clinical picture after a pre-patent period of five days post-infection (dpi). Parasitaemia levels in the untreated mice and those given different

teas developed exponentially at similar rates reaching similar densities at the peak of parasitaemia eight dpi. Between nine and 13 dpi parasitaemia decreased more rapidly in tea treated compared with the untreated mice which indicated that tea lowered parasitaemia level. Anaemia indicated by a fall in erythrocyte packed cell volume (PCV) occurred within four dpi and remained below the normal levels until the terminal stages of the disease. A significant difference ($p<0.05$) was observed 11 dpi between the tea treated and the untreated mice indicating that tea enhanced resistance to erythrocyte destruction. Mice treated with tea exhibited significantly ($p<0.01$) reduced parasite-induced hypoalbuminaemia as compared to the untreated. Since albumin is a negative acute phase protein, it shows a decrease during inflammatory conditions and therefore its elevation in the mice given tea in this study clearly demonstrated that tea ameliorated inflammation induced by *T. b. brucei*. Although green and white teas were superior in most of these characteristics, black tea, which is the principal tea product from Kenya, displayed remarkable properties, some even comparable to those of green tea. Interestingly, tea was more efficacious than dexamethasone an established anti-inflammatory drug, demonstrating its therapeutic potential.

14647. **Lemos, K. R., Marques, L. C., Aquino, L. P., Alessi, A. C. & Zacarias, R. Z., 2008**. Astrocytic and microglial response and histopathological changes in the brain of horses with experimental chronic *Trypanosoma evansi* infection. *Revista do Instituto de Medicina Tropical de São Paulo,* **50** (4): 243-249.

Departamento de Medicina Veterinaria, UNICENTRO, Guarapuava, PR, Brasil. [krlemos@yahoo.com.br]

This study aimed to characterize astrocytic and microglial response in the central nervous system (CNS) of equines experimentally infected with *T. evansi*. The experimental group comprised males and females with various degrees of crossbreeding, ages between four and seven years. The animals were inoculated intravenously with 10^6 trypomastigotes of *T. evansi* originally isolated from a naturally infected dog. All equines inoculated with *T. evansi* were observed until they presented symptoms of CNS disturbance, characterized by motor incoordination of the pelvic limbs, which occurred 67 days after inoculation (dai) and 124 dai. The animals in the control group did not present any clinical symptom and were observed up to the 125^{th} dai. For this purpose the HE histochemical stain and the avidin biotin peroxidase method was used. Lesions in the CNS of experimentally infected horses were those of a widespread non-suppurative meningoencephalomyelitis.The severity of lesions varied in different parts of the nervous system, reflecting an irregular distribution of inflammatory vascular changes. The infiltration of mononuclear cells was associated with anisomorphic gliosis and reactive microglia was identified. The intensity of the astrocytic response in the CNS of the equines infected with *T. evansi* characterizes the importance of the performance of these cells in this trypanosomiasis. The characteristic gliosis observed in the animals in this experiment suggests the ability of these cells as mediators of immune response. The parasite, *T. evansi*, was not identified in the nervous tissues.

14648. **Li, S. Q., Yang, W. B., Lun, Z. R., Ma, L. J., Xi, S. M., Chen, Q. L., Song, X. W., Kang, J. & Yang, L. Z., 2008**. Immunization with recombinant actin from *Trypanosoma evansi* induces protective immunity against *T. evansi, T. equiperdum* and *T. b. brucei* infection. *Parasitology Research*. **e - Publication ahead of print 16 October 2008.**

Department of Biochemistry and Molecular Biology, Medical College, Henan University of Science and Technology, Luoyang, 471003, People's Republic of China. [sanqiangli2001@163.com].

The actin gene of *Trypanosoma evansi* (STIB 806) was cloned and expressed in *Escherichia coli*. The predicted amino acid sequence of *T. evansi* actin shows 100 percent, 98.7 percent, and 93.1 percent, homology with *Trypanosoma equiperdum, Trypanosoma brucei brucei*, and *Trypanosoma cruzi*. Recombinant actin was expressed as inclusion bodies in *E. coli*. It was purified and renatured for immunological studies. Mice immunized with the renatured recombinant actin were protected from lethal challenge with *T. evansi* STIB 806, *T. equiperdum* STIB 818, and *T. b. brucei* STIB 940, showing 63.3 percent, 56.7 percent, and 53.3 percent protection, respectively. Serum collected from the rabbit immunized with recombinant actin inhibited the growth of *T. evansi, T. equiperdum*, and *T. b. brucei in vitro* cultivation. Serum from mice and rabbits immunized with recombinant actin only recognized *T. evansi* actin but not mouse actin. The results of this study suggest that the recombinant *T. evansi* actin induces protective immunity against *T. evansi, T. equiperdum*, and *T. b. brucei* infection and may be useful in the development of a vaccine with other cytoskeletal proteins to prevent animal trypanosomiasis caused by these three trypanosome species.

14649. **Lopez, R., Demick, K. P., Mansfield, J. M. & Paulnock, D. M., 2008**. Type I IFNs play a role in early resistance, but subsequent susceptibility, to the African trypanosomes. *Journal of Immunology,* **181** (7): 4908-4917.

Department of Medical Microbiology and Immunology, University of Wisconsin Medical School, Madison, WI 53706, USA. [paulnock@wisc.edu].

Macrophages express a spectrum of proinflammatory and regulatory mediators during African trypanosomiasis. Microarray analyses revealed similar profiles of induced genes in macrophages stimulated with the trypanosome soluble variant surface glycoprotein *in vitro* and in macrophages taken from infected mice. Genes associated with the acute phase response and with type 1 IFN responses were prominent components of the macrophage activation profiles expressed within 72 h *in vitro* and *in vivo.* Thus, induction of proinflammatory gene expression is a characteristic of early trypanosome infection that is driven primarily by soluble variant surface glycoprotein exposure, and it may be that IFN-alpha/beta plays a central role in regulation of early resistance to trypanosomes. To test this hypothesis, we assessed parameters of infection in mouse strains with genetic alterations in the IFN-alpha/beta response pathway. We found that Ifnar1(-/-) mice, which lack the receptor for type 1 IFNs, exhibited delayed control of parasite burden during the first week of infection and died earlier than did wild-type controls. However, infection of Ubp43(-/-) mice, which are hyperresponsive to type 1 IFNs, did not exhibit enhanced resistance to trypanosomes. Instead, these animals also failed to control parasite burden and were more susceptible than wild-type animals. Additionally, the Ubp43(-/-) mice exhibited a significant defect in IFN-gamma production, which is definitively linked to host resistance in trypanosomiasis. These results show that type 1 IFNs play a role in early control of parasites in infected mice but may contribute to down-regulation of IFN-gamma production and subsequent loss of host resistance later in infection.

14650. **Magez, S., Schwegmann, A., Atkinson, R., Claes, F., Drennan, M., De Baetselier, P. & Brombacher, F., 2008**. The role of B-cells and IgM antibodies in parasitaemia, anaemia, and VSG switching in *Trypanosoma brucei*-infected mice. *PLoS Pathogens,* **4** (8): e1000122.

Division of Immunology, Institute for Infectious Diseases and Molecular Medicine (IIDMM), Health Science Faculty, University of Cape Town, and International Centre for Genetic Engineering and Biotechnology (ICGEB), Cape Town, South Africa. [fbrombac@mweb.co.za].

African trypanosomes are extracellular parasitic protozoa, predominantly transmitted by the bite of the haematophagic tsetse fly. The main mechanism considered to mediate parasitaemia control in a mammalian host is the continuous interaction between antibodies and the parasite surface, covered by variant-specific surface glycoproteins. Early experimental studies have shown that B-cell responses can be strongly protective but are limited by their VSG-specificity. We have used B-cell (µMT) and IgM-deficient (IgM(-/-)) mice to investigate the role of B-cells and IgM antibodies in parasitaemia control and the *in vivo* induction of trypanosomiasis-associated anaemia. These infection studies revealed that that the initial setting of peak levels of parasitaemia in *Trypanosoma brucei*-infected µMT and IgM(-/-) mice occurred independent of the presence of B-cells. However, B-cells helped to periodically reduce circulating parasites levels and were required for long term survival, while IgM antibodies played only a limited role in this process. Infection-associated anaemia, hypothesized to be mediated by B-cell responses, was induced during infection in µMT mice as well as in IgM(-/-) mice, and as such occurred independently from the infection-induced host antibody response. Antigenic variation, the main immune evasion mechanism of African trypanosomes, occurred independently from host antibody responses against the parasite's ever-changing antigenic glycoprotein coat. Collectively, these results demonstrated that in murine experimental *T. brucei* trypanosomiasis, B-cells were crucial for periodic peak parasitaemia clearance, whereas parasite-induced IgM antibodies played only a limited role in the outcome of the infection.

14651. **Manarin, R., Bottasso, E., Bottasso, O., Serra, E. & Revelli, S., 2008**. Beneficial effects of benznidazole during an infectious-based situation of systemic inflammatory response: caecal ligation and puncture. *American Journal of Tropical Medicine and Hygiene,* **79** (5): 793-796.

Instituto de Inmunologia, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Rosario, Argentina. [r.manarin@yahoo.com.ar].

We have shown that benznidazole (BZL), a drug used to treat Chagas disease, markedly reduced the production of pro-inflammatory cytokines and NO-derived metabolites in experimentally *Trypanosoma cruzi*-infected rats. Treatment with BZL exerted beneficial effects in a model of inflammation-based pathology like murine experimental endotoxaemia. Based on these findings, we wished to ascertain the effect of BZL in a closer situation to sepsis: the caecal ligation and puncture (CLP) model in C57BL/6 mice. We analyzed clinical course, survival, circulating levels of inflammation-related compounds (NO, tumour necrosis factor [TNF]-alpha), and bacteraemia. Recipients of BZL, 25 mg/kg, had an increased survival rate at 24 hours after CLP, showing a better clinical situation and a significant reduction of TNF-alpha levels and bacteraemia, with respect to the other groups. BZL failed

to inhibit *in vitro* bacterial growth, suggesting that these effects may be partly caused by the immunomodulatory effects of BZL.

14652. **Masocha, W., Amin, D. N., Kristensson, K. & Rottenberg, M. E., 2008**. Differential invasion of *Trypanosoma brucei brucei* and lymphocytes into the brain of C57BL/6 and 129Sv/Ev mice. *Scandanavian Journal of Immunology,* **68** (5): 484-491.

Department of Applied Therapeutics, Faculty of Pharmacy, Kuwait University, Safat, Kuwait. [masocha@hsc.edu.kw]

Trypanosoma brucei subspecies invade the brain parenchyma at late stages of human and experimental rodent infections. In this study, we compared the outcome of infection with *T. b. brucei* in MHC-matched (H-2b) C57BL/6 (B6) and 129Sv/Ev (Sv-129) mice. Sv-129 showed higher parasitaemia and lower specific IgM (but not IgG) antibody levels than B6 mice. The number of trypanosomes, CD4+ and CD8+ T cells in the brain parenchyma was higher in B6 mice. B6 mice lost weight and showed higher cumulative mortality when compared with Sv-129 mice. Higher levels of IL-1beta, IL-6, IL-10, TNF-alpha, IFN-gamma, ICAM-1 and E-selectin, but low levels of TGF-beta mRNA were present in brains of B6 when compared with Sv-129-infected mice. Thus, host genetics differentially determine the invasion of *T. b. brucei* into the brain parenchyma, which is paralleled by the severity of inflammation in the brain and course of the disease, but not by parasitaemia nor by antibody titres.

14653. **Mendoza, M., Uzcanga, G. L., Pacheco, R., Rojas, H., Carrasquel, L. M., Garcia-Marchan, Y., Serrano-Martin, X., Benaim, G., Bubis, J. & Mijares, A., 2008**. Anti-VSG antibodies induce an increase in *Trypanosoma evansi* intracellular Ca^{2+} concentration. *Parasitology,* **135** (11): 1303-1315.

Centro de Estudios Biomédicos y Veterinarios, Universidad Nacional Experimental Simón Rodriguez, Caracas, Venezuela. [memendoza@cantv.net].

Trypanosoma evansi and *Trypanosoma vivax* have shown a very high immunological cross-reactivity. Anti-*T. vivax* antibodies were used to monitor changes in the *T. evansi* intracellular Ca^{2+} concentration ($[Ca^{2+}]$) by fluorometric ratio imaging from single parasites. A short-time exposure of *T. evansi* parasites to sera from *T. vivax*-infected bovines induced an increase in $[Ca^{2+}]$, which generated their complete lysis. The parasite $[Ca^{2+}]$ boost was reduced but not eliminated in the absence of extracellular Ca^{2+} or following serum decomplementation. Decomplemented anti-*T. evansi* VSG antibodies also produced an increase in the parasite $[Ca^{2+}]$, in the presence of extracellular Ca^{2+}. Furthermore, this Ca^{2+} signal was reduced following blockage with Ni^{2+} or in the absence of extracellular Ca^{2+}, suggesting that this response was a combination of an influx of Ca^{2+} throughout membrane channels and a release of this ion from intracellular stores. The observed Ca^{2+} signal was specific since (i) it was completely eliminated following pre-incubation of the anti-VSG antibodies with the purified soluble VSG, and (ii) affinity-purified anti-VSG antibodies also generated an increase in $[Ca^{2+}]$ by measurements on single cells or parasite populations. We also showed that an increase of the *T. evansi* $[Ca^{2+}]$ by the calcium A-23187 ionophore led to VSG release from the parasite surface. In addition, *in vivo* immunofluorescence labelling

revealed that anti-VSG antibodies induced the formation of raft patches of VSG on the parasite surface. This is the first study to identify a ligand that is coupled to calcium flux in salivarian trypanosomes.

14654. **Molina-Portela, M. P., Samanovic, M. & Raper, J., 2008**. Distinct roles of apolipoprotein components within the trypanosome lytic factor complex revealed in a novel transgenic mouse model. *Journal of Experimental Medicine,* **205** (8): 1721-1728.

Department of Medical Parasitology, New York University Langone Medical Center/School of Medicine, New York, NY 10010, USA. [jr57@nyu.edu].

Humans express a unique subset of high-density lipoproteins (HDLs) called trypanosome lytic factors (TLFs) that kill many *Trypanosoma* parasite species. The proteins apolipoprotein (apo) A-I, apoL-I, and haptoglobin-related protein, which are involved in TLF structure and function, were expressed through the introduction of transgenes in mice to explore their physiological roles *in vivo*. Transgenic expression of human apolipoprotein L-I alone conferred trypanolytic activity *in vivo*. Co-expression of human apolipoprotein A-I and haptoglobin-related protein (Hpr) had an effect on the integration of apolipoprotein L-I into HDL, and both proteins were required to increase the specific activity of TLF, which was measurable *in vitro*. Unexpectedly, truncated apolipoprotein L-I devoid of the serum resistance gene interacting domain, which was previously shown to kill human infective trypanosomes, was not trypanolytic in transgenic mice despite being co-expressed with human apolipoprotein A-I and Hpr and incorporated into HDLs. We conclude that all three human apolipoproteins act cooperatively to achieve maximal killing capacity and that truncated apolipoprotein L-I does not function in transgenic animals.

14655. **Namangala, B., Yokoyama, N., Ikehara, Y., Taguchi, O., Tsujimura, K., Sugimoto, C. & Inoue, N., 2008**. Effect of CD4+CD25+ T cell-depletion on acute lethal infection of mice with *Trypanosoma congolense*. *Journal of Veterinary Medical Science,* **70** (8): 751-759.

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan. [ircpmi@obihiro.ac.jp].

Despite the immense socio-economic repercussions of African trypanosomosis (AT), there is currently no effective control measure against the disease. Characterization of mechanisms governing resistance and/or susceptibility to AT could suggest interventions that might lead to more effective disease control. The present study was designed in an attempt to address the possible role of CD4+CD25+ T cells during an acute lethal infection of mice with *Trypanosoma congolense*, the causative agent of AT in domestic animals, through selective depletion using anti-CD25 monoclonal antibody. Accordingly, CD4+CD25+ T-cell-depletion resulted in a significant reduction or delay in parasitaemia, pathology, and mortality, as compared to controls. The apparent resistance in CD4+CD25+-T-cell-depleted mice correlated with a profound suppression of Th2 cytokines *in vitro* and *in vivo*, culminating in a net Th1 cytokine environment. Cumulatively, these findings suggest that CD4+CD25+ T-cell- depletion improves the trypanotolerance of highly susceptible BALB/c mice acutely infected with the lethal *T. congolense*.

14656. **Ny, L., Li, H., Mukherjee, S., Persson, K., Holmqvist, B., Zhao, D., Shtutin, V., Huang, H., Weiss, L. M., Machado, F. S., Factor, S. M., Chan, J., Tanowitz, H. B. & Jelicks, L. A., 2008**. A magnetic resonance imaging study of intestinal dilation in *Trypanosoma cruzi*-infected mice deficient in nitric oxide synthase. *American Journal of Tropical Medicine and Hygiene,* **79** (5): 760-767.

Department of Oncology, Sahlgrenska University Hospital, SE-413 45 Goteborg, Sweden. [tanowitz@aecom.yu].

Infection with *Trypanosoma cruzi* causes megasyndromes of the gastrointestinal (GI) tract. We used magnetic resonance imaging (MRI) to monitor alterations in the GI tract of *T. cruzi*-infected mice, and to assess the role of nitric oxide (NO) in the development of intestinal dilation. Brazil strain-infected C57BL/6 wild-type (WT) mice exhibited dilatation of the intestines by 30 days post-infection. Average intestine lumen diameter increased by 72 percent. Levels of intestinal NO synthase (NOS) isoforms NOS2 and NOS3 were elevated in infected WT mice. Inflammation and ganglionitis were observed in all infected mice. Intestinal dilation was observed in infected WT, NOS1, NOS2, and NOS3 knockout mice. This study demonstrates that MRI is a useful tool to monitor intestinal dilation in living mice and that these alterations may begin during acute infection. Furthermore, our data strongly suggests that NO may not be the sole contributor to intestinal dysfunction resulting from this infection.

14657 **Osman, N. M., Kaila, G. J., Eltahir, H. A. & Abdel-Rahman, A. H., 2008**. Susceptibility of Sudanese Nubian goats, Nilotic dwarf goats and Garag ewes to experimental infection with a mechanically transmitted *Trypanosoma vivax* stock. *Pakistan Journal of Biological Science,* **11** (3): 472-475.

Central Veterinary Research Laboratories, Khartoum, Sudan.

The present study was conducted to study the susceptibility of two different types of Sudanese goats namely: Black Nubian, the Nilotic dwarf goats and ewes of the Garag type to experimental infection with *Trypanosoma vivax* stock isolated from cattle outside a tsetse area. The infection caused parasitaemia, anaemia and pyrexia in the infected goats. However, the Nilotic dwarf goats were more tolerant to the infection than the Nubian goats, showing significantly higher values of packed cell volume, haemoglobin concentration, total red and white blood cells counts and significantly lower parasitaemias and body temperatures. Garag ewes which were found to be susceptible to *T. vivax* infection showed different signs of anaemia and pyrexia; it is recommended that comparative studies should be conducted on the sensitivity of this and other Sudanese types of sheep to trypanosomosis.

14658. **Peck, R. F., Shiflett, A. M., Schwartz, K. J., McCann, A., Hajduk, S. L. & Bangs, J. D., 2008**. The LAMP-like protein p67 plays an essential role in the lysosome of African trypanosomes. *Molecular Microbiology,* **68** (4): 933-946.

Department of Medical Microbiology & Immunology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53706, USA. [jdbangs@wisc.edu].

RNAi knockdown was employed to study the function of p67, a lysosome-associated membrane protein (LAMP)-like type I transmembrane lysosomal glycoprotein in African trypanosomes. Conditional induction of p67 dsRNA resulted in specific approximately 90 percent reductions in *de novo* p67 synthesis in both mammalian bloodstream and procyclic insect-stage parasites. Bloodstream cell growth was severely retarded with extensive death after > 24 h of induction. Biosynthetic trafficking of residual p67 and of the soluble lysosomal protease trypanopain were unimpaired. Endocytosis of tomato lectin, a surrogate receptor-mediated cargo, was only mildly impaired (approximately 20 percent), but proper lysosomal targeting was unaffected. p67 ablation had dramatic effects on lysosomal morphology with gross enlargement (four- to five-fold) and internal membrane profiles reminiscent of autophagic vacuoles. Ablation of p67 expression rendered bloodstream trypanosomes refractory to lysis by human trypanolytic factor (TLF), a lysosomally activated host innate immune mediator. Similar effects on lysosomal morphology and TLF sensitivity were also obtained by two pharmacological agents that neutralize lysosomal pH--chloroquine and bafilomycin A1. Surprisingly, however, lysosomal pH was not affected in ablated cells suggesting that other physiological alterations must account for increased resistance to TLF. These results indicate p67 plays an essential role in maintenance of normal lysosomal structure and physiology in bloodstream-stage African trypanosomes.

14659. **Radwanska, M., Guirnalda, P., De Trez, C., Ryffel, B., Black, S. & Magez, S., 2008**. Trypanosomiasis-induced B cell apoptosis results in loss of protective anti-parasite antibody responses and abolishment of vaccine-induced memory responses. *PLoS Pathogens,* **4** (5): e1000078.

Laboratoire de Parasitologie, Université Libre de Bruxelles, ULB, Brussels, Belgium. [stemagez@vub.ac.be].

African trypanosomes of the *Trypanosoma brucei* species are extra-cellular parasites that cause human African trypanosomiasis (HAT) as well as infections in game animals and livestock. Trypanosomes are known to evade the immune response of their mammalian host by continuous antigenic variation of their surface coat. Here, we aim to demonstrate that in addition, trypanosomes (i) cause the loss of various B cell populations, (ii) disable the hosts' capacity to raise a long-lasting specific protective anti-parasite antibody response, and (iii) abrogate vaccine-induced protective response to a non-related human pathogen such as *Bordetella pertussis*. Using a mouse model for *T. brucei*, various B cell populations were analyzed by FACS at different time points of infection. The results show that during early onset of a *T. brucei* infection, spleen remodelling results in the rapid loss of the IgM(+) marginal zone (IgM(+)MZ) B cell population characterized as B220(+)IgM(High)IgD(Int) CD21(High)CD23(Low)CD1d(+)CD138(-). These cells, when isolated during the first peak of infection, stained positive for Annexin V and had increased caspase-3 enzyme activity. Elevated caspase-3 mRNA levels coincided with decreased mRNA levels of the anti-apoptotic Bcl-2 protein and BAFF receptor (BAFF-R), indicating the onset of apoptosis. Moreover, affected B cells became unresponsive to stimulation by BCR cross-linking with anti-IgM Fab fragments. *In vivo*, infection-induced loss of IgM(+) B cells coincided with the disappearance of protective variant-specific T-independent IgM responses, rendering the host rapidly susceptible to re-challenge with previously encountered parasites. Finally, using the well-established human diphtheria, tetanus, and *B. pertussis* (DTPa) vaccination model in

mice, we show that *T. brucei* infections abrogate vaccine-induced protective responses to a non-related pathogen such as *B. pertussis*. Infections with *T. brucei* parasites result in the rapid loss of T-cell independent IgM(+)MZ B cells that are normally functioning as the primary immune barrier against blood-borne pathogens. In addition, ongoing trypanosome infections results in the rapid loss of B cell responsiveness and prevent the induction of protective memory responses. Finally, trypanosome infections disable the host's capacity to recall vaccine-induced memory responses against non-related pathogens. In particular, this last result calls for detailed studies of the effect of HAT on memory recall responses in humans, prior to the planning of any mass vaccination campaign in HAT endemic areas.

14660. **Rosenkranz, V. & Wink, M., 2008**. Alkaloids induce programmed cell death in bloodstream forms of trypanosomes (*Trypanosoma b. brucei*). *Molecules,* **13** (10): 2462-2473.

Heidelberg University, Institute of Pharmacy and Molecular Biotechnology, INF 364, 69120 Heidelberg, Germany. [wink@uni-hd.de].

The potential induction of a programmed cell death (PCD) in *Trypanosoma b. brucei* by 55 alkaloids of the quinoline, quinolizidine, isoquinoline, indole, terpene, tropane, steroid, and piperidine type was studied by measuring DNA fragmentation and changes in mitochondrial membrane potential. For comparison, the induction of apoptosis by the same alkaloids in human leukaemia cells (Jurkat APO-S) was tested. Several alkaloids of the isoquinoline, quinoline, indole and steroidal type (berberine, chelerythrine, emetine, sanguinarine, quinine, ajmalicine, ergotamine, harmine, vinblastine, vincristine, colchicine, chaconine, demissidine and veratridine) induced programmed cell death, whereas quinolizidine, tropane, terpene and piperidine alkaloids were mostly inactive. Effective PCD induction (EC(50) below 10 µM) was caused in *T. brucei* by chelerythrine, emetine, sanguinarine, and chaconine. The active alkaloids can be characterized by their general property to inhibit protein biosynthesis, to intercalate DNA, to disturb membrane fluidity or to inhibit microtubule formation.

14661. **Scharfstein, J., Monteiro, A. C., Schmitz, V. & Svensjo, E., 2008**. Angiotensin-converting enzyme limits inflammation elicited by *Trypanosoma cruzi* cysteine proteases: a peripheral mechanism regulating adaptive immunity via the innate kinin pathway. *Biological Chemistry,* **389** (8): 1015-1024.

Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, 21949-900 Rio de Janeiro, Brazil. [scharf@biof.ufrj.br]

Tissue injury by pathogens induces a stereotyped inflammatory response that alerts the innate immune system of the potential threat to host integrity. Here, we review knowledge emerging from investigations of the role of the kinin system in the mechanisms that link innate to the adaptive phase of immunity. Progress in this field started with results demonstrating that bradykinin is an endogenous danger signal that induces dendritic cell (DC) maturation via G protein-coupled bradykinin B2 receptors (B2R). The immunostimulatory role of kinins was recently confirmed in two different mouse models of *Trypanosoma cruzi* infection, a parasitic protozoan equipped with kinin-releasing cysteine proteases (cruzipain). Infection by the intraperitoneal route showed that DCs from B2R-/-

mice (susceptible phenotype) failed to sense kinin "danger" signals proteolytically released by parasites, explaining why these mutant mice display lower frequencies of interferon-gamma-producing effector T-cells. Studies of the dynamics of inflammation in the subcutaneous model of infection revealed that the balance between cruzipain and angiotensin-converting enzyme, respectively acting as kinin-generating and degrading enzymes, governs extent of DC maturation and TH1 development via the B2R-dependent innate pathway. Studies of the kinin role in immunity may shed light on the relationship between proteolytic networks and the cytokine circuits that guide T-cell development.

14662. **Stijlemans, B., Vankrunkelsven, A., Brys, L., Magez, S. & De Baetselier, P., 2008.** Role of iron homeostasis in trypanosomiasis-associated anaemia. *Immunobiology*, **213** (9-10): 823-835.

Laboratory of Cellular and Molecular Immunology, VIB Department of Molecular and Cellular Interactions, Vrije Universiteit Brussel (VUB), Building E, Level 8, Pleinlaan 2, B-1050 Brussels, Belgium. [bstijlem@vub.ac.be].

Anaemia is a well-established infection-associated immunopathological feature of trypanosomiasis and the degree of the anaemia is a reliable indicator of the severity of infection. Since infections with trypanosomes trigger a strong cytokine production and a type I immune response, the trypanosome-elicited anaemia may be type I cytokine driven. This type of anaemia termed anaemia of chronic disease is characterized by an imbalance between erythrophagocytosis and erythropoiesis that is linked to a perturbed iron homeostasis including altered iron recycling by macrophages and iron sequestration. To further unravel the mechanisms underlying trypanosome-elicited anaemia the expression profile of genes involved in erythrophagocytosis, uptake of iron-containing complexes and iron homeostasis was performed during the acute and chronic phase of experimental *Trypanosoma brucei* infections in a murine model. The results suggest that liver-associated erythrophagocytosis mediated by cytokine-activated macrophages (M1 cells) is the most likely main initiating event of aggressive anaemia during the acute phase of infection. Persistence of strong type 1 cytokine production during the chronic phase of infection leads to hyper-activated M1 cells and a more progressive anaemia. RT-PCR analysis of liver tissue demonstrates a strong increase of cell surface receptors involved in uptake of RBC and iron-containing compounds. For genes involved in iron processing we found an increase of ferroportin-1 (FPN-1), transferrin (Tf) and ceruloplasmin (CP) only in the acute phase, suggesting that export of iron is hampered in the chronic phase of infection. Our results suggest that in the chronic phase of trypanosomiasis, the iron-processing pathway is skewed towards iron sequestration, as evidenced by increased ferritin expression, while enhanced uptake of RBC/iron-containing compounds is maintained.

14663. **Stockdale, C., Swiderski, M. R., Barry, J. D. & McCulloch, R., 2008**. Antigenic variation in *Trypanosoma brucei*: joining the DOTs. *PLoS Biology,* **6** (7): e185.

Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow Biomedical Research Centre, Glasgow, UK. [rmc9z@udcf.gla.ac.uk]

Abstract not available

14664. **Tabel, H., Wei, G. & Shi, M., 2008**. T cells and immunopathogenesis of experimental African trypanosomiasis. *Immunological Reviews,* **225** (1): 128-139.

Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK, Canada. [henry.tabel@usask.ca]

African trypanosomes are pathogens for humans and livestock. They are single-cell, extra-cellular parasites that cause persistent infections of the blood and induce profound immunosuppression. Here, we review recent work on experimental African trypanosomiasis, especially infections with *Trypanosoma congolense*, in mice with regard to mechanisms of immunosuppression and immunopathology. The centre of the immunopathology is the T-cell-independent production of antibodies to the variant surface glycoprotein (VSG) of trypanosomes, the anti-VSG antibody-mediated phagocytosis of trypanosomes by macrophages, and the subsequent profound dysregulation of the macrophage system. Depending on the genetics of the host and the parasite load, the malfunction of the macrophage system is enhanced by interferon-gamma produced by parasite-specific, major histocompatibility complex class II-restricted, matrix-adherent CD4(+) T cells or downregulated by interleukin-10 produced by parasite-specific, CD4(+)CD25(high) Forkhead box protein 3(+) regulatory T cells. There is a physiological conflict of the two relevant cytokines interleukin-10 and interferon-gamma in regulating the immunopathology versus regulating the induction and effect of protective immune responses. On the basis of very recent work in our laboratory, we propose a hypothetical model suggesting a cross-regulation of natural killer T cells and CD4(+)CD25(high) Forkhead box protein 3(+) regulatory T cells in experimental infections with *T. congolense*.

14665. **Thuita, J. K., Kagira, J. M., Mwangangi, D., Matovu, E., Turner, C. M. & Masiga, D., 2008**. *Trypanosoma brucei rhodesiense* transmitted by a single tsetse fly bite in vervet monkeys as a model of human African trypanosomiasis. *PLoS Neglected Tropical Diseases,* **2** (5): e238.

Trypanosomiasis Research Centre, (KARI-TRC), Kikuyu, Kenya. [dmasiga@icipe.org].

We have investigated the pathogenicity of tsetse (*Glossina pallidipes*)-transmitted cloned strains of *Trypanosoma brucei rhodesiense* in vervet monkeys. Tsetse flies were confirmed to have mature trypanosome infections by xenodiagnosis, after which nine monkeys were infected via the bite of a single infected fly. Chancres developed in five of the nine (55.6 percent) monkeys within four to eight days post infection (dpi). All nine individuals were successfully infected, with a median pre-patent period of four (range = 4-10) days, indicating that trypanosomes migrated from the site of fly bite to the systemic circulation rapidly and independently of the development of the chancre. The time lag to detection of parasites in cerebrospinal fluid (CSF) was a median 16 (range = 8-40) days, marking the onset of central nervous system (CNS, late) stage disease. Subsequently, CSF white cell numbers increased above the pre-infection median count of two (range = 0-9) cells/µL, with a positive linear association between their numbers and that of CSF trypanosomes. Haematological changes showed that the monkeys experienced an early microcytic-hypochromic anaemia and severe progressive thrombocytopaenia. Despite a three-fold increase in granulocyte numbers by four dpi, leucopaenia occurred early (8 dpi) in

the monkey infection, determined mainly by reductions in lymphocyte numbers. Terminally, leucocytosis was observed in three of nine (33 percent) individuals. The duration of infection was a median of 68 (range = 22-120) days. Strain and individual differences were observed in the severity of the clinical and clinical pathology findings, with two strains (KETRI 3741 and 3801) producing a more acute disease than the other two (KETRI 3804 and 3928). The study shows that the fly-transmitted model accurately mimics the human disease and is therefore a suitable gateway to understanding human African trypanosomiasis (HAT; sleeping sickness).

14666. **Vasconi, M. D., Malfante, P., Bassi, A., Giudici, C., Revelli, S., Di Masso, R., Font, M. T. & Hinrichsen, L., 2008**. Phenotypic differences on the outcome of the host-parasite relationship: behaviour of mice of the CBi stock in natural and experimental infections. *Veterinary Parasitology,* **153** (1-2): 157-163.

Cátedra de Parasitologia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina. [lhinrich@unr.edu.ar].

Investigation of defined animal models may help to elucidate the role of the host genetic background in the development and establishment of a parasitic infection. Four lines of mice obtained by disruptive selection for body conformation (CBi+, CBi-, CBi/C and CBi/L) and the unselected control line CBi were examined in their response to different parasites to assess whether these distinct genotypes showed differences in their resistance to natural and experimental parasitosis. Protozoans (*Trichomonas muris* and *Spironucleus muris*) and nemathelminths (*Syphacia obvelata* and *Aspiculurus tetraptera*) were found naturally parasitising the mices' intestines. CBi/C and CBi were the only genotypes in which *T. muris* was found. CBi- was least resistant to *S. muris*. The helminth parasitic burden showed differences between sexes within genotypes (males had a higher burden than females) and among genotypes (CBi/L males had the lowest burden). CBi/L animals were also most resistant to experimental challenge with *Heligmosomoides polygyrus* and *Trypanosoma cruzi*. Since all the animals examined shared a common habitat throughout the study and were equally exposed to infection, the phenotypic differences in the natural enteroparasitism herein described evince genetic differences among lines in the host-parasite relationship. This interpretation is further supported by the differences in the response to the experimental challenge to *H. polygyrus* and *T. cruzi.*

14667. **Vitelli-Avelar, D. M., Sathler-Avelar, R., Teixeira-Carvalho, A., Pinto Dias, J. C., Gontijo, E. D., Faria, A. M., Eloi-Santos, S. M. & Martins-Filho, O. A., 2008**. Strategy to assess the overall cytokine profile of circulating leukocytes and its association with distinct clinical forms of human Chagas disease. *Scandanavian Journal of Immunology,* **68** (5): 516-525.

Laboratório de Biomarcadores de Diagnóstico e Monitoração, Instituto René Rachou, Fundação Oswaldo Cruz, Belo Horizontem, Minas Gerais, Brazil. [vitelli@cpqrr.fiocruz.br]

An alternative strategy was employed to assess the cytokine patterns of circulating leukocytes and correlate dominant cytokine profiles with indeterminate-IND and cardiac-CARD clinical forms of Chagas disease. We first calculated the median percentages of cytokine-positive leukocytes of our study sample to establish, for each cytokine-positive cell

population, the cut-off edge that would segregate "low" and "high" cytokine producers to build colour diagrams and draw a panoramic cytokine chart. Using this approach we demonstrated that most IND individuals presented a dominant regulatory cytokine profile, whereas CARD individuals displayed a dominant inflammatory cytokine pattern. In addition, radar chart analysis confirmed the dichotomic cytokine balance between IND and CARD groups and further allowed the identification of the relative contribution of each cell population for the global cytokine pattern. Data analysis demonstrated that CD4+ T cells were the major cell population defining the regulatory profile in IND, whereas monocytes and CD4+ T cells determined the inflammatory cytokine pattern in CARD individuals. Interestingly, *in vitro* stimulation with trypomastigote *Trypanosoma cruzi* antigen was able to invert the cytokine balances in IND and CARD groups. Upon antigenic stimulation, changes in the frequencies of IL-10-producing CD4+ T cells and monocytes drove IND individuals towards an inflammatory pattern and CARD towards a regulatory cytokine profile. A similar inversion could be found after *in vivo* treatment of IND and CARD individuals with benzonidazole. These findings shed some light into the complex cytokine network underlying the immunopathogenesis of Chagas disease and provide putative immunological biomarkers of disease severity and therapeutic response.

14668. **Young, R., Taylor, J. E., Kurioka, A., Becker, M., Louis, E. J. & Rudenko, G., 2008**. Isolation and analysis of the genetic diversity of repertoires of VSG expression site containing telomeres from *Trypanosoma brucei gambiense, T. b. brucei* and *T. equiperdum*. *BMC Genomics,* **9**: 385.

Peter Medawar Building for Pathogen Research, University of Oxford, South Parks Road, Oxford, OX1 3SY, UK. [rosanna.young@green.ox.ac]

African trypanosomes (including *Trypanosoma brucei*) are unicellular parasites which multiply in the mammalian bloodstream. *T. brucei* has about twenty telomeric bloodstream form variant surface glycoprotein (VSG) expression sites (BESs), of which one is expressed at a time in a mutually exclusive fashion. BESs are polycistronic transcription units, containing a variety of families of expression site associated genes (ESAGs) in addition to the telomeric VSG. These polymorphic ESAG families are thought to play a role in parasite-host adaptation, and it has been proposed that ESAG diversity might be related to host range. Analysis of the genetic diversity of these telomeric gene families has been confounded by the underrepresentation of telomeric sequences in standard libraries. We have previously developed a method to selectively isolate sets of trypanosome BES containing telomeres using transformation associated recombination (TAR) cloning in yeast. Here we describe the isolation of repertoires of BES containing telomeres from three trypanosome subspecies: *Trypanosoma brucei gambiense* DAL 972 (causative agent of West African trypanosomiasis), *T. b. brucei* EATRO 2340 (a non-human infective strain) and *T. equiperdum* STIB 818 (which causes a sexually transmitted disease in equines). We have sequenced and analysed the genetic diversity at four BES loci (BES promoter region, ESAG6, ESAG5 and ESAG2) from these three trypanosome BES repertoires. With the exception of ESAG2, the BES sequence repertoires derived from *T. b. gambiense* are both less diverse than and nearly reciprocally monophyletic relative to those from *T. b. brucei* and *T. equiperdum*. Furthermore, although we find evidence for adaptive evolution in all three ESAG repertoires in *T. b. brucei* and *T. equiperdum*, only ESAG2 appears to be under diversifying selection in *T. b. gambiense.* This low level of variation in the *T. b. gambiense*

BES sequence repertoires is consistent both with the relatively narrow host range of this subspecies and its apparent long-term clonality. However, our data does not show a clear correlation between size of trypanosome host range and either number of BESs or extent of ESAG genetic diversity.

(c) CHEMOTHERAPEUTICS

[See also **31**: 14709, 14713, 14714, 14717, 14719, 14738, 14768, 14770, 14772, 14791, 14792]

14669 **Aderbauer, B., Clausen, P. H., Kershaw, O. & Melzig, M. F., 2008**. *In vitro* and *in viv*o trypanocidal effect of lipophilic extracts of medicinal plants from Mali and Burkina Faso. *Journal of Ethnopharmacology,* **119** (2): 225-231.

Department of Pharmacy, Freie Universität Berlin, Konigin-Luise-Street, 2+4, D-14195 Berlin, Germany. [melzig@zedat.fu-berlin.de].

To determine the *in vitro* and *in vivo* anti-trypanosomal activity of extracts of traditionally used plants, 47 dichloromethane extracts were tested *in vitro* in the long-term viability assay (LtVA) on *Trypanosoma brucei brucei*. The most active ones were also tested *in vivo* using a standardised mouse test. Thirteen extracts (28 percent) were active *in vitro* with MIC-values < or = 100 µg/ml, six extracts showed MIC-values < or = 50 µg/ml. The root extract of *Securidaca longepedunculata* Fresen. (*Polygalaceae*) and the leaf extract of *Guiera senegalensis* J. F. Gmel. (*Combretaceae*) were able to reduce parasitaemia in mice, experimentally infected with *Trypanosoma brucei brucei* by 48 percent and 42 percent at the dose of 150 mg/kg b.w. intraperitoneally, two times daily for three days. The extract of *Acacia nilotica* Delile (*Mimosaceae*) stem bark showed immunosuppressive effect *in vivo*. The results confirm an effect of the ethnobotanically used plants. Further investigation is needed to optimize the effectiveness of the extracts.

14670. **Arafa, R. K., Ismail, M. A., Munde, M., Wilson, W. D., Wenzler, T., Brun, R. & Boykin, D. W., 2008**. Novel linear triaryl guanidines, N-substituted guanidines and potential prodrugs as antiprotozoal agents. *European Journal of Medicinal Chemistry*. **In press, corrected proof.**

Department of Chemistry, Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303-3083, USA. [dboykin@gsu.edu].

A series of triaryl guanidines and N-substituted guanidines designed to target the minor groove of DNA were synthesized and evaluated as anti-protozoal agents. Selected carbamate prodrugs of these guanidines were assayed for their oral efficacy. The linear triaryl bis-guanidines 6a,b were prepared from their corresponding diamines 4a,b through the intermediate BOC protected bis-guanidines 5a,b followed by acid catalyzed deprotection. The N-substituted guanidino analogues 9c-f were obtained in three steps starting by reacting the diamines 4a,b with ethyl isothiocyanatoformate to give the carbamoyl thioureas 7a,b. Subsequent condensation of 7a,b with various amines in the presence of EDCI provided the carbamoyl N-substituted guanidine intermediates 8a-f which can also be regarded as potential prodrugs for the guanidino derivatives. Compounds 9c-f were obtained via the base catalyzed

decarbamoylation of 8a-f. The DNA binding affinities for the target dicationic bis-guanidines were assessed by DeltaT(m) values. *In vitro* anti-protozoal screening of the new compounds showed that derivatives 6a, 9c and 9e possess high to moderate activity against *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum*. While the prodrugs did not yield cures upon oral administration in the antitrypanosomal STIB900 mouse model, compounds 8a and 8c prolonged the survival of the treated mice.

14671. **Arrighi, R. B., Ebikeme, C., Jiang, Y., Ranford-Cartwright, L., Barrett, M. P., Langel, U. & Faye, I., 2008**. Cell-penetrating peptide TP10 shows broad spectrum activity against both *Plasmodium falciparum* and *Trypanosoma brucei brucei*. *Antimicrobial Agents and Chemotherapy,* **52** (9): 3414-3417.

Department of Genetics, Stockholm University, Stockholm, Sweden. [Ingrid.Faye@gmt.su.se].

Malaria and trypanosomiasis are diseases which afflict millions and for which novel therapies are urgently required. We have tested two well-characterized cell-penetrating peptides (CPPs) for antiparasitic activity. One CPP, designated TP10, has broad-spectrum antiparasitic activity against *Plasmodium falciparum*, both blood and mosquito stages, and against blood-stage *Trypanosoma brucei brucei.*

14672. **Bakshi, R. P., Sang, D., Morrell, A., Cushman, M. & Shapiro, T. A., 2008**. Activity of indenoisoquinolines against African trypanosomes. *Antimicrobial Agents and Chemotherapy*. **Published online ahead of print 29 September 2008**

Division of Clinical Pharmacology, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; Department of Medicinal Chemistry and Molecular Pharmacology and the Purdue Cancer Center, Purdue University, West Lafayette, IN 47907, USA.

African trypanosomiasis (sleeping sickness), caused by protozoan *Trypanosoma brucei* species, is a debilitating disease that is lethal if untreated. Available drugs are antiquated, toxic and compromised by emerging resistance. The indenoisoquinolines are a class of non-camptothecin topoisomerase IB poisons that are under development as anti-cancer agents. We tested a variety of indenoisoquinolines for their ability to kill *T. brucei*. Indenoisoquinolines proved trypanocidal at submicromolar concentrations *in vitro*. Structure-activity analysis yielded motifs that enhanced potency including alkylamino substitutions on N6, methoxy groups on C2 and C3, and a methylenedioxy bridge between C8 and C9. Detailed analysis of eight water-soluble indenoisoquinolines demonstrated that in trypanosomes the compounds inhibited DNA synthesis and acted as topoisomerase poisons. Testing these compounds on L1210 mouse leukaemia cells revealed that all eight were more effective against trypanosomes than against mammalian cells. In preliminary *in vivo* experiments one compound delayed parasitaemia and extended survival in mice subjected to a lethal trypanosome challenge. The indenoisoquinolines provide a promising lead for developing drugs against sleeping sickness.

14673. **Bakunov, S. A., Bakunova, S. M., Wenzler, T., Barszcz, T., Werbovetz, K. A., Brun, R. & Tidwell, R. R., 2008**. Synthesis and antiprotozoal activity of cationic 2-phenylbenzofurans. *Journal of Medicinal Chemistry,* **51** (21): 6927-6944.

Department of Pathology and Laboratory Medicine, School of Medicine, The University of North Carolina, Chapel Hill, North Carolina 27599-7525, USA. [Tidwell@med.unc.edu].

A series of cationically substituted 2-phenylbenzofurans 1- 49 have been synthesized, and their *in vitro* antiprotozoal properties against *Trypanosoma brucei rhodesiense, Plasmodium falciparum*, and *Leishmania donovani*, as well as cytotoxicity against mammalian cells, have been evaluated. Eight dications exhibited antitrypanosomal activities comparable to that of pentamidine and melarsoprol. Twenty six compounds were more active than pentamidine, and seven demonstrated increased activities against *P. falciparum* than artemisinin. Five congeners were more active against *L. donovani* than pentamidine. Introduction of methoxy or hydroxy groups in the 7- and/or 2'-position afforded derivatives that were highly selective against *T. b. rhodesiense, P. falciparum*, and *L. donovani*. Fourteen 2-phenylbenzofurans displayed excellent *in vivo* efficacies in the acute mouse model of trypanosomiasis, curing 3/4 or 4/4 animals at 4 x 5 mg/kg. Diamidine 1 and di (N-isopropyl)amidine 45, administered at 4 x 1 mg/kg, exhibited potency comparable to that of melarsoprol, providing 3/4 and 2/4 cures, respectively.

14674. **Barbaras, D., Kaiser, M., Brun, R. & Gademann, K., 2008**. Potent and selective antiplasmodial activity of the cyanobacterial alkaloid nostocarboline and its dimers. *Bioorganic and Medicinal Chemistry Letters,* **18** (15): 4413-4415.

Laboratorium fur Organische Chemie, Swiss Federal Institute of Technology (ETH), 8093 Zurich, Switzerland. [karl.gademann@epfl.ch].

The quaternary beta-carbolinium alkaloid nostocarboline from the cyanobacterium Nostoc 78-12A and 10 bis-cationic dimeric derivatives were evaluated against four protozoan parasites and low micromolar values against *Trypanosoma brucei*, submicromolar values against *Leishmania donovani* and low nanomolar values against *Plasmodium falciparum* K1 were determined. Selectivity against rat myoblasts (L6 cells) was found to be up to >2 500-fold.

14675. **Bawm, S., Matsuura, H., Elkhateeb, A., Nabeta, K., Subeki, Nonaka, N., Oku, Y. & Katakura, K., 2008**. *In vitro* antitrypanosomal activities of quassinoid compounds from the fruits of a medicinal plant, *Brucea javanica*. *Veterinary Parasitology*. **e - Publication ahead of print 26 September 2008.**

Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan. [kenkata@vetmed.hokudai.ac.jp].

The medicinal plant *Brucea javanica* (L.) Merr. (Simaroubaceae) is widely distributed throughout Asia where its bitter fruits have been used in traditional medicine for various ailments. Fifteen C-20 quassinoids were isolated from the fruits of *B. javanica* and examined

for their *in vitro* antitrypanosomal activities against trypomastigotes of *Trypanosoma evansi*. Bruceine A, bruceantinol, bruceine C, brusatol, and bruceine B showed strong anti-trypanosomal activities with IC(50) values in the range of 2.9-17.8nM, which compared well with the standard trypanocidal drugs diminazene aceturate (IC(50)=8.8nM) and suramin (IC(50)=43.2nM). However, dehydrobruceine A, dehydrobruceine B, and dehydrobrusatol were about 2 100, 900, and 1 200 times less active, respectively, than bruceine A, bruceine B, and brusatol. The relationship of the structure and antitrypanosomal activity of these quassinoid compounds suggested that the presence of a diosphenol moiety in ring A and the nature of the C-15 side chain are important for their activities against *T. evansi*. This is the first report on the antitrypanosomal activity of isolated quassinoids.

14676. **Bielawski, K., Bielawska, A., Poplawska, B. & Bolkun-Skornicka, U., 2008**. Synthesis, DNA-binding affinity and cytotoxicity of the dinuclear platinum(II) complexes with berenil and amines ligands. *Acta Poloniae Pharmaceutica,* **65** (3): 363-370.

Department of Medicinal Chemistry and Drug Technology, Medical University of Bialystok, Bialystok, Poland. [kbiel@amb.edu.pl]

14677. **Cerecetto, H. & González, M., 2008**. Anti-*T. cruzi* agents: Our experience in the evaluation of more than five hundred compounds. *Mini Reviews in Medicinal Chemistry,* **8** (13): 1355-1383.

Departamento de Química Orgánica. Facultad de Química y Facultad de Ciencias, Universidad de la República. Igúa 4225. 11400 Montevideo Uruguay. [hcerecet@fq.edu.uy/megonzal@fq.edu.uy].

Chagas disease is the major endemic disease in South and Central America caused by a trypanosomatid parasite (*Trypanosoma cruzi*). The current treatment relies on two old and non-specific chemotherapeutic agents, Nifurtimox and Benznidazole. Despite the major advances that have been made in the identification of specific targets that afford selectivity, the drugs used today have serious side effects. Furthermore, differences in drug susceptibility among different *T. cruzi* isolates have led to varied parasitological cure rates depending on the geographical region. There is, therefore, an urgent need for the development of new anti-chagasic drugs. In this regard we have spent more than a decade in the search for more effective agents able to compromise the proliferation of *T. cruzi*. We began our research with our own compounds and then continued with compounds from other research groups. We systematically characterized representatives of a wide range of different chemical families. In this review we summarize our ongoing efforts to identify potential anti-*T. cruzi* agents using our compound-library. The structure-activity relationship observed among the different groups of chemical families is presented and discussed.

14678. **Dua, V. K., Verma, G. & Dash, A. P., 2008**. *In vitro* antiprotozoal activity of some xanthones isolated from the roots of *Andrographis paniculata*. *Phytotherapy Research*. **Published online 6 August 2008.**

National Institute of Malaria Research, Field Unit, Sector III, BHEL, Hardwar 249 403, India. [vkdua51@gmail.com].

Four xanthones isolated from the roots of *Andrographis paniculata* were tested *in vitro* for antiprotozoal activity against *Trypanosoma brucei brucei, Trypanosoma cruzi* and *Leishmania infantum*. Compound TDR13011 (1,2-dihydroxy-6,8-dimethoxy-xanthone) showed good activity against *T. b. brucei* and *L. infantum* with a 50 percent inhibitory concentration (IC(50)) of 4.6 µg/ml and 8 µg/ml respectively. Xanthones from the roots of *Andrographis paniculata* exhibited promising anti-protozoal activity and these compounds could be chemically modified to obtain a more potent product.

14679. **Gallo, M. B., Marques, A. S., Vieira, P. C., da Silva, M. F., Fernandes, J. B., Silva, M., Guido, R. V., Oliva, G., Thiemann, O. H., Albuquerque, S. & Fairlamb, A. H., 2008**. Enzymatic inhibitory activity and trypanocidal effects of extracts and compounds from *Siphoneugena densiflora* O. Berg and *Vitex polygama* Cham. *Zeitschrift für Naturforschung,* **63** (5-6): 371-382.

Departamento de Química, Universidade Federal de São Carlos, CP 676, 13565-905, São Carlos-SP, Brazil. [margareth.gallo@gmail.com]

14680. **Ge, J. F., Arai, C., Kaiser, M., Wittlin, S., Brun, R. & Ihara, M., 2008**. Synthesis and *in vitro* antiprotozoal activities of water-soluble, inexpensive 3,7-bis(dialkylamino)phenoxazin-5-ium derivatives. *Journal of Medicinal Chemistry,* **51** (12): 3654-3658.

Institute of Medicinal Chemistry, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan. [m-ihara@hoshi.ac.jp].

14681. **Goeminne, A., Berg, M., McNaughton, M., Bal, G., Surpateanu, G., Van der Veken, P., De Prol, S., Versees, W., Steyaert, J., Haemers, A. & Augustyns, K., 2008.** N-arylmethyl substituted iminoribitol derivatives as inhibitors of a purine specific nucleoside hydrolase. *Bioorganic and Medicinal Chemistry,* **16** (14): 6752-6763.

Department of Medicinal Chemistry, University of Antwerp, Universiteitsplein 1, Antwerp B-2610, Belgium. [koen.augustyns@ua.ac.be].

14682. **Jansson, R., Malm, M., Roth, C. & Ashton, M., 2008**. Enantioselective and nonlinear intestinal absorption of eflornithine in the rat. *Antimicrobial Agents and Chemotherapy,* **52** (8): 2842-2848.

Sahlgrenska Academy at Goteborg University, Unit for Pharmacokinetics and Drug Metabolism, Medicinaregatan 13, 41390 Goteborg, Sweden. [Michael.Ashton@pharm.gu.se].

This study aimed to investigate if the absorption of the human African trypanosomiasis agent eflornithine was stereospecific and dose dependent after oral administration. Male Sprague-Dawley rats were administered single doses of racemic eflornithine hydrochloride as an oral solution (750, 1 500, 2 000, or 3 000 mg/kg body weight) or intravenously (375 or 1 000 mg/kg body weight). Small blood samples were obtained for determination of eflornithine enantiomers by liquid chromatography with evaporative light-scattering detection (lower limit of quantification [LLOQ], 83 µM for 300 µL plasma). The full plasma concentration-time profile of racemic eflornithine following frequent sampling was determined for another group of rats, using a high-performance liquid chromatography-UV method (LLOQ, 5 µM for 50 µL plasma). Pharmacokinetic data were analyzed in NONMEM for the combined racemic and enantiomeric concentrations. Upon intravenous administration, the plasma concentration-time profile of eflornithine was biphasic, with marginal differences in enantiomer kinetics (mean clearances of 14.5 and 12.6 ml/min/kg for L- and D-eflornithine, respectively). The complex absorption kinetics were modelled with a number of transit compartments to account for delayed absorption, transferring the drug into an absorption compartment from which the rate of influx was saturable. The mean bioavailabilities for L- and D-eflornithine were 41 percent and 62 percent, respectively, in the dose range of 750 to 2 000 mg/kg body weight, with suggested increases to 47 percent and 83 percent, respectively, after a dose of 3 000 mg/kg body weight. Eflornithine exhibited enantioselective absorption, with the more potent L-isomer being less favoured, a finding which may help to explain why clinical attempts to develop an oral treatment have hitherto failed. The mechanistic explanation for the stereoselective absorption remains unclear.

14683. **Lepesheva, G. I., Hargrove, T. Y., Kleshchenko, Y., Nes, W. D., Villalta, F. & Waterman, M. R., 2008**. CYP51: A major drug target in the cytochrome P450 superfamily. *Lipids*.**e - Publication ahead of print 4 September 2008.**

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

The cytochrome P540 (CYP) superfamily currently includes about 9 000 proteins forming more than 800 families. The enzymes catalyze monooxygenation of a vast array of compounds and play essentially two roles. They provide biodefence (detoxification of xenobiotics, antibiotic production) and participate in biosynthesis of important endogenous molecules, particularly steroids. Based on these two roles, sterol 14|*alpha*|-demethylases (CYP51) belong to the second group of P450s. The CYP51 family, however, is very special as its members preserve strict functional conservation in enzyme activity in all biological kingdoms. At amino acid identity across the kingdoms as low as 25 percent-30 percent, they all catalyze essentially the same three-step reaction of oxidative removal of the 14|*alpha*|-methyl group from the lanostane frame. This reaction is the required step in sterol biosynthesis of pathogenic microbes. We have shown that specific inhibition of protozoan CYP51 can potentially provide treatment for human trypanosomiases. Three sets of CYP51 inhibitors tested *in vitro* and in trypanosomal cells in this study include azoles [best results being 50 percent cell growth inhibition at <1 and at 1.3 mµM for *Trypanosoma cruzi* (TC) and *Trypanosoma brucei* (TB), respectively], non-azole compounds (50 percent TC cell growth inhibition at 5 mµM) and substrate analogues of the 14|*alpha*|-demethylase reaction. 32-methylene cyclopropyl lanost-7-enol exhibited selectivity toward TC with 50 percent cell growth inhibition at 3 mµM.

14684. **Ojo, K. K., Gillespie, J. R., Riechers, A. J., Napuli, A. J., Verlinde, C. L., Buckner, F. S., Gelb, M. H., Domostoj, M. M., Wells, S. J., Scheer, A., Wells, T. N. & Van Voorhis, W. C., 2008**. Glycogen synthase kinase 3 is a potential drug target for African trypanosomiasis therapy. *Antimicrobial Agents and Chemotherapy*, **52** (10): 3710-3717.

Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington, Seattle, WA 98195-7185, USA. [wesley@u.washington.edu]

Development of a safe, effective, and inexpensive therapy for African trypanosomiasis is an urgent priority. In this study, we evaluated the validity of *Trypanosoma brucei* glycogen synthase kinase 3 (GSK-3) as a potential drug target. Interference with the RNA of either of two GSK-3 homologues in bloodstream-form *T. brucei* parasites led to growth arrest and altered parasite morphology, demonstrating their requirement for cell survival. Since the growth arrest after RNA interference appeared to be more profound for *T. brucei* GSK-3 "short" (*Tb*10.161.3140) than for *T. brucei* GSK-3 "long" (*Tb*927.7.2420), we focused on *T. brucei* GSK-3 short for further studies. *T. brucei* GSK-3 short with an N-terminal maltose-binding protein fusion was cloned, expressed, and purified in a functional form. The potency of a GSK-3-focused inhibitor library against the recombinant enzyme of *T. brucei* GSK-3 short, as well as bloodstream-form parasites, was evaluated with the aim of determining if compounds that inhibit enzyme activity could also block the parasites' growth and proliferation. Among the compounds active against the cell, there was an excellent correlation between activity inhibiting the *T. brucei* GSK-3 short enzyme and the inhibition of *T. brucei* growth. Thus, there is reasonable genetic and chemical validation of GSK-3 short as a drug target for *T. brucei*. Finally, selective inhibition may be required for therapy targeting the GSK-3 enzyme, and a molecular model of the *T. brucei* GSK-3 short enzyme suggests that compounds that selectively inhibit *T. brucei* GSK-3 short over the human GSK-3 enzymes can be found.

14685. **Portmann, C., Blom, J. F., Kaiser, M., Brun, R., Juttner, F. & Gademann, K., 2008**. Isolation of aerucyclamides C and D and structure revision of microcyclamide 7806A: Heterocyclic ribosomal peptides from *Microcystis aeruginosa* PCC 7806 and their antiparasite evaluation. *Journal of Natural Products,* **71***:* 1193-1196.

École Polytechnique Fédérale de Lausanne (EPFL), Institute of Chemistry and Chemical Engineering (ISIC), Chemical Synthesis Laboratory, 1015 Lausanne, Switzerland. [karl.gademann@epfl.ch].

Aerucyclamides C and D were isolated from the cyanobacterium *Microcystis aeruginosa* PCC 7806, and their structures established by NMR spectroscopy and chemical transformation and degradation. Acidic hydrolysis of aerucyclamide C (CF 3CO 2H, H_2O) resulted in microcyclamide 7806A. This chemical evidence combined with spectroscopic and physical data suggests a structure revision for microcyclamide 7806A, which incorporates an O-acylated Thr ammonium residue instead of the originally proposed methyl oxazoline ring. We have prepared microcyclamide 7806B upon basic and acidic treatment of microcyclamide

7806A, which suggests that both these compounds are hydrolysis products of aerucyclamide C and that the aerucyclamides A-D are the actual metabolites produced via ribosomal peptide synthesis in *M. aeruginosa* PCC 7806. Antiplasmodial evaluation established submicromolar IC 50 values for aerucyclamide B against *Plasmodium falciparum*; low micromolar values for aerucyclamide C were found against *Trypanosoma brucei rhodesiense*. The compounds were selective for the parasites over a cell line of L6 rat myoblasts and are thus considered for further study as antimalarial agents.

14686. **Reguera, R. M., Díaz-González, R., Pérez-Pertejo, Y. & Balaña-Fouce, R., 2008.** Characterizing the bi-subunit type IB DNA topoisomerase of *Leishmania* parasites: A novel scenario for drug intervention in trypanosomatids. *Current Drug Targets*, **9** (11): 966-978.

Dpto. Farmacologia y Toxicologia (INTOXCAL), Universidad de León, Campus de Vegazana s/n, 24071 Leon, Spain. [rbalf@unileon.es].

African and South American trypanosomes and leishmanias are unicellular protozoan parasites, forming part of the order Kinetoplastida. These ancient eukaryotes are causative agents of some of the most devastating neglected tropical diseases called trypanosomiasis and leishmaniasis. Despite the efforts to develop effective vaccines, immunoprophylaxis is not even a method of prevention of these diseases at present. Current antiprotozoal chemotherapy is often expensive, has side or toxic effects and it does not provide economic profits to the pharmaceutical industry, which has scant enthusiasm in R & D investments in this field. The surprising finding of unusual bi-subunit type IB DNA-topoisomerase in kinetoplastids adds a new promising drug target to antiprotozoal chemotherapy. The remarkable differences between trypanosomal and leishmanial DNA-topoisomerase IB with respect to the one in the mammalian hosts, have provided a new lead in the study of structural determinants that can be effectively targeted. This review provides an update on recent progress in research in kinetoplastid's topoisomerase IB as potential chemotherapeutic target against this group of parasitic diseases.

14687. **Reid, C. M., Ebikeme, C., Barrett, M. P., Patzewitz, E. M., Muller, S., Robins, D. J. & Sutherland, A., 2008**. Synthesis of novel benzamidine- and guanidine-derived polyazamacrocycles: Selective anti-protozoal activity for human African trypanosomiasis. *Bioorganic and Medicinal Chemistry Letters,* **18** (20): 5399-5401.

WestCHEM, Department of Chemistry, The Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, UK. [andrews@chem.gla.ac.uk].

Efficient synthetic routes have been developed for the preparation of two new polyazamacrocycles tagged with structural motifs recognised by the *Trypanosoma brucei* P2 aminopurine transporter. Biological testing of these compounds showed highly selective anti-protozoal activity against trypanosomes.

14688. **Reid, C. M., Ebikeme, C., Barrett, M. P., Patzewitz, E. M., Muller, S., Robins, D. J. & Sutherland, A., 2008**. Synthesis and anti-protozoal activity of C2-substituted

polyazamacrocycles. *Bioorganic and Medicinal Chemistry Letters,* **18** (7): 2455-2458.

WestCHEM, Department of Chemistry, The Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, UK. [andrews@chem.gla.ac.uk].

A focused library of C2-substituted-1,4,7,10-tetraazacyclododecanes was synthesised and the compounds were tested for their ability to kill trypanosome and malaria parasites. Several compounds showed significant *in vitro* activity and were selectively active against the parasites over human embryonic kidney cells used as a control.

14689. **Rodrigues, J. C. & de Souza, W., 2008**. Ultrastructural alterations in organelles of parasitic protozoa induced by different classes of metabolic inhibitors. *Current Pharmaceutical Design,* **14** (9): 925-938.

Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, CCS, Bloco G, subsolo, Ilha do Fundão, Rio de Janeiro, RJ, 21.941-902, Brazil.[julycola@biof.ufrj.br].

Parasitic protozoa such as *Leishmania, Trypanosoma, Plasmodium, Toxoplasma gondii, Giardia* and *Trichomonas* are able to cause several diseases affecting millions of people around the world with dramatic consequences to the socio-economic life of the affected countries. Diseases like malaria, leishmaniasis and trypanosomiasis have been classified by the World Health Organization as neglected diseases, because they have been almost completely forgotten by the governments as well as the pharmaceutical companies. The specific chemotherapy currently employed for the treatment of these diseases has serious limitations due to lack of efficacy, toxic side effects, growth of drug-resistance and high costs. Thus, it is urgent to develop new chemotherapeutic agents that are more effective, safe and accessible. In this context, several works have been focused on understanding the effect of different drug-treatments on these parasitic protozoa. Organelles and structures such as mitochondrion, kinetoplast, apicoplast, glycosome, acidocalcisome, hydrogenosome, plasma membrane and the cytoskeleton have been studied using different approaches to identify new targets for the development of new chemotherapeutic agents that are required. Some studies on alterations in the fine structure, as assayed using electron microscopy, have indicated the nature of lesions induced by several drugs, allowing deductions on possible modes of action. Here, we briefly review the available data of the effects of several drugs on the ultrastructure of parasitic protozoa and show how electron microscopy can contribute to elucidate the different mechanisms of these anti-parasitic drugs.

14690. **Sanderson, L., Dogruel, M., Rodgers, J., Bradley, B. & Thomas, S. A., 2008**. The blood-brain barrier significantly limits eflornithine entry into *Trypanosoma brucei brucei* infected mouse brain. *Journal of Neurochemistry,* **107** (4): 1136-1146.

Pharmaceutical Sciences Research Division, King's College London, London, UK.

Drugs to treat African trypanosomiasis are toxic, expensive and subject to parasite resistance. New drugs are urgently being sought. Although the existing drug, eflornithine, is

assumed to reach the brain in high concentrations, little is known about how it crosses the healthy and infected blood-brain barrier. This information is essential for the design of drug combinations and new drugs. This study used novel combinations of animal models to address these omissions. Eflornithine crossed the healthy blood-CNS interfaces poorly, but this could be improved by co-administering suramin, but not nifurtimox, pentamidine or melarsoprol. Work using a murine model of sleeping sickness demonstrated that *Trypanosoma brucei brucei* crossed the blood-CNS interfaces, which remained functional, early in the course of infection. Concentrations of brain parasites increased during the infection and this resulted in detectable blood-brain barrier, but not choroid plexus, dysfunction at day 28 post-infection with resultant increases in eflornithine brain delivery. Barrier integrity was never restored and the animals died at day 37.9 +/- 1.2. This study indicates why an intensive treatment regimen of eflornithine is required (poor blood-brain barrier penetration) and suggests a possible remedy (combining eflornithine with suramin). The blood-brain barrier retains functionality until a late, possibly terminal stage, of *Trypanosoma* infection.

14691. **Schad, G. J, Allanson, A., Mackay, S. P., Cannavan, A. & Tettey, J. N. A., 2008**. Development and validation of an improved HPLC method for the control of potentially counterfeit isometamidium products. *Journal of Pharmaceutical and Biomedical Analysis*, **46:** 45-51.

Strathclyde Institute for Pharmacy & Biomedical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, UK; Agrochemicals Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, IAEA Laboratories, A-2444 Seibersdorf, Austria [Justice.TETTEY@unodc.org].

Isometamidium, a mixture of related substances of which 8-(3-m-amidinophenyl-2-triazeno)-3-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (M&B4180A) is the principal active component, is the only chemical agent available for prophylaxis of veterinary trypanosomiasis. A method for the simultaneous quantitation of the major constituents M&B4180A, 3-(3-m-amidinophenyl-2-triazeno)-8-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (M&B38897), 7-(m-amidinophenyldiazo)-3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride (M&B4250) and 3,8-di(3-m-amidinophenyltriazeno)-5-ethyl-6-phenylphenanthridinium chloride dihydrochloride (M&B4596) is described. The related substances are resolved on a Gemini C18 column (150 x 4.6 mm, 5 μm) using a mobile phase composed of a mixture of acetonitrile and 50 mM ammonium formate buffer pH 2.8 (25:75 v/v) at a flow rate of 1 ml/min with UV detection at 320 nm. The method is compatible with electrospray ionisation mass spectrometry and provides a tool for the control of substandard and counterfeit commercial preparations of isometamidium.

14692. **Schlapper, C., Seebacher, W., Faist, J., Kaiser, M., Brun, R., Saf, R. & Weis, R., 2008**. Antiplasmodial and antitrypanosomal activities of aminobicyclo[2.2.2]octyl omega-aminoalkanoates. *European Journal of Medicinal Chemistry*. **In press, corrected proof.**

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

Several 4-aminobicyclo[2.2.2]octyl esters of omega-dialkylamino acids were prepared. Their activities against the multidrug-resistant K(1) strain of *Plasmodium falciparum* and *Trypanosoma brucei rhodesiense* (STIB 900) were determined using microplate assays and compared to those of formerly prepared analogues. The biological activity was influenced by the relative configuration in ring position 2, by the chain length of the acid moiety and by the amino substitution. The most active antiplasmodial ester was as active as chloroquine. One of the new compounds exhibited the highest antitrypanosomal activity and selectivity of all bicyclo-octane derivatives prepared so far.

14693. **Sienkiewicz, N., Jaroslawski, S., Wyllie, S. & Fairlamb, A. H., 2008**. Chemical and genetic validation of dihydrofolate reductase-thymidylate synthase as a drug target in African trypanosomes. *Molecular Microbiology,* **69** (2): 520-533.

Division of Biological Chemistry & Drug Discovery, College of Life Sciences, University of Dundee, Dundee, UK. [a.h.fairlamb@dundee.ac.uk].

The phenotypes of single- (SKO) and double-knockout (DKO) lines of dihydrofolate reductase-thymidylate synthase (DHFR-TS) of bloodstream *Trypanosoma brucei* were evaluated *in vitro* and *in vivo*. Growth of SKO *in vitro* is identical to wild-type (WT) cells, whereas DKO has an absolute requirement for thymidine. Removal of thymidine from the medium triggers growth arrest in S phase, associated with gross morphological changes, followed by cell death after 60 h. DKO is unable to infect mice, whereas the virulence of SKO is similar to WT. Normal growth and virulence could be restored by transfection of DKO with *T. brucei* DHFR-TS, but not with *Escherichia coli* TS. As pteridine reductase (PTR1) levels are unchanged in SKO and DKO cells, PTR1 is not able to compensate for loss of DHFR activity. Drugs such as raltitrexed or methotrexate with structural similarity to folic acid are up to 300-fold more potent inhibitors of WT cultured in a novel low-folate medium, unlike hydrophobic antifols such as trimetrexate or pyrimethamine. DKO trypanosomes show reduced sensitivity to these inhibitors ranging from twofold for trimetrexate to >10 000-fold for raltitrexed. These data demonstrate that DHFR-TS is essential for parasite survival and represents a promising target for drug discovery.

14694. **Stump, B., Eberle, C., Kaiser, M., Brun, R., Krauth-Siegel, R. L. & Diederich, F., 2008**. Diaryl sulphide-based inhibitors of trypanothione reductase: inhibition potency, revised binding mode and antiprotozoal activities. *Organic and Biomolecular Chemistry*, **6** (21): 3935-3947.

Laboratorium fur Organische Chemie, ETH Zurich, Honggerberg, HCI 8093, Zurich, Switzerland.

Trypanothione reductase (TR) is an essential enzyme of trypanosomatids and therefore a promising target for the development of new drugs against African sleeping sickness and Chagas disease. Diaryl sulphides with a central anilino moiety, decorated with a flexible N-alkyl side chain bearing a terminal ammonium ion, are a known class of inhibitors. Using

computer modelling, we revised the binding model for this class of TR inhibitors predicting simultaneous interactions of the ammonium ion-terminated N-alkyl chain with Glu18 as well as Glu465'/Glu466' of the second subunit of the homodimer, whereas the hydrophobic substituent of the aniline ring occupies the "mepacrine binding site" near Trp21 and Met113. Systematic alteration of the carboxylate-binding fragments and the diaryl sulphide core of the inhibitor scaffold provided evidence for the proposed binding mode. *In vitro* studies showed IC(50) values in the low micromolar to submicromolar range against *Trypanosoma brucei rhodesiense* as well as the malaria parasite *Plasmodium falciparum*.

14695. **Thuita, J. K., Karanja, S. M., Wenzler, T., Mdachi, R. E., Ngotho, J. M., Kagira, J. M., Tidwell, R. & Brun, R., 2008**. Efficacy of the diamidine DB75 and its prodrug DB289, against murine models of human African trypanosomiasis. *Acta Tropica,* **108** (1): 6-10.

Trypanosomiasis Research Centre, Kenya Agricultural Research Institute (TRC-KARI), P.O. Box 362, Kikuyu, Kenya. [reto.brun@unibas.ch].

The choice of drugs for the treatment of sleeping sickness is extremely limited. To redress this situation, the recently synthesised diamidine, 2,5-bis(4-amidinophenyl)-furan (DB75, furamidine) and its methamidoxime prodrug, 2,5-bis(4-amidinophenyl)-furan-bis-O-methylamidoxime (DB289, pafuramidine) were, together with pentamidine, evaluated for efficacy in acute rodent models. The activity was compared in three common mouse models that mimic the first stage of human African trypanosomiasis. The mice were infected with the pleomorphic *T.b. rhodesiense* strains KETRI2537 and STIB900 or with the monomorphic *T. b. brucei* strain STIB795. Importantly, DB75 showed activity superior to that of pentamidine at comparable doses in all three mouse models. Complete cures were achieved with oral dosing of the prodrug DB289 in all three models without any overt toxicity. This shows that the prodrug strategy was successful in terms of reducing toxicity and increasing efficacy and oral bioavailability.

14696. **Vanhamme, L., 2008**. Trypanosome RNA polymerases and transcription factors: sensible trypanocidal drug targets? *Current Drug Targets,* **9** (11): 979-996.

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

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

dedicated molecular players. The first of them have been recently characterized. They are reviewed and their suitability as drug targets is discussed.

14697. **Wang, X., Beckham, T. H., Morris, J. C., Chen, F. & Gangemi, J. D., 2008**. Bioactivities of gossypol, 6-methoxygossypol, and 6,6'-dimethoxygossypol. *Journal of Agriculture and Food Chemistry,* **56** (12): 4393-4398.

Department of Genetic and Biochemistry, Department of Food Science and Human Nutrition, and Institute for Nutraceutical Research, Clemson University, Clemson, South Carolina 29634, USA. [xiw@clemson.edu]

14698. **Weis, R., Berger, H., Kaiser, M., Brun, R., Saf, R. & Seebacher, W., 2008**. Synthesis of bicyclic amines and their activities against *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum* K1. *Archives of Pharmaceutical Research*, **31** (6): 688-697.

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

New alkenes, aziridines, and diamines were prepared from antiprotozoal 4-dialkylaminobicyclo[2.2.2]octan-2-imines to investigate the influence of several functional groups in position 2 of the ring skeleton on the antitrypanosomal and antiplasmodial activities. They were synthesized from 4-dialkylaminobicyclo[2.2.2]octan-2-imines and tested for their activities against *Trypanosoma b. rhodesiense* and *Plasmodium falciparum* K1 (resistant to chloroquine and pyrimethamine) using *in vitro* microplate assays. 4-aminobicyclo[2.2.2]oct-2-enes and 3-azatricyclo[3.2.2.0(2,4)]nonylamines exhibit similar antiprotozoal activities as 4-aminobicyclo[2.2.2] octanes. 4-aminobicyclo[2.2.2]oct-2-ylamines and their N-benzyl derivatives showed decreased antiplasmodial but enhanced antitrypanosomal (IC50 = 0.22-0.41 μM) activities compared to their parent oximes and to formerly synthesized 4-amino-2-azabicyclo[3.2.2]nonanes. Some of the 4-aminobicyclo[2.2.2]oct-2-ylamines exhibit moderate *in vivo* activity in mice against *Trypanosoma brucei brucei*.

14699. **Zoidis, G., Tsotinis, A., Kolocouris, N., Kelly, J. M., Prathalingam, S. R., Naesens, L. & De Clercq, E., 2008**. Design and synthesis of bioactive 1,2-annulated adamantane derivatives. *Organic and Biomolecular Chemistry,* **6** (17): 3177-3185.

Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Athens, Panepistimioupoli-Zografou, GR-15771, Athens, Greece.

Adamantanopyrrolidines 8, 9 and 10, adamantanopyrrolidines 16 and 18, adamantanoxazolone 20, adamantanopyrazolone 23, adamantanopyrazolothione 24 and adamantanocyclopentanamine 32 were synthesized and tested for anti-influenza A virus and trypanocidal activity. The stereoelectronic requirements for optimal antiviral and trypanocidal potency were investigated. Pyrrolidine 16 proved to be the most active of the compounds tested against influenza A virus, being four-fold more active than amantadine, equipotent to

rimantadine and 19-fold more potent than ribavirin. Oxazolone 20 showed significant trypanocidal activity against bloodstream forms of the African trypanosome, *Trypanosoma brucei*, being approximately three times more potent than rimantadine and almost 50-fold more active than amantadine.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

(b) TAXONOMY; CHARACTERISATION OF ISOLATES

14700. **Balmer, O., Palma, C., Macleod, A. & Caccone, A., 2006**. Characterization of di-, tri-, and tetranucleotide microsatellite markers with perfect repeats for *Trypanosoma brucei* and related species. *Molecular Ecology Notes,* **6** (2): 508-510.

Yale University, Department of Ecology and Evolutionary Biology, New Haven, USA. [oliver.balmer@pronet.ch].

Trypanosoma brucei, unicellular parasites causing human sleeping sickness and animal nagana, have a great impact on the socio-economic environment of sub-Saharan Africa. The dynamics of the parasite are still poorly understood. We have characterized 14 polymorphic di-, tri-, and tetranucleotide microsatellite loci with perfect repeats (only one motif) exhibiting between five and 16 alleles in *T. brucei* isolates from all over Africa and from all described subspecies. The microsatellites will be useful in addressing population genetic questions in *T. brucei* to better understand the population structure and spread of this important parasite.

14701. **Gibson, W., 2008**. Molecular epidemiology of African trypanosomiasis: The contributions of David George Godfrey OBE to the biochemical characterization of trypanosomes. *Parasite,* **15** (3): 233-236.

School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK. [w.gibson@bris.ac.uk].

The accurate identification of the causative organisms of disease is fundamental to the study of epidemiology. Hence molecular tools are now widely used to detect and distinguish pathogens, and have greatly improved our understanding of epidemiology. David Godfrey pioneered the use of molecular markers in the epidemiology of African trypanosomiasis, thus enabling the light of reliable evidence to shine on this previously problematic and controversial subject area. From the early 1970's David's group employed first isoenzyme electrophoresis and subsequently DNA-based characterization methods to aid identification of typanosomes collected from a range of endemic countries across Africa. These investigations had a major impact on our understanding of the zoonotic nature of human trypanosomiasis in Africa and of the genetic diversity of African trypanosomes.

14702. **Morrison, L. J., Tait, A., McCormack, G., Sweeney, L., Black, A., Truc, P., Likeufack, A. C., Turner, C. M. & Macleod, A., 2008**. *Trypanosoma brucei*

gambiense Type 1 populations from human patients are clonal and display geographical genetic differentiation. *Infection, Genetics and Evolution,* **8** (6): 847-854

Wellcome Centre for Molecular Parasitology, University of Glasgow, Biomedical Research Centre, 120 University Place, Glasgow G12 8TA, UK. [lm78y@udcf.gla.ac.uk].

We have rigorously tested the hypothesis that *Trypanosoma brucei gambiense* Type 1 is composed of genetically homogenous populations by examining the parasite population present in Human African Trypanosomiasis (HAT) patients from the Democratic Republic of Congo (DRC) and Cameroon (CAM). We amplified eight microsatellite markers by PCR directly from blood spots on FTA filters, thereby avoiding the significant parasite selection inherent in the traditional isolation techniques of rodent inoculation or *in vitro* culture. All microsatellite markers were polymorphic, although for four markers there was only polymorphism between the DRC and CAM populations, not within populations, suggesting very limited genetic exchange. Within the largest population from the DRC, Hardy-Weinberg equilibrium is not evident at any loci. This evidence suggests a clonal population. However, there was significant sub-structuring between the DRC and CAM samples (F(ST)=0.32), indicating that *Trypanosoma brucei gambiense* Type 1 has genetically distinct clades. The data combine to indicate that genetic exchange plays a very limited role. The finding of distinct clades in different places suggests the possibility that samples from humans with clinical signs represent clonal expansions from an underlying population that requires identifying and characterising.

14703. **Rodrigues, A. C., Neves, L., Garcia, H. A., Viola, L. B., Marcili, A., Da Silva, F. M., Sigauque, I., Batista, J. S., Paiva, F. & Teixeira, M. M., 2008**. Phylogenetic analysis of *Trypanosoma vivax* supports the separation of South American/West African from East African isolates and a new *T. vivax*-like genotype infecting a nyala antelope from Mozambique. *Parasitology,* **135** (11): 1317-1328.

Departamento de Parasitologia, Universidade de São Paulo, São Paulo, SP, Brazil.

In this study, we addressed the phylogenetic and taxonomic relationships of *Trypanosoma vivax* and related trypanosomes nested in the subgenus *Duttonella* through combined morphological and phylogeographical analyses. We previously demonstrated that the clade *T. vivax* harbours a homogeneous clade comprising West African/South American isolates and the heterogeneous East African isolates. Herein we characterized a trypanosome isolated from a nyala antelope (*Tragelaphus angasi*) wild-caught in Mozambique (East Africa) and diagnosed as *T. vivax*-like based on biological, morphological and molecular data. Phylogenetic relationships, phylogeographical patterns and estimates of genetic divergence were based on SSU and ITS rDNA sequences of *T. vivax* from Brazil and Venezuela (South America), Nigeria (West Africa), and from *T. vivax*-like trypanosomes from Mozambique, Kenya and Tanzania (East Africa). Despite being well-supported within the *T. vivax* clade, the nyala trypanosome was highly divergent from all other *T. vivax* and *T. vivax*-like trypanosomes, even those from East Africa. Considering its host origin, morphological features, behaviour in experimentally infected goats, phylogenetic placement,

and genetic divergence this isolate represents a new genotype of trypanosome closely phylogenetically related to *T. vivax.* This study corroborated the high complexity and the existence of distinct genotypes yet undescribed within the subgenus *Duttonella*.

14704. **Roellig, D. M., Brown, E. L., Barnabe, C., Tibayrenc, M., Steurer, F. J. & Yabsley, M. J., 2008**. Molecular typing of *Trypanosoma cruzi* isolates, United States. *Emerging Infectious Diseases,* **14** (7): 1123-1125.

University of Georgia, Athens, Georgia, USA. [droellig@uga.edu].

Studies have characterized *Trypanosoma cruzi* from parasite-endemic regions. With new human cases, increasing numbers of veterinary cases, and influx of potentially infected immigrants, understanding the ecology of this organism in the United States is imperative. We used a classic typing scheme to determine the lineage of 107 isolates from various hosts.

14705. **Viola, L. B., Almeida, R. S., Ferreira, R. C., Campaner, M., Takata, C. S., Rodrigues, A. C., Paiva, F., Camargo, E. P. & Teixeira, M. M., 2008**. Evolutionary history of trypanosomes from South American caiman (*Caiman yacare*) and African crocodiles inferred by phylogenetic analyses using SSU rDNA and gGAPDH genes. *Parasitology.* **Published online 4 November 2008.**

Departamento de Parasitologia, Instituto de Ciências Biomedicas, Universidade de São Paulo, São Paulo, SP, 05508-900, Brazil.[mmgteix@icb.usp.br].

In this study, using a combined data set of SSU rDNA and gGAPDH gene sequences, we provide phylogenetic evidence that supports clustering of crocodilian trypanosomes from the Brazilian *Caiman yacare* (Alligatoridae) and *Trypanosoma grayi*, a species that circulates between African crocodiles (Crocodilydae) and tsetse flies. In a survey of trypanosomes in *Caiman yacare* from the Brazilian Pantanal, the prevalence of trypanosome infection was 35 percent as determined by microhaematocrit and haemoculture, and nine cultures were obtained. The morphology of trypomastigotes from caiman blood and tissue imprints was compared with those described for other crocodilian trypanosomes. Differences in morphology and growth behaviour of caiman trypanosomes were corroborated by molecular polymorphism that revealed two genotypes. Eight isolates were ascribed to genotype Cay01 and one to genotype Cay02. Phylogenetic inferences based on concatenated SSU rDNA and gGAPDH sequences showed that caiman isolates are closely related to *T. grayi*, constituting a well-supported monophyletic assemblage (clade *T. grayi*). Divergence time estimates based on clade composition, and biogeographical and geological events were used to discuss the relationships between the evolutionary histories of crocodilian trypanosomes and their hosts.

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

14706. **Abdille, M. H., Li, S. Y., Ding, J. & Suo, X., 2008**. *Trypanosoma evansi*: Paraflagellar rod protein 1 and 2 are similar but lack common B cell epitopes. *Experimental Parasitology,* **120** (4): 411-416.

Parasitology Laboratory, College of Veterinary Medicine, China Agricultural University, Beijing 100193, PR China; Centre for Biotechnology Research and Development, Kenya Medical Research Institute (KEMRI), PO Box 54840-00200, Nairobi, Kenya. [suoxun@cau.edu.cn].

In an attempt to identify invariant proteins with vaccine potential against African trypanosomes, we investigated the existence of PFR1 protein in *Trypanosoma evansi* and compared its B cell epitope with that of PFR2 protein of *T. evansi* using Western blotting and immuno-precipitation assays. The PFR1 gene of *T. evansi* was amplified by RT-PCR using primers designed based on the open reading frame of PFR1 gene of *Trypanosoma brucei*. The cloned PFR1 gene of *T. evansi* was similar to PFR1 genes of *T. brucei* and *Trypanosoma cruzi*. The expressed protein from the PFR1 gene was 68.4 percent homologous to the PFR2 protein of *T. evansi*, and showed 99.8 percent, 87 percent, 77.9 percent and 77.5 percent homologous to the PFR1 protein of *T. brucei, T. cruzi, Leishmania mexicana* and *Leishmania major*, respectively. Western blot and immuno-precipitation assays showed that antibodies raised against PFR1 and 2 proteins in BALB/c mice recognized the PFR1 and 2 proteins, respectively, with no cross-reactivity. Immuno-agglutination assay showed trypanolytic properties of the anti-PFR1, anti-PFR2 and anti-native PFR sera. These results suggest that PFR1 and PFR2 proteins are components of native PFR antigen and do not share common B cell epitopes.

14707. **Absalon, S., Blisnick, T., Bonhivers, M., Kohl, L., Cayet, N., Toutirais, G., Buisson, J., Robinson, D. & Bastin, P., 2008**. Flagellum elongation is required for correct structure, orientation and function of the flagellar pocket in *Trypanosoma brucei*. *Journal of Cell Science,* **121** (Pt 22): 3704-3716.

Trypanosome Cell Biology Unit, Pasteur Institute and CNRS, 25 rue du Docteur Roux, 75015 Paris, France. [philippe.bastin@pasteur.fr].

In trypanosomes, the flagellum is rooted in the flagellar pocket, a surface micro-domain that is the sole site for endocytosis and exocytosis. By analysis of anterograde or retrograde intraflagellar transport in IFT88(RNAi) or IFT140(RNAi) mutant cells, we show that elongation of the new flagellum is not required for flagellar pocket formation but is essential for its organisation, orientation and function. Transmission electron microscopy revealed that the flagellar pocket exhibited a modified shape (smaller, distorted and/or deeper) in cells with abnormally short or no flagella. Scanning electron microscopy analysis of intact and detergent-extracted cells demonstrated that the orientation of the flagellar pocket collar was more variable in trypanosomes with short flagella. The structural protein BILBO1 was present but its localisation and abundance were altered. The membrane flagellar pocket protein CRAM leaked out of the pocket and reached the short flagella. CRAM also accumulated in intracellular compartments, indicating defects in routing of resident flagellar pocket proteins. Perturbations of vesicular trafficking were obvious; vesicles were observed in the lumen of the flagellar pocket or in the short flagella, and fluid-phase endocytosis was drastically diminished in non-flagellated cells. We propose a model to explain the role of flagellum elongation in correct flagellar pocket organisation and function.

14708. **Ackermann, A. A., Carmona, S. J. & Agüero, F., 2008**. *Tc*SNP: A database of genetic variation in *Trypanosoma cruzi*. *Nucleic Acids Research*. **e - Publication ahead of print 18 October 2008.**

Instituto de Investigaciones Biotecnólogicas, Universidad de San Martín - CONICET, San Martín, 1650, Argentina. [fernan@unsam.edu.ar].

The *Tc*SNP database (http://snps.tcruzi.org) integrates information on genetic variation (polymorphisms and mutations) for different stocks, strains and isolates of *Trypanosoma cruzi*, the causative agent of Chagas disease. The database incorporates sequences (genes from the *T. cruzi* reference genome, mRNAs, ESTs and genomic sequences); multiple sequence alignments obtained from these sequences; and single-nucleotide polymorphisms and small indels identified by scanning these multiple sequence alignments. Information in *Tc*SNP can be readily interrogated to arrive at gene sets, or SNP sets of interest based on a number of attributes. Sequence similarity searches using BLAST are also supported. This first release of *Tc*SNP contains nearly 170 000 high-confidence candidate SNPs, derived from the analysis of annotated coding sequences. As new sequence data become available, *Tc*SNP will incorporate these data, mapping new candidate SNPs onto the reference genome sequences.

14709. **Alphey, M. S., Konig, J. & Fairlamb, A. H., 2008**. Structural and mechanistic insights into type II trypanosomatid tryparedoxin-dependent peroxidases. *Biochemical Journal,* **414** (3): 375-381.

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

*Tb*TDPX (*Trypanosoma brucei* tryparedoxin-dependent peroxidase) is a genetically validated drug target in the fight against African sleeping sickness. Despite its similarity to members of the GPX (glutathione peroxidase) family, *Tb*TDPX2 is functional as a monomer, lacks a selenocysteine residue and relies instead on peroxidatic and resolving cysteine residues for catalysis and uses tryparedoxin rather than glutathione as electron donor. Kinetic studies indicate a saturable ping pong mechanism, unlike selenium-dependent GPXs, which display infinite K(m) and V(max) values. The structure of the reduced enzyme at 2.1 A (0.21 nm) resolution reveals that the catalytic thiol groups are widely separated [19 A (0.19 nm)] and thus unable to form a disulphide bond without a large conformational change in the secondary-structure architecture, as reported for certain plant GPXs. A model of the oxidized enzyme structure is presented and the implications for small-molecule inhibition are discussed.

14710. **Alsford, S. & Horn, D., 2008**. Single-locus targeting constructs for reliable regulated RNAi and transgene expression in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology,* **161** (1): 76-79.

London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK. [david.horn@lshtm.ac.uk].

A major obstacle to reproducible expression of recombinant transcripts lies in the epigenetic effects of the flanking chromatin following integration. We previously presented a strategy to overcome this problem in bloodstream form *Trypanosoma brucei*, using a reporter to identify a ribosomal-spacer locus that supports optimal expression and then marking that locus for subsequent targeting. Advantages include elimination of variable-expression position-effects and the easy confirmation of correct integration. We now report a set of validated constructs that exploit this system for expression of dsRNA or recombinant protein. The current construct-set allows expression of intramolecular dsRNA for RNA interference knockdown or expression of proteins that can incorporate c-Myc epitope(s) or a fluorescent-tag for subcellular localisation, interaction and/or other functional analysis. The constructs are integrated at a single, marked locus and deliver reliable and reproducible expression.

14711. **Alvarez, V. E., Kosec, G., Sant Anna, C., Turk, V., Cazzulo, J. J. & Turk, B., 2008**. Blocking autophagy to prevent parasite differentiation: a possible new strategy for fighting parasitic infections? *Autophagy,* **4** (3): 361-363.

Instituto de Investigaciones Biotecnológicas (IIB/INTECH, UNSAM/CONICET), Buenos Aires, Argentina.

14712. **Amaro, R. E., Schnaufer, A., Interthal, H., Hol, W., Stuart, K. D. & McCammon, J. A., 2008**. Discovery of drug-like inhibitors of an essential RNA-editing ligase in *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences USA,* **105** (45): 17278-17283.

Department of Chemistry and Biochemistry and Center for Theoretical Biological Physics, University of California at San Diego, La Jolla, CA 92093-0365, USA. [achim.schnaufer@ed.ac.uk].

Trypanosomatid RNA editing is a unique process and essential for these organisms. It therefore represents a drug target for a group of protozoa that includes the causative agents for African sleeping sickness and other devastating tropical and subtropical diseases. Here, we present drug-like inhibitors of a key enzyme in the editing machinery, RNA-editing ligase 1 (REL1). These inhibitors were identified through a strategy employing molecular dynamics to account for protein flexibility. A virtual screen of the REL1 crystal structure against the National Cancer Institute Diversity Set was performed by using AutoDock4. The top 30 compounds, predicted to interact with REL1's ATP-binding pocket, were further refined by using the relaxed complex scheme (RCS), which redocks the compounds to receptor structures extracted from an explicitly solvated molecular dynamics trajectory. The resulting reordering of the ligands and filtering based on drug-like properties resulted in an initial recommended set of eight ligands, two of which exhibited micromolar activity against REL1. A subsequent hierarchical similarity search with the most active compound over the full National Cancer Institute database and RCS rescoring resulted in an additional set of six ligands, two of which were confirmed as REL1 inhibitors with IC(50) values of approximately 1 muM. Tests of the three most promising compounds against the most closely related bacteriophage T4 RNA ligase 2, as well as against human DNA ligase III beta, indicated a considerable degree of selectivity for RNA ligases. These compounds are promising scaffolds for future drug design and discovery efforts against these important pathogens.

14713. **Ammerman, M. L., Fisk, J. C. & Read, L. K., 2008**. gRNA/pre-mRNA annealing and RNA chaperone activities of RBP16. *Rna,* **14** (6): 1069-1080.

Department of Microbiology and Immunology, School of Medicine, State University of New York at Buffalo, Buffalo, New York 14214, USA. [lread@acsu.buffalo.edu].

Editing in trypanosomes involves the addition or deletion of uridines at specific sites to produce translatable mitochondrial mRNAs. RBP16 is an accessory factor from *Trypanosoma brucei* that affects mitochondrial RNA editing *in vivo* and also stimulates editing *in vitro*. We report here experiments aimed at elucidating the biochemical activities of RBP16 involved in modulating RNA editing. *In vitro* RNA annealing assays demonstrate that RBP16 significantly stimulates the annealing of gRNAs to cognate pre-mRNAs. In addition, RBP16 also facilitates hybridization of partially complementary RNAs unrelated to the editing process. The RNA annealing activity of RBP16 is independent of its high-affinity binding to gRNA oligo(U) tails, consistent with the previously reported *in vitro* editing stimulatory properties of the protein. *In vivo* studies expressing recombinant RBP16 in mutant *Escherichia coli* strains demonstrate that RBP16 is an RNA chaperone and that in addition to RNA annealing activity, it contains RNA unwinding activity. Our data suggest that the mechanism by which RBP16 facilitates RNA editing involves its capacity to modulate RNA secondary structure and promote gRNA/pre-mRNA annealing.

14714. **Andreeva, A. V. & Kutuzov, M. A., 2008**. Protozoan protein tyrosine phosphatases. *International Journal of Parasitology,* **38** (11): 1279-1295.

Department of Pharmacology (MC 868), University of Illinois at Chicago, Chicago, IL 60612, USA. [aandrev@uic.edu].

The aim of this review is to provide a synthesis of the published experimental data on protein tyrosine phosphatases from parasitic protozoa, *in silico* analysis based on the availability of completed genomes and to place available data for individual phosphatases from different unicellular parasites into the comparative and evolutionary context. We analysed the complement of protein tyrosine phosphatases (PTP) in several species of unicellular parasites that belong to Apicomplexa *(Plasmodium; Cryptosporidium, Babesia, Theileria,* and *Toxoplasma*), kinetoplastids (*Leishmania* and *Trypanosoma* spp.), as well as *Entamoeba histolytica, Giardia lamblia, Trichomonas vaginalis* and a microsporidium *Encephalitozoon cuniculi*. The analysis shows distinct distribution of the known families of tyrosine phosphatases in different species. Protozoan tyrosine phosphatases show considerable levels of divergence compared with their mammalian homologues, both in terms of sequence similarity between the catalytic domains and the structure of their flanking domains. This potentially makes them suitable targets for development of specific inhibitors with minimal effects on physiology of mammalian hosts.

14715. **Arsenieva, D., Appavu, B. L., Mazock, G. H. & Jeffery, C. J., 2008**. Crystal structure of phosphoglucose isomerase from *Trypanosoma brucei* complexed with glucose-6-phosphate at 1.6 A resolution. *Proteins,* **74**(1): 72-80.

Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois, Chicago, Illinois 60607, USA. [cjeffery@uic.edu].

14716. **Barker, A. R., Wickstead, B., Gluenz, E. & Gull, K., 2008**. Bioinformatic insights to the ESAG5 and GRESAG5 gene families in kinetoplastid parasites. *Molecular and Biochemical Parasitology,* **162** (2): 112-122.

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK. [keith.gull@path.ox.ac.uk].

Trypanosoma brucei, the causative agent of African sleeping sickness, evades the immune response by expressing a coat of variant surface glycoprotein (VSG). VSG is expressed from a single telomeric expression site (ES), along with a number of expression site associated genes (ESAGs). Thus far, the function of most ESAGs is unknown. One ES contains the serum resistance associated gene (SRA), which confers resistance to trypanosome lytic factor in *T. b. rhodesiense*. Only three other ESAGs -5, 6 and 7 - are present in this ES. ESAGs 6 and 7 encode a heterodimeric transferrin receptor, but the function of ESAG5 has not been identified. We present here a bioinformatic analysis of ESAG5 and distinguish between *T. brucei*-specific ESAGs and Genes Related to ESAG5 (GRESAGs), which occur outside of ESs in chromosomal-internal contexts. Further, a genome-wide survey of these genes across kinetoplastids identifies a family of GRESAG5s in a number of species. Analysis of phylogenetic relationships indicates that this family may have evolved from a single ancestral copy. Predicted properties of GRESAG5 proteins indicate a glycosylated protein containing either a signal peptide or transmembrane domain. Further analysis indicates a possible relationship to the lipid transfer/lipopolysaccharide-binding family which includes the bactericidal/permeability increasing (BPI) protein. Together, these results provide insights into the structure and evolution of an important extended gene family, and present a number of testable hypotheses which will aid in elucidating the function of ESAG5.

14717. **Bonhivers, M., Landrein, N., Decossas, M. & Robinson, D. R., 2008**. A monoclonal antibody marker for the exclusion-zone filaments of *Trypanosoma brucei*. *Parasite Vectors,* **1** (1): 21.

UMR-CNRS 5234, University of Bordeaux, 233076 Bordeaux, France. [Drobinso@u-bordeaux2.fr].

Trypanosoma brucei is a haemoflagellate pathogen of man, wild animals and domesticated livestock in central and southern Africa. In all life cycle stages this parasite has a single mitochondrion that contains a uniquely organised genome that is condensed into a flat disk-like structure called the kinetoplast. The kinetoplast is essential for insect form procyclic cells and therefore is a potential drug target. The kinetoplast is unique in nature because it consists of novel structural proteins and thousands of circular, interlocking, DNA molecules (kDNA). Secondly, kDNA replication is critically timed to coincide with nuclear S phase and new flagellum biogenesis. Thirdly, the kinetoplast is physically attached to the flagellum basal bodies via a structure called the tripartite attachment complex (TAC). The TAC consists of unilateral filaments (within the mitochondrion matrix), differentiated mitochondrial membranes and exclusion-zone filaments that extend from the distal end of the

basal bodies. To date only one protein, p166, has been identified to be a component of the TAC. In the work presented here we provide data based on a novel EM technique developed to label and characterise cytoskeleton structures in permeabilised cells without extraction of mitochondrion membranes. We used this protocol to provide data on a new monoclonal antibody reagent (Mab 22) and illustrate the precise localisation of basal body-mitochondrial linker proteins. Mab 22 binds to these linker proteins (exclusion-zone filaments) and provides a new tool for the characterisation of cytoskeleton mediated kinetoplast segregation. The antigen(s) recognised by Mab 22 are cytoskeletal, insensitive to extraction by high concentrations of non-ionic detergent, extend from the proximal region of basal bodies and bind to the outer mitochondrial membrane. This protein(s) is the first component of the TAC exclusion-zone fibres to be identified. Mab 22 will therefore be important in characterising TAC biogenesis.

14718. **Bonhivers, M., Nowacki, S., Landrein, N. & Robinson, D. R., 2008**. Biogenesis of the trypanosome endo-exocytotic organelle is cytoskeleton mediated. *PLoS Biology,* **6** (5): e105.

Microbiologie Cellulaire et Moléculaire et Pathogénicité, UMR-CNRS 5234, University Bordeaux 2, Bordeaux, Cedex France. [Drobinso@u-bordeaux2.fr].

Trypanosoma brucei is a protozoan parasite that is used as a model organism to study such biological phenomena as gene expression, protein trafficking, and cytoskeletal biogenesis. In *T. brucei*, endocytosis and exocytosis occur exclusively through a sequestered organelle called the flagellar pocket (FP), an invagination of the pellicular membrane. The pocket is the sole site for specific receptors thus maintaining them inaccessible to components of the innate immune system of the mammalian host. The FP is also responsible for the sorting of protective parasite glycoproteins targeted to, or recycling from, the pellicular membrane, and for the removal of host antibodies from the cell surface. Here, we describe the first characterisation of a flagellar pocket cytoskeletal protein, BILBO1. BILBO1 functions to form a cytoskeleton framework upon which the FP is made and which is also required and essential for FP biogenesis and cell survival. Remarkably, RNA interference (RNAi)-mediated ablation of BILBO1 in insect procyclic-form parasites prevents FP biogenesis and induces vesicle accumulation, Golgi swelling, the aberrant repositioning of the new flagellum, and cell death. Cultured bloodstream-form parasites are also nonviable when subjected to BILBO1 RNAi. These results provide the first molecular evidence for cytoskeletally mediated FP biogenesis.

14719. **Borst, P. & Sabatini, R., 2008**. Base J: discovery, biosynthesis, and possible functions. *Annual Review of Microbiology,* **62**: 235-251.

Center of Biomedical Genetics, Division of Molecular Biology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands. [p.borst@nki.nl].

In 1993, a new base, beta-d-glucopyranosyloxymethyluracil (base J), was identified in the nuclear DNA of *Trypanosoma brucei*. Base J is the first hypermodified base found in eukaryotic DNA. It is present in all kinetoplastid flagellates analyzed and some unicellular flagellates closely related to trypanosomatids, but it has not been found in other protozoa or

in metazoa. J is invariably present in the telomeric repeats of all organisms analyzed. Whereas in *Leishmania* nearly all J is telomeric, there are other repetitive DNA sequences containing J in *T. brucei* and *T. cruzi*, and most J is outside telomeres in *Euglena*. The biosynthesis of J occurs in two steps: First, a specific thymidine in DNA is converted into hydroxymethyldeoxyuridine (HOMedU), and then this HOMedU is glycosylated to form J. This review discusses the identification and localization of base J in the genome of kinetoplastids, the enzymes involved in J biosynthesis, possible biological functions of J, and J as a potential target for chemotherapy of diseases caused by kinetoplastids.

14720. **Bosetto, M. C., Mortara, R. A., Ambrosio, D. L., Passerini, G. D., da Silva, M. T., Okuda, E. S. & Cicarelli, R. M., 2008**. Detection and localization of small nuclear ribonucleoproteins (snRNPs) in trypanosomatids using anti-m3G antibodies. *Japanese Journal of Infectious Diseases,* **61** (2): 95-99.

Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, São Paulo, Brazil.

This work reports for the first time the identification and immunolocalization, by confocal and conventional indirect immunofluorescence, of m3G epitopes present in ribonucleoproteins of the following trypanosomatids: *Trypanosoma cruzi* epimastigotes of three different strains, *Blastocrithidia* ssp., and *Leishmania major* promastigotes. The identity of these epitopes and hence the specificity of the anti-m3G monoclonal antibody were ascertained through competition reaction with 7-methylguanosine that blocks the Ig binding sites, abolishing the fluorescence in all the parasites tested and showing a specific perinuclear localization of the snRNPs, which suggests their nuclear reimport in the parasites. Using an immunoprecipitation technique, it was also possible to confirm the presence of the trimethylguanosine epitopes in trypanosomatids.

14721. **Bringaud, F., Ghedin, E., El-Sayed, N. M. & Papadopoulou, B., 2008**. Role of transposable elements in trypanosomatids. *Microbes and Infection,* **10** (6): 575-581.

Laboratoire de Résonance Magnétique des Systêmes Biologiques, Université Victor Segalen Bordeaux 2, UMR-5536 CNRS, 146 rue Leo Saignat, 33076 Bordeaux Cedex, France. [bringaud@u-bordeaux2.fr].

Transposable elements constitute 2 percent-5 percent of the genome content in trypanosomatid parasites. Some of them are involved in critical cellular functions, such as the regulation of gene expression *in Leishmania* spp. In this review, we highlight the remarkable role extinct transposable elements can play as the source of potential new functions.

14722. **Cao, R., Chen, C. K., Guo, R. T., Wang, A. H. & Oldfield, E., 2008**. Structures of a potent phenylalkyl bisphosphonate inhibitor bound to farnesyl and geranylgeranyl diphosphate synthases. *Proteins,* **73** (2): 431-439.

Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA. [eo@chad.scs.uiuc.edu].

14723. **Carrero-Lérida, J., Pérez-Moreno, G., Castillo-Acosta, V. M., Ruiz-Pérez, L. M. & González-Pacanowska, D., 2008**. Intracellular location of the early steps of the isoprenoid biosynthetic pathway in the trypanosomatids *Leishmania major* and *Trypanosoma brucei*. *International Journal of Parasitology*. **In press, corrected proof.**

Instituto de Parasitología y Biomedicina "López-Neyra", Parque Tecnológico de Ciencias de la Salud, Consejo Superior de Investigaciones Cientificas, Avenida del Conocimiento s/n, 18100-Armilla, Granada, Spain.

The isoprenoid biosynthetic pathway is a very complex route that entails multiple steps and generates a high number of end-products that are essential for cell viability such as sterols, dolichols, coenzyme Q, haeme and prenylated proteins. In parasites from the Trypanosomatidae family this pathway provides new potential drug targets for exploitation in the search for improved therapies, and indeed compounds such as ketoconazole, aminobisphosphonates or terbinafine have been shown to have antiprotozoal activity both *in vitro* and *in vivo*. However, despite the high therapeutic importance of the pathway, the subcellular compartmentalization of the different steps of isoprenoid biosynthesis is not known in detail. Here we have analysed the intracellular location of the enzymes 3-hydroxy-3-methyl-glutaryl Coenzyme A (HMG-CoA) synthase (HMGS) and mevalonate kinase (MVAK) in *Leishmania major* promastigotes as well as in *Trypanosoma brucei* procyclic and bloodstream forms. For this purpose we generated specific polyclonal antibodies against both highly purified recombinant proteins and used those in indirect immunofluorescence and digitonin titration experiments. Results show that sterol biosynthesis is distributed in multiple intracellular compartments and provide evidence indicating that in trypanosomatids the production of HMG-CoA from acetyl Coenzyme A and generation of mevalonate occur mainly in the mitochondrion while further mevalonate phosphorylation is almost exclusively located in glycosomes. Furthermore, we have determined that peroxin 2 (PEX2) is involved in efficient targeting of MVAK and that the enzyme is relocated to the cytosol upon depletion of this peroxin involved in glycosomal matrix protein import.

14724. **Castillo-Acosta, V. M., Estévez, A. M., Vidal, A. E., Ruiz-Perez, L. M. & González-Pacanowska, D., 2008**. Depletion of dimeric all-alpha dUTPase induces DNA strand breaks and impairs cell cycle progression in *Trypanosoma brucei*. *International Journal of Biochemistry and Cell Biology,* **40** (12): 2901-2913.

Instituto de Parasitología y Biomedicina "López-Neyra", Parque Tecnológico de Ciencias de la Salud, Avenida del Conocimiento, s/n 18100-Armilla, Granada, Spain. [dgonzalez@ipb.csic.es].

The enzyme deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPase) is responsible for the control of intracellular levels of dUTP thus controlling the incorporation of uracil into DNA during replication. Trypanosomes and certain eubacteria contain a dimeric dUTP-dUDPase belonging to the recently described superfamily of all-alpha NTP pyrophosphatases which bears no resemblance with typical eukaryotic trimeric dUTPases and presents unique properties regarding substrate specificity and product inhibition. While the biological trimeric enzymes have been studied in detail and the human enzyme has been proposed as a promising novel target for anticancer chemotherapeutic strategies, little is

known regarding the biological function of dimeric proteins. Here, we show that in *Trypanosoma brucei*, the dimeric dUTPase is a nuclear enzyme and that down-regulation of activity by RNAi greatly reduces cell proliferation and increases the intracellular levels of dUTP. Defects in growth could be partially reverted by the addition of exogenous thymidine. dUTPase-depleted cells presented hypersensitivity to methotrexate, a drug that increases the intracellular pools of dUTP, and enhanced uracil-DNA glycosylase activity, the first step in base excision repair. The knockdown of activity produces numerous DNA strand breaks and defects in both S and G2/M progression. Multiple parasites with a single enlarged nucleus were visualized together with an enhanced population of anucleated cells. We conclude that dimeric dUTPases are strongly involved in the control of dUTP incorporation and that adequate levels of enzyme are indispensable for efficient cell cycle progression and DNA replication.

14725. **Castro, H. & Tomas, A. M., 2008**. Peroxidases of trypanosomatids. *Antioxidants and Redox Signaling,* **10** (9): 1593-1606.

Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal.

This article provides an overview about the recent advances in the dissection of the peroxide metabolism of Trypanosomatidae. This family of protozoan organisms comprises the medically relevant parasites *Trypanosoma brucei, Trypanosoma cruzi,* and *Leishmania* spp. Over the past 10 years, three major families of peroxidases have been identified in these organisms: (a) 2-cysteine peroxiredoxins, (b) non-selenium glutathione peroxidases, and (c) ascorbate peroxidases. In trypanosomatids, these enzymes display the unique feature of using reducing equivalents derived from trypanothione, a dithiol found exclusively in these protozoa. The electron transfer between trypanothione and the peroxidases is mediated by a redox shuttle, which can either be tryparedoxin, ascorbate, or even glutathione. The preference for the intermediate molecule differs among each peroxidase and so does the specificity for the peroxide substrate. These observations, added to the fact that these peroxidases are distributed throughout different subcellular compartments, point to the existence of an elaborate peroxide metabolism in trypanosomatids. With the completion of the trypanosomatids genome, other molecules displaying peroxidase activity might be added to this list in the future.

14726. **Chandler, J., Vandoros, A. V., Mozeleski, B. & Klingbeil, M. M., 2008**. Stem-loop silencing reveals a third mitochondrial DNA polymerase, POLID, is required for kinetoplast DNA replication in trypanosomes. *Eukaryotic Cell*. **Published online ahead of print 10 October 2008.**

Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA. [klingbeil@microbio.umass.edu].

Kinetoplast DNA (kDNA), the mitochondrial genome of trypanosomes, is a catenated network containing thousands of minicircles and tens of maxicircles. The topological complexity dictates some unusual features including a topoisomerase mediated release and reattachment mechanism for minicircle replication, and at least six mitochondrial DNA polymerases (pol) for kDNA transactions. Previously, we identified four family A DNA pols

from *Trypanosoma brucei* with similarity to bacterial DNA pol I and demonstrated that two (POLIB and POLIC) were essential for maintaining the kDNA network while POLIA was not. Here, we used RNA interference to investigate the function of POLID in procyclic *T. brucei*. Stem-loop silencing of POLID resulted in growth arrest and progressive loss of the kDNA network. Additional defects in kDNA replication included a rapid decline in minicircle and maxicircle abundance, and a transient accumulation of minicircle replication intermediates before loss of the kDNA network. These results demonstrate that POLID is a third essential DNA pol required for kDNA replication. While other eukaryotes utilize a single DNA pol (pol gamma) for replication of mitochondrial DNA, *T. brucei* requires at least three to maintain the complex kDNA network.

14727. **Chung, W. L., Leung, K. F., Carrington, M. & Field, M. C., 2008**. Ubiquitylation is required for degradation of transmembrane surface proteins in trypanosomes. *Traffic,* **9** (10): 1681-1697.

Department of Pathology, University of Cambridge, Cambridge, UK. [mcf34@cam.ac.uk].

The surface of *Trypanosoma brucei* is dominated by glycosyl-phosphatidylinositol (GPI)-anchored proteins, and endocytosis is clathrin dependent. The vast majority of internalized GPI-anchored protein is efficiently recycled, while the processes by which transmembrane domain (TMD) proteins are internalized and sorted are unknown. We demonstrate that internalization of invariant surface glycoprotein (ISG)65, a trypanosome TMD protein, involves ubiquitylation and also requires clathrin. We find a hierarchical requirement for cytoplasmic lysine residues in internalization and turnover, and a single position-specific lysine is sufficient for degradation, surface removal and attachment of oligoubiquitin chains. Ubiquitylation is context dependent as provision of additional lysine residues by C-terminal fusion of neuronal precursor cell-expressed developmentally downregulated protein (NEDD)8 fails to support ubiquitylation. Attachment of NEDD8 leads to degradation by a second ubiquitin-independent pathway. Moreover, degradation of ubiquitylated or NEDDylated substrate takes place in an acidic compartment and is proteosome independent. Significantly, in non-opisthokont lineages, Rsp5p or c-Cbl, the E3 ubiquitin ligases acting on endocytic cargo, are absent but Uba1 class genes are present and are required for cell viability and ISG65 ubiquitylation. Hence, ubiquitylation is an evolutionarily conserved mechanism for internalization of surface proteins, but aspects of the machinery differ substantially between the major eukaryotic lineages.

14728. **Clayton, C., Schwede, A., Stewart, M., Robles, A., Benz, C., Po, J., Wurst, M., Quieroz, R. & Archer, S., 2008.** Control of mRNA degradation in trypanosomes. *Biochemical Society Transactions,* **36** (3): 520-521.

ZMBH (Zentrum fur Molekulare Biologie der Universitat Heidelberg), Im Neuenheimer Feld 282, D69120 Heidelberg, Germany. [cclayton@zmbh.uni-heidelberg.de].

Control of gene expression in trypanosomes relies almost exclusively on post-transcriptional mechanisms. Trypanosomes have the normal enzymes for mRNA decay: both the exosome and a 5'-3'-exoribonuclease are important in the degradation of very unstable

transcripts, whereas the CAF1/NOT complex plays a major role in the degradation of all mRNAs tested. Targeted RNA interference screening was used to identify RNA-binding proteins that regulate mRNA degradation, and it revealed roles for proteins with RNA recognition motifs or pumilio domains.

14729. **Comini, M. A., Rettig, J., Dirdjaja, N., Hanschmann, E. M., Berndt, C. & Krauth-Siegel, R. L., 2008**. Monothiol glutaredoxin-1 is an essential iron-sulphur protein in the mitochondrion of African trypanosomes. *Journal of Biological Chemistry,* **283** (41): 27785-27798.

Centre of Biochemistry, Heidelberg University, Im Neuenheimer Feld 504, D-69120 Heidelberg, Germany. [mcomini@pateur.edu.uy].

African trypanosomes encode three monothiol glutaredoxins (1-C-Grx). 1-C-Grx1 occurs exclusively in the mitochondrion, and 1-C-Grx2 and -3 are predicted to be mitochondrial and cytosolic proteins, respectively. All three 1-C-Grx are expressed in both the mammalian bloodstream and the insect procyclic form of *Trypanosoma brucei*, with the highest levels found in stationary phase and starving parasites. In the rudimentary mitochondrion of bloodstream cells, 1-C-Grx1 reaches concentrations above 200 µm/subunit. Recombinant *T. brucei* 1-C-Grx1 exists as a noncovalent homodimer, whereas 1-C-Grx2 and 1-C-Grx3 are monomeric proteins. *In vitro*, dimeric 1-C-Grx1 coordinated an H_2O_2-sensitive [2Fe-2S] cluster that required GSH as an additional ligand. Both bloodstream and procyclic trypanosomes were refractory to down-regulation of 1-C-Grx1 expression by RNA interference. In procyclic parasites, the 1-C-grx1 alleles could only be deleted if an ectopic copy of the gene was expressed. A five-ten-fold overexpression of 1-C-Grx1 in both parasite forms did not yield a growth phenotype under optimal culture conditions. However, exposure of these cells to the iron chelator deferoxamine or H_2O_2, but not to iron or menadione, impaired cell growth. Treatment of wild-type bloodstream parasites with deferoxamine and H_2O_2 caused a two-fold down- and up-regulation of 1-C-Grx1, respectively. The results point to an essential role of the mitochondrial 1-C-Grx1 in the iron metabolism of these parasites.

14730. **Cooper, A., Tait, A., Sweeney, L., Tweedie, A., Morrison, L., Turner, C. M. & MacLeod, A., 2008**. Genetic analysis of the human infective trypanosome *Trypanosoma brucei gambiense*: chromosomal segregation, crossing over, and the construction of a genetic map. *Genome Biology,* **9** (6): R103.

Wellcome Centre for Molecular Parasitology, Glasgow Biomedical Research Centre, University Place, Glasgow G12 8TA, UK. [acc15p@udcf.gla.ac.uk]

Trypanosoma brucei is the causative agent of human sleeping sickness and animal trypanosomiasis in sub-Saharan Africa, and it has been subdivided into three subspecies: *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, which cause sleeping sickness in humans, and the nonhuman infective *Trypanosoma brucei brucei*. *T. b. gambiense* is the most clinically relevant subspecies, being responsible for more than 90 percent of all trypanosomal disease in humans. The genome sequence is now available, and a Mendelian genetic system has been demonstrated in *T. brucei*, facilitating genetic analysis in this diploid protozoan parasite. As an essential step toward identifying loci that determine important traits in the human-infective subspecies, we report the construction of a high-resolution genetic

map of the STIB 386 strain of *T. b. gambiense*. The genetic map was determined using 119 microsatellite markers assigned to the 11 megabase chromosomes. The total genetic map length of the linkage groups was 733.1 cM, covering a physical distance of 17.9 megabases with an average map unit size of 24 kilobases/cM. Forty-seven markers in this map were also used in a genetic map of the nonhuman infective *T. b. brucei* subspecies, permitting comparison of the two maps and showing that synteny is conserved between the two subspecies. The genetic linkage map presented here is the first available for the human-infective trypanosome *T. b. gambiense*. In combination with the genome sequence, this opens up the possibility of using genetic analysis to identify the loci responsible for *T. b. gambiense* specific traits such as human infectivity as well as comparative studies of parasite field populations.

14731. **Costas, M., Rodríguez-Larrea, D., De Maria, L., Borchert, T. V., Gómez-Puyou, A. & Sanchez-Ruiz, J. M., 2008**. Between-species variation in the kinetic stability of TIM proteins linked to solvation-barrier free energies. *Journal of Molecular Biology*. **e - Publication ahead of print 28 October 2008.**

Laboratorio de Biofisicoquimica, Departamento de Fisicoquimica, Facultad de Quimica, Universidad Nacional Autónoma de México, Cd Universitaria, México DF 04510, México.

14732. **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,* **283** (24): 16342-16354.

Laboratoire de Microbiologie Cellulaire et Moléculaire et Pathogénicité, UMR-5234 CNRS, Bordeaux, France. [bringaud@rmsb.u-bordeaux2.fr].

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 investigated 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 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 RNA interference-harbouring or knock-out 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 F(0)/F(1)-ATP synthase activity by RNA interference led to cell death in glucose-depleted medium, but not in glucose-rich medium. This implies that, in the presence of d-glucose, the importance of the F(0)/F(1)-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.

14733. **de Graffenried, C. L., Ho, H. H. & Warren, G., 2008**. Polo-like kinase is required for Golgi and bilobe biogenesis in *Trypanosoma brucei*. *Journal of Cell Biology,* **181** (3): 431-438.

Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06520, USA. [chris.degraffenried@univie.ac.at].

A bilobed structure marked by *Tb*Centrin2 regulates Golgi duplication in the protozoan parasite *Trypanosoma brucei*. This structure must itself duplicate during the cell cycle for Golgi inheritance to proceed normally. We show here that duplication of the bilobed structure is dependent on the single polo-like kinase (PLK) homologue in *T. brucei* (*Tb*PLK). Depletion of *Tb*PLK leads to malformed bilobed structures, which is consistent with an inhibition of duplication and an increase in the number of dispersed Golgi structures with associated endoplasmic reticulum exit sites. These data suggest that the bilobe may act as a scaffold for the controlled assembly of the duplicating Golgi.

14734. **Degrasse, J. A., Chait, B. T., Field, M. C. & Rout, M. P., 2008**. High-yield isolation and subcellular proteomic characterization of nuclear and subnuclear structures from trypanosomes. *Methods in Molecular Biology,* **463**: 77-92.

Laboratory of Mass Spectrometry and Gaseous Ion Chemistry, The Rockefeller University, New York, NY, USA.

The vast evolutionary distance between the Opisthokonta (animals and yeast) and the Excavata (a major group of protists, including *Giardia* and *Trypanosoma*) presents a significant challenge to *in silico* functional genomics and orthologue identification. Subcellular proteomic identification of the constituents of highly enriched organelles can alleviate this problem by both providing localization evidence and yielding a manageably sized proteome for detailed *in silico* functional assignment. We describe a method for the high-yield isolation of nuclei from the kinetoplastid *Trypanosoma brucei*. We also describe the subsequent purification of subnuclear compartments, including the nuclear envelope and nucleolus. Finally, using several proteomic strategies, we survey the proteome of a subcellular structure or organelle, using the nuclear pore complex as an example.

14735. **Emes, R. D. & Yang, Z., 2008**. Duplicated paralogous genes subject to positive selection in the genome of *Trypanosoma brucei*. *PLoS ONE,* **3** (5): e2295.

Institute for Science and Technology in Medicine, Keele University, Stoke-on-Trent, UK. [r.d.emes@hfac.keele.ac.uk].

Whole genome studies have highlighted duplicated genes as important substrates for adaptive evolution. We have investigated adaptive evolution in this class of genes in the human parasite *Trypanosoma brucei*, as indicated by the ratio of non-synonymous (amino-acid changing) to synonymous (amino acid retaining) nucleotide substitution rates. We have identified duplicated genes that are most rapidly evolving in this important human parasite. This is the first attempt to investigate adaptive evolution in this species at the codon level. We identify 109 genes within 23 clusters of paralogous gene expansions to be subject to positive selection. Genes identified include surface antigens in both the mammalian and insect host life cycle stage suggesting that competitive interaction is not solely with the

adaptive immune system of the mammalian host. Also surface transporters related to drug resistance and genes related to developmental progression are detected. We discuss how adaptive evolution of these genes may highlight lineage specific processes essential for parasite survival. We also discuss the implications of adaptive evolution of these targets for parasite biology and control.

14736. **Estevez, A. M., 2008**. The RNA-binding protein *Tb*DRBD3 regulates the stability of a specific subset of mRNAs in trypanosomes. *Nucleic Acids Research,* **36** (14): 4573-4586.

Instituto de Parasitologia y Biomedicina López-Neyra, CSIC Avda. del Conocimiento s/n, Armilla, 18100-Granada, Spain. [aestevez@ipb.csic.es].

In trypanosomes, the apparent lack of regulation of RNA polymerase II-dependent transcription initiation poses a challenge to understand how these eukaryotes adjust gene expression to adapt to the contrasting environments they find during their life cycles. Evidence so far indicates that mRNA turnover and translation are the major control points in which regulation is exerted in trypanosomes. However, very little is known about which proteins are involved, and how do they regulate the abundance and translation of different mRNAs in different life stages. In this work, an RNA-binding protein, *Tb*DRBD3, has been identified by affinity chromatography, and its function addressed using RNA interference, microarray analysis and immunoprecipitation of mRNA-protein complexes. The results obtained indicate that *Tb*DRBD3 binds to a subset of developmentally regulated mRNAs encoding membrane proteins, and that this association promotes the stabilization of the target transcripts. These observations raise the possibility that *Tb*DRBD3-mRNA complexes act as a post-transcriptional operon, and provide a framework to interpret how trypanosomes regulate gene expression in the absence of transcriptional control.

14737. **Etheridge, R. D., Aphasizheva, I., Gershon, P. D. & Aphasizhev, R., 2008**. 3' adenylation determines mRNA abundance and monitors completion of RNA editing in *T. brucei* mitochondria. *Embo Journal,* **27** (11): 1596-1608.

Department of Microbiology and Molecular Genetics, School of Medicine, University of California, Irvine, CA 92697, USA. [ruslan@uci.edu]

Expression of the mitochondrial genome in protozoan parasite *Trypanosoma brucei* is controlled post-transcriptionally and requires extensive U-insertion/deletion mRNA editing. In mitochondrial extracts, 3' adenylation reportedly influences degradation kinetics of synthetic edited and pre-edited mRNAs. We have identified and characterized a mitochondrial poly(A) polymerase, termed KPAP1, and determined major polypeptides in the polyadenylation complex. Inhibition of KPAP1 expression abrogates short and long A-tails typically found in mitochondrial mRNAs, and decreases the abundance of never-edited and edited transcripts. Pre-edited mRNAs are not destabilized by the lack of 3' adenylation, whereas short A-tails are required and sufficient to maintain the steady-state levels of partially edited, fully edited, and never-edited mRNAs. The editing directed by a single guide RNA is sufficient to impose a requirement for the short A-tail in edited molecules. Upon completion of the editing process, the short A-tails are extended as (A/U) heteropolymers into structures previously thought to be long poly(A) tails. These data provide the first direct

evidence of functional interactions between 3' processing and editing of mitochondrial mRNAs in trypanosomes.

14738. **Feistel, T., Hodson, C. A., Peyton, D. H. & Landfear, S. M., 2008**. An expression system to screen for inhibitors of parasite glucose transporters. *Molecular and Biochemical Parasitology,* **162** (1): 71-76.

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

Chemotherapy of parasitic protists is limited by general toxicity, high expense and emergence of resistance to currently available drugs. Thus methods to identify new leads for further drug development are increasingly important. Previously, glucose transporters have been validated as new drug targets for protozoan parasites including *Plasmodium falciparum, Leishmania mexicana* and *Trypanosoma brucei*. A recently derived glucose transporter null mutant (Deltalmgt) of *L. mexicana* was used to functionally express various heterologous glucose transporters including those from *T. brucei* THT1, *P. falciparum* PfHT and human GLUT1-resulting in recovery of growth of the Deltalmgt null mutant in glucose replete medium. This heterologous expression system can be employed to screen for compounds that retard growth by inhibiting the expressed glucose transporter. The ability of this expression system to identify specific glucose transporter inhibitors was demonstrated using 3-O-undec-10-enyl-d-glucose, a previously described specific inhibitor of PfHT.

14739. **Ferella, M., Li, Z. H., Andersson, B. & Docampo, R., 2008**. Farnesyl diphosphate synthase localizes to the cytoplasm of *Trypanosoma cruzi* and *T. brucei*. *Experimental Parasitology,* **119** (2): 308-312.

Department of Cell and Molecular Biology (CMB), Karolinska Institutet, Berzelius vag 35, 171 77 Stockholm, Sweden. [marcela.ferella@ki.se]

The farnesyl diphosphate synthase (FPPS) has previously been characterized in trypanosomes as an essential enzyme for their survival and as the target for bisphosphonates, drugs that are effective both *in vitro* and *in vivo* against these parasites. Enzymes from the isoprenoid pathway have been assigned to different compartments in eukaryotes, including trypanosomatids. We here report that FPPS localizes to the cytoplasm of both *Trypanosoma cruzi* and *T. brucei*, and is not present in other organelles such as the mitochondria and glycosomes.

14740. **Field, M. C. & O'Reilly, A. J., 2008**. How complex is GTPase signalling in trypanosomes? *Trends in Parasitology,* **24** (6): 253-257.

The Molteno Building, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, UK. [mcf34@cam.ac.uk]

Many signalling pathways in higher eukaryotes use Ras-like small GTPases. Here, we ask how complex are these small GTPase signalling pathways in trypanosomes? We seek to address this issue by comparisons with the representation of both the GTPase molecules and their accessory factors in several genomes.

14741. **Figueiredo, L. M., Janzen, C. J. & Cross, G. A., 2008**. A histone methyltransferase modulates antigenic variation in African trypanosomes. *PLoS Biology*, **6** (7): e161.

Laboratory of Molecular Parasitology, Rockefeller University, New York, USA. [george.cross@rockefeller.edu].

To evade the host immune system, several pathogens periodically change their cell-surface epitopes. In the African trypanosomes, antigenic variation is achieved by tightly regulating the expression of a multigene family encoding a large repertoire of variant surface glycoproteins (VSGs). Immune evasion relies on two important features: exposing a single type of VSG at the cell surface and periodically and very rapidly switching the expressed VSG. Transcriptional switching between resident telomeric VSG genes does not involve DNA rearrangements, and regulation is probably epigenetic. The histone methyltransferase DOT1B is a nonessential protein that trimethylates lysine 76 of histone H3 in *Trypanosoma brucei*. Here we report that transcriptionally silent telomeric VSGs become partially derepressed when DOT1B is deleted, whereas nontelomeric loci are unaffected. DOT1B also is involved in the kinetics of VSG switching: in DeltaDOT1B cells, the transcriptional switch is so slow that cells expressing two VSGs persist for several weeks, indicating that monoallelic transcription is compromised. We conclude that DOT1B is required to maintain strict VSG silencing and to ensure rapid transcriptional VSG switching, demonstrating that epigenetics plays an important role in regulating antigenic variation in *T. brucei*.

14742. **Fisk, J. C., Ammerman, M. L., Presnyak, V. & Read, L. K., 2008**. *Tb*RGG2, an essential RNA editing accessory factor in two *Trypanosoma brucei* life cycle stages. *Journal of Biological Chemistry,* **283** (34): 23016-23025.

Department of Microbiology and Immunology, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, New York 14214, USA. [lread@buffalo.edu].

In the mitochondria of kinetoplastid protozoa, including *Trypanosoma brucei*, RNA editing inserts and/or deletes uridines from pre-mRNAs to produce mature, translatable mRNAs. RNA editing is carried out by several related multiprotein complexes known as editosomes, which contain all of the enzymatic components required for catalysis of editing. In addition, noneditosome accessory factors necessary for editing of specific RNAs have also been described. Here, we report the *in vitro* and *in vivo* characterization of the mitochondrial *Tb*RGG2 protein (originally termed *Tb*RGGm) and demonstrate that it acts as an editing accessory factor. *Tb*RGG2 is an RNA-binding protein with a preference for poly(U). *Tb*RGG2 protein levels are up-regulated ten-fold in procyclic form *T. brucei* compared with bloodstream forms. Nevertheless, the protein is essential for growth in both life cycle stages. *Tb*RGG2 associates with RNase-sensitive and RNase-insensitive mitochondrial complexes, and a small fraction of the protein co-immunoprecipitates with editosomes. RNA interference-mediated depletion of *Tb*RGG2 in both the procyclic and bloodstream forms of *T. brucei* leads to a dramatic decrease in pan-edited RNAs and in some cases a corresponding increase in the pre-edited RNA. *Tb*RGG2 down-regulation also results in moderate stabilization of never-edited and minimally edited RNAs. Thus, our data are consistent with a model in which *Tb*RGG2 is multifunctional, strongly facilitating the editing of pan-edited RNAs and modestly destabilizing minimally edited and never-edited RNAs. This is the first

example of an RNA editing accessory factor that functions in the mammalian infective *T. brucei* life cycle stage.

14743. **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.

Department of Pathology and Microbiology-Immunology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA. [d-engman@northwestern.edu].

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*.

14744. **Gibellini, F., Hunter, W. N. & Smith, T. K., 2008**. Biochemical characterization of the initial steps of the Kennedy pathway in *Trypanosoma brucei*: The ethanolamine and choline kinases. *Biochemical Journal,* **415** (1): 135-144.

Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK. [tks1@st-andrews.ac.uk].

Ethanolamine and choline are major components of the trypanosome membrane phospholipids, in the form of GPEtn (glycerophosphoethanolamine) and GPCho (glycerophosphocholine). Ethanolamine is also found as an integral component of the GPI

(glycosylphosphatidylinositol) anchor that is required for membrane attachment of cell-surface proteins, most notably the variant-surface glycoproteins. The *de novo* synthesis of GPEtn and GPCho starts with the generation of phosphoethanolamine and phosphocholine by ethanolamine and choline kinases via the Kennedy pathway. Database mining revealed two putative C/EKs (choline/ethanolamine kinases) in the *Trypanosoma brucei* genome, which were cloned, overexpressed, purified and characterized. *Tb*EK1 (*T. brucei* ethanolamine kinase 1) was shown to be catalytically active as an ethanolamine-specific kinase, i.e. it had no choline kinase activity. The K(m) values for ethanolamine and ATP were found to be 18.4+/-0.9 and 219+/-29 µM respectively. TbC/EK2 (*T. brucei* choline/ethanolamine kinase 2), on the other hand, was found to be able to phosphorylate both ethanolamine and choline, even though choline was the preferred substrate, with a K(m) 80 times lower than that of ethanolamine. The K(m) values for choline, ethanolamine and ATP were 31.4+/-2.6 µM, 2.56+/-0.31 mM and 20.6+/-1.96 µM respectively. Further substrate specificity analysis revealed that both *Tb*EK1 and *TbC*/EK2 were able to tolerate various modifications at the amino group, with the exception of a quaternary amine for *Tb*EK1 (choline) and a primary amine for *TbC*/EK2 (ethanolamine). Both enzymes recognized analogues with substituents on C-2, but substitutions on C-1 and elongations of the carbon chain were not well tolerated.

14745. **Gluenz, E., Sharma, R., Carrington, M. & Gull, K., 2008**. Functional characterization of cohesin subunit SCC1 in *Trypanosoma brucei* and dissection of mutant phenotypes in two life cycle stages. *Molecular Microbiology,* **69** (3): 666-680.

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK. [eva.gluenz@path.ox.ac.uk].

In yeast and metazoa, structural maintenance of chromosome (SMC) complexes plays key roles in chromosome segregation, architecture and DNA repair. The main function of the cohesin complex is to hold replicated sister chromatids together until segregation at anaphase, which is dependent on proteolytic cleavage of the cohesin subunit SCC1. Analysis of trypanosomatid genomes showed that the core cohesin and condensin complexes are conserved, but SMC5/6 is absent. To investigate the functional conservation of cohesin in eukaryotes distantly related to yeast and metazoa, we characterized the *Trypanosoma brucei* SCC1 orthologue. *Tb*SCC1 is expressed prior to DNA synthesis at late G1, remains in the nucleus throughout S- and G2-phases of the cell cycle and disappears at anaphase. Depletion of SCC1 by RNAi or expression of a non-cleavable SCC1 resulted in karyokinesis failure. Using the dominant negative phenotype of non-cleavable SCC1 we investigated checkpoint regulation of cytokinesis in response to mitosis failure at anaphase. In the absence of chromosome segregation, procyclic trypanosomes progressed through cytokinesis to produce one nucleated and one anucleate cell (zoid). In contrast, cytokinesis was incomplete in bloodstream forms, where cleavage was initiated but cells failed to progress to abscission. Kinetoplast duplication was uninterrupted resulting in cells with multiple kinetoplasts and flagella.

14746. **Goldshmidt, H., Sheiner, L., Butikofer, P., Roditi, I., Uliel, S., Gunzel, M., Engstler, M. & Michaeli, S., 2008**. Role of protein translocation pathways across the endoplasmic reticulum in *Trypanosoma brucei*. *Journal of Biological Chemistry,* **283** (46): 32085-32098.

The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel, the Institutes of Biochemistry and Molecular Medicine and Cell Biology, University of Bern, 3012 Bern, Switzerland. [michaes@mail.biu.ac.il].

The translocation of secretory and membrane proteins across the endoplasmic reticulum (ER) membrane is mediated by co-translational (via the signal recognition particle (SRP)) and post-translational mechanisms. In this study, we investigated the relative contributions of these two pathways in trypanosomes. A homologue of SEC71, which functions in the post-translocation chaperone pathway in yeast, was identified and silenced by RNA interference. This factor is essential for parasite viability. In SEC71-silenced cells, signal peptide (SP)-containing proteins traversed the ER, but several were mislocalized, whereas polytopic membrane protein biogenesis was unaffected. Surprisingly trypanosomes can interchangeably utilize two of the pathways to translocate SP-containing proteins except for glycosylphosphatidylinositol-anchored proteins, whose level was reduced in SEC71-silenced cells but not in cells depleted for SRP68, an SRP-binding protein. Entry of SP-containing proteins to the ER was significantly blocked only in cells co-silenced for the two translocation pathways (SEC71 and SRP68). SEC63, a factor essential for both translocation pathways in yeast, was identified and silenced by RNA interference. SEC63 silencing affected entry to the ER of both SP-containing proteins and polytopic membrane proteins, suggesting that, as in yeast, this factor is essential for both translocation pathways *in vivo*. This study suggests that, unlike bacteria or other eukaryotes, trypanosomes are generally promiscuous in their choice of mechanism for translocating SP-containing proteins to the ER, although the SRP-independent pathway is favoured for glycosylphosphatidylinositol-anchored proteins, which are the most abundant surface proteins in these parasites.

14747. **Guo, X., Ernst, N. L. & Stuart, K. D., 2008**. The KREPA3 zinc finger motifs and OB-fold domain are essential for RNA editing and survival of *Trypanosoma brucei*. *Molecular and Cellular Biology,* **28** (22): 6939-6953.

Seattle Biomedical Research Institute, 307 Westlake Avenue North, Suite 500, Seattle, WA 98109, USA. [ken.stuart@sbri.org].

Three types of editosomes, each with an identical core containing six related KREPA proteins, catalyze the U insertion and deletion RNA editing of mitochondrial mRNAs in trypanosomes. Repression of expression of one of these, KREPA3 (also known as *Tb*MP42), shows that it is essential for growth and *in vivo* editing in both procyclic (PF) and bloodstream (BF) life cycle stages of *Trypanosoma brucei*. RNA interference knockdown results in editosome disruption and altered *in vitro* editing in PFs, while repression by regulatable double knockout results in almost complete loss of editosomes in BFs. Mutational analysis shows that the KREPA3 zinc fingers and OB-fold domain are each essential for growth and *in vivo* editing. Nevertheless, KREPA3 with mutated zinc fingers incorporates into editosomes that catalyze *in vitro* editing and thus is not essential for editosome integrity, although stability is affected. In contrast, the OB-fold domain is essential for editosome integrity. Overall, KREPA3, especially its OB-fold, functions in editosome integrity, and its zinc fingers are essential for editing *in vivo* but not for the central catalytic steps. KREPA3 may function in editosome organization and/or RNA positioning.

14748. **Hernandez, A., Panigrahi, A., Cifuentes-Rojas, C., Sacharidou, A., Stuart, K. & Cruz-Reyes, J., 2008**. Determinants for association and guide RNA-directed endonuclease cleavage by purified RNA editing complexes from *Trypanosoma brucei. Journal of Molecular Biology,* **381** (1): 35-48.

Department of Biochemistry and Biophysics, Texas A&M University, 2128 TAMU, College Station, TX 77843, USA. [cruzrey@tamu.edu].

U-insertion/deletion RNA editing in the single mitochondrion of kinetoplastids, an ancient lineage of eukaryotes, is a unique mRNA maturation process needed for translation. Multisubunit editing complexes recognize many pre-edited mRNA sites and modify them via cycles of three catalytic steps: guide RNA (gRNA)-directed cleavage, insertion or deletion of uridylates at the 3'-terminus of the upstream cleaved piece, and ligation of the two mRNA pieces. While catalytic and many structural protein subunits of these complexes have been identified, the mechanisms and basic determinants of substrate recognition are still poorly understood. This study defined relatively simple single- and double-stranded determinants for association and gRNA-directed cleavage. To this end, we used an electrophoretic mobility shift assay to directly score the association of purified editing complexes with RNA ligands, in parallel with UV photocrosslinking and functional studies. The cleaved strand required a minimal 5' overhang of 12 nt and an approximately 15-bp duplex with gRNA to direct the cleavage site. A second protruding element in either the cleaved or the guide strand was required unless longer duplexes were used. Importantly, the single-stranded RNA requirement for association can be upstream or downstream of the duplex, and the binding and cleavage activities of purified editing complexes could be uncoupled. The current observations together with our previous reports in the context of purified native editing complexes show that the determinants for association, cleavage and full-round editing gradually increase in complexity as these stages progress. The native complexes in these studies contained most, if not all, known core subunits in addition to components of the MRP complex. Finally, we found that the endonuclease KREN1 in purified complexes photocrosslinks with a targeted editing site. A model is proposed whereby one or more RNase III-type endonucleases mediate the initial binding and scrutiny of potential ligands and subsequent catalytic selectivity triggers either insertion or deletion editing enzymes.

14749. **Hertz-Fowler, C., Figueiredo, L. M., Quail, M. A., Becker, M., Jackson, A., Bason, N., Brooks, K., Churcher, C., Fahkro, S., Goodhead, I., Heath, P., Kartvelishvili, M., Mungall, K., Harris, D., Hauser, H., Sanders, M., Saunders, D., Seeger, K., Sharp, S., Taylor, J. E., Walker, D., White, B., Young, R., Cross, G. A., Rudenko, G., Barry, J. D., Louis, E. J. & Berriman, M., 2008**. Telomeric expression sites are highly conserved in *Trypanosoma brucei. PLoS ONE,* **3** (10): e3527.

Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK. [chf@sanger.ac.uk].

Subtelomeric regions are often under-represented in genome sequences of eukaryotes. One of the best known examples of the use of telomere proximity for adaptive purposes are the bloodstream expression sites (BESs) of the African trypanosome *Trypanosoma brucei*. To enhance our understanding of BES structure and function in host adaptation and immune

evasion, the BES repertoire from the Lister 427 strain of *T. brucei* was independently tagged and sequenced. BESs are polymorphic in size and structure but reveal a surprisingly conserved architecture in the context of extensive recombination. Very small BESs do exist and many functioning BESs do not contain the full complement of expression site associated genes (ESAGs). The consequences of duplicated or missing ESAGs, including ESAG9, a newly named ESAG12, and additional variant surface glycoprotein genes (VSGs) were evaluated by functional assays after BESs were tagged with a drug-resistance gene. Phylogenetic analysis of constituent ESAG families suggests that BESs are sequence mosaics and that extensive recombination has shaped the evolution of the BES repertoire. This work opens important perspectives in understanding the molecular mechanisms of antigenic variation, a widely used strategy for immune evasion in pathogens, and telomere biology.

14750. **Hide, G., 2008**. Visualizing trypanosome sex. *Trends in Parasitology,* **24** (10): 425-428.

Centre for Parasitology and Disease, Biomedical Sciences Research Institute, University of Salford, Salford, M5 4WT, UK. [g.hide@salford.ac.uk].

Genetic exchange in *Trypanosoma brucei* is now well characterized. It is a key tool that has enabled an understanding of important parasite genetic traits and underpinned the *Trypanosoma brucei* genome project. However, a key aspect that has eluded us is the point in the trypanosome life cycle where genetic exchange occurs. Research using green and red fluorescent trypanosomes for visualizing genetic crosses has now identified this stage.

14751. **Kamleh, A., Barrett, M. P., Wildridge, D., Burchmore, R. J., Scheltema, R. A. & Watson, D. G., 2008**. Metabolomic profiling using Orbitrap Fourier transform mass spectrometry with hydrophilic interaction chromatography: a method with wide applicability to analysis of biomolecules. *Rapid Communications in Mass Spectrometry,* **22** (12): 1912-1918.

Strathclyde Institute for Pharmacy and Biomedical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, UK. [d.g.watson@strath.ac.uk].

It was shown that coupling hydrophilic interaction chromatography (HILIC) to Orbitrap Fourier transform mass spectrometery (FT-MS) provided an excellent tool for metabolic profiling, principally due to rapid elution of lipids in advance of most metabolites entering the mass spectrometer. We used *in vitro* cultivated procyclic forms of the protozoan parasite *Trypanosoma brucei* as a source of metabolites to test the performance of the HILIC column and the mass accuracy of MS. The mass accuracy achieved fell within 2 ppm for all the metabolites identified within samples. It was, for example, possible to identify the signature metabolite of the trypanosome, trypanothione, and also glutathione which were well retained by the HILIC column. By comparing trypanosomes grown in two different media we were able to clearly distinguish the samples in terms of the relative abundance of a number of metabolites using Sieve 1.1 software.

14752. **Kawahara, T., Siegel, T. N., Ingram, A. K., Alsford, S., Cross, G. A. & Horn, D., 2008**. Two essential MYST-family proteins display distinct roles in histone H4K10

acetylation and telomeric silencing in trypanosomes. *Molecular Microbiology,* **69** (4): 1054-1068.

London School of Hygiene and Tropical Medicine, London, UK. [david.horn@lshtm.ac.uk].

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

14753. **Koumandou, V. L., Natesan, S. K., Sergeenko, T. & Field, M. C., 2008**. The trypanosome transcriptome is remodelled during differentiation but displays limited responsiveness within life stages. *BMC Genomics,* **9**: 298.

The Molteno Building, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, UK. [vk219@cam.ac.uk].

Trypanosomatids utilize polycistronic transcription for production of the vast majority of protein-coding mRNAs, which operates in the absence of gene-specific promoters. Resolution of nascent transcripts by polyadenylation and trans-splicing, together with specific rates of mRNA turnover, serve to generate steady state transcript levels that can differ in abundance across several orders of magnitude and can be developmentally regulated. We used a targeted oligonucleotide microarray, representing the strongly developmentally-regulated *T. brucei* membrane trafficking system and approximately 10 percent of the *T. brucei* genome, to investigate both between-stage, or differentiation-dependent, transcriptome changes and within-stage flexibility in response to various challenges. 6 percent of the gene cohort are developmentally regulated, including several small GTPases, SNAREs, vesicle coat factors and protein kinases both consistent with and extending previous data. Therefore substantial differentiation-dependent remodelling of the trypanosome transcriptome is associated with membrane transport. Both the microarray and qRT-PCR were then used to analyse transcriptome changes resulting from specific gene overexpression, knockdown, altered culture conditions and chemical stress. Firstly, manipulation of Rab5 expression results in co-ordinate changes to clathrin protein expression levels and endocytotic activity, but no detectable changes to steady-state mRNA levels, which indicates that the effect is mediated post-transcriptionally. Secondly, knockdown of clathrin or the variant surface

glycoprotein failed to perturb transcription. Thirdly, exposure to dithiothreitol or tunicamycin revealed no evidence for a classical unfolded protein response, mediated in higher eukaryotes by transcriptional changes. Finally, altered serum levels invoked little transcriptome alteration beyond changes to expression of ESAG6/7, the transferrin receptor. While trypanosomes regulate mRNA abundance to effect the major changes accompanying differentiation, a given differentiated state appears transcriptionally inflexible. The implications of the absence of a transcriptome response in trypanosomes for both virulence and models of life cycle progression are discussed.

14754. **Krauth-Siegel, R. L. & Comini, M. A., 2008**. Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. *Biochimica and Biophysica Acta,* **1780** (11): 1236-1248.

Biochemie-Zentrum der Universitat Heidelberg, 69120 Heidelberg, Germany. [luise.krauth-siegel@bzh.uni-heidelberg.de].

14755. **Li, S. Q., Reid, S. A., Fung, M. C., Inoue, N. & Lun, Z. R., 2008**. Analysis of gene expression profiles in the liver and spleen of mice infected with *Trypanosoma evansi* by using a cDNA microarray. *Parasitology Research*. **Published online 9 October, 2008.**

Center for Parasitic Organisms, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-Sen (Zhongshan) University, Guangzhou, 510275, People's Republic of China.[lsslzr@mail.sysu.edu.cn].

Trypanosoma evansi, the cause of the disease surra in livestock, is the most widely geographically distributed pathogenic trypanosome occurring in Africa, South and Central America, and Asia, where it causes significant economic loss. Although many studies have described the histopathology induced in the organs of mice infected with *T. evansi,* few studies have been conducted on gene expression in these organs. Here we used complementary DNA microarray to analyze the gene expression profiles in the liver and spleen of mice infected with *T. evansi* (STIB 806) at the peak parasitaemia (seven days after infection). A total of 14 000 sequences including full length and partial complementary DNAs representing novel, known, and control genes of mouse were analyzed. Results from GeneOntology annotation showed that 158 genes in the liver and 73 genes in the spleen were up-regulated in the infected mice and that 178 genes in the liver and 117 genes in the spleen of infected mice were down-regulated compared with control (non-infected) mice. Most of these genes are metabolism, transport, protein biosynthesis, transcription factors, and nucleic acid binding protein-related genes. The changes of some important genes, such as heat shock protein 70 and proliferating cell nuclear antigen, were confirmed by quantitative reverse transcriptase polymerase chain reaction and immunohistochemistry. TdT-mediated dUTP-digoxigenin nick end labelling analysis results revealed that extensive apoptosis occurred in the liver of infected mice at the peak of parasitaemia. Our results provide a comprehensive profile of changes in gene expression in the liver and spleen of mice infected with *T. evansi* and may be helpful in understanding the pathogenesis of surra at a molecular level.

14756. **Li, Z. & Wang, C. C., 2008**. KMP-11, a basal body and flagellar protein, is required for cell division in *Trypanosoma brucei*. *Eukaryotic Cell,* **7** (11): 1941-1950.

Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94158-2280, USA. [ccwang@cgl.ucsf.edu].

Kinetoplastid membrane protein 11 (KMP-11) has been identified as a flagellar protein and is conserved among kinetoplastid parasites, but its potential function remains unknown. In a recent study, we identified KMP-11 as a microtubule-bound protein localizing to the flagellum as well as the basal body in both procyclic and bloodstream forms of *Trypanosoma brucei*. Silencing of KMP-11 by RNA interference inhibited basal body segregation and cytokinesis in both forms and resulted in multiple nuclei of various sizes, indicating a continuous, albeit somewhat defective, nuclear division while cell division was blocked. KMP-11 knockdown in the procyclic form led to severely compromised formation of the new flagellum attachment zone (FAZ) and detachment of the newly synthesized flagellum. However, a similar phenotype was not observed in the bloodstream form depleted of KMP-11. Thus, KMP-11 is a flagellar protein playing critical roles in regulating cytokinesis in both forms of the trypanosomes. Its distinct roles in regulating FAZ formation in the two forms may provide a clue to the different mechanisms of cytokinetic initiation in procyclic and bloodstream trypanosomes.

14757. **Li, Z., Lee, J. H., Chu, F., Burlingame, A. L., Gunzl, A. & Wang, C. C., 2008**. Identification of a novel chromosomal passenger complex and its unique localization during cytokinesis in *Trypanosoma brucei*. *PLoS ONE,* **3** (6): e2354.

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

Aurora B kinase is a key component of the chromosomal passenger complex (CPC), which regulates chromosome segregation and cytokinesis. An orthologue of Aurora B was characterized in *Trypanosoma brucei* (*Tb*AUK1), but other conserved components of the complex have not been found. Here we identified four novel *Tb*AUK1 associated proteins by tandem affinity purification and mass spectrometry. Among these four proteins, *Tb*KIN-A and *Tb*KIN-B are novel kinesin homologues, whereas *Tb*CPC1 and *Tb*CPC2 are hypothetical proteins without any sequence similarity to those known CPC components from yeasts and metazoans. RNAi-mediated silencing of each of the four genes led to loss of spindle assembly, chromosome segregation and cytokinesis. *Tb*KIN-A localizes to the mitotic spindle and *Tb*KIN-B to the spindle midzone during mitosis, whereas *Tb*CPC1, *Tb*CPC2 and *Tb*AUK1 display the dynamic localization pattern of a CPC. After mitosis, the CPC disappears from the central spindle and re-localizes at a dorsal mid-point of the mother cell, where the anterior tip of the daughter cell is tethered, to start cell division toward the posterior end, indicating a most unusual CPC-initiated cytokinesis in a eukaryote.

14758. **Long, S., Jirku, M., Ayala, F. J. & Lukes, J., 2008**. Mitochondrial localization of human frataxin is necessary but processing is not for rescuing frataxin deficiency in *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences USA,* **105** (36): 13468-13473.

Biology Centre, Institute of Parasitology, Czech Academy of Sciences, and Faculty of Natural Sciences, University of South Bohemia, 37005 Ceske Budejovice, Czech Republic. [jula@paru.cas.cz].

Trypanosoma brucei, the agent of human sleeping sickness and ruminant nagana, is the most genetically tractable representative of the domain Excavata. It is evolutionarily very distant from humans, with a last common ancestor over 1 billion years ago. Frataxin, a highly conserved small protein involved in iron-sulphur cluster synthesis, is present in both organisms, and its deficiency is responsible for Friedreich's ataxia in humans. We have found that *T. brucei* growth-inhibition phenotype caused by down-regulated frataxin is rescued by means of human frataxin. The rescue is fully dependent on the human frataxin being imported into the trypanosome mitochondrion. Processing of the imported protein by mitochondrial processing peptidase can be blocked by mutations in the signal peptide, as in human cells. Although in human cells frataxin must be processed to execute its function, the same protein in the *T. brucei* mitochondrion is functional even in the absence of processing. Our results illuminate remarkable conservation of the mechanisms of mitochondrial protein import and processing.

14759. **Long, S., Vavrova, Z. & Lukes, J., 2008**. The import and function of diatom and plant frataxins in the mitochondrion of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology,* **162** (1): 100-104.

Biology Centre, Institute of Parasitology, Czech Academy of Sciences, and Faculty of Natural Sciences, University of South Bohemia, Branisovska 31, 37005 Ceske Budejovice (Budweis), Czech Republic. [jula@paru.cas.cz].

14760. **Machado-Silva, A., Teixeira, S. M., Franco, G. R., Macedo, A. M., Pena, S. D., McCulloch, R. & Machado, C. R., 2008**. Mismatch repair in *Trypanosoma brucei:* heterologous expression of MSH2 from *Trypanosoma cruzi* provides new insights into the response to oxidative damage. *Gene,* **411** (1-2): 19-26.

Instituto de Ciêencias Biológicas, Universidade Federal de Minas Gerais, Departamento de Bioquímica e Imunologia, Belo Horizonte, Brazil.

Trypanosomes are unicellular eukaryotes that cause disease in humans and other mammals. *Trypanosoma cruzi* and *Trypanosoma brucei* are the causative agents, respectively, of Chagas disease in the Americas and sleeping sickness in sub-Saharan Africa. To better comprehend the interaction of these parasites with their hosts, understanding the mechanisms involved in the generation of genetic variability is critical. One such mechanism is mismatch repair (MMR), which has a crucial, evolutionarily conserved role in maintaining the fidelity of DNA replication, as well as acting in other cellular processes, such as DNA recombination. Here we have attempted to complement *T. brucei* MMR through the expression of MSH2 from *T. cruzi*. Our results show that *T. brucei* MSH2-null mutants are more sensitive to hydrogen peroxide (H_2O_2) than wild type cells, suggesting the involvement of MSH2 in the response to oxidative stress in this parasite. This phenotype is reverted by the expression of either the *T. cruzi* or the *T. brucei* MSH2 protein in the MSH2-null mutants. In contrast, MMR complementation, as assessed by resistance to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and microsatellite instability, was not achieved by the

heterologous expression of *T. cruzi* MSH2. This finding, associated to the demonstration that mutation of MLH1, another component of the MMR system, did not affect sensitivity of *T. brucei* cells to H2O2, suggests an additional role of MSH2 in dealing with oxidative damage in these parasites, which may occur independently of MMR.

14761. **Maier, A. & Steverding, D., 2008**. Expression and purification of non-glycosylated *Trypanosoma brucei* transferrin receptor in insect cells. *Experimental Parasitology,* **120** (2): 205-207.

Department of Parasitology, Ruprecht-Karls-University, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany. [dsteverding@hotmail.com].

The transferrin receptor of the parasite *Trypanosoma brucei* is a heterodimeric protein complex encoded by the two expression site-associated genes (ESAGs) 6 and 7. ESAG6 is a heterogeneously glycosylated protein of 50-60kDa modified by a glycosylphosphatidylinositol anchor at the C-terminus, while ESAG7 is a 40-42kDa glycoprotein carrying an unmodified C-terminus. In order to determine whether glycosylation is necessary for dimer formation and ligand binding, the receptor was expressed in insect cells in the presence of tunicamycin. When insect cells were infected with recombinant ESAG6/ESAG7 double expressor baculovirus and grown in the presence of tunicamycin, non-glycosylated forms of ESAG6 and ESAG7 of 46 and 36kDa, respectively, were synthesized. The non-glycosylated ESAG6 and ESAG7 were capable of forming a heterodimer and of binding transferrin. This result shows that glycosylation is not necessary for synthesis of a functional *T. brucei* transferrin receptor.

14762. **Mallick, B., Ghosh, Z. & Chakrabarti, J., 2008**. MicroRNA switches in *Trypanosoma brucei*. *Biochemical and Biophysical Research Communications,* **372** (3): 459-463.

Computational Biology Group, Indian Association for the Cultivation of Science, 2A & 2B, Raja S.C. Mullick Road, Jadavpur, Calcutta 700 032, India. [tpbm@iacs.res.in].

Trypanosoma brucei develops chronic infection in mammalian hosts due to a sophisticated strategy of antigenic variation of variant surface glycoprotein (VSG) coat to escape antibody-mediated lysis. MicroRNAs are a class of non-coding RNAs with presumed post-transcriptional regulatory activity. Homology based informatic approach is used to identify the microRNA (miRNA) genes of *T. brucei* and their target mRNAs. Our observation reveals a set of microRNAs targeting mRNAs corresponding to VSGs. Further, a number of miRNA hairpins have been found in clusters of multiple identical copies. The target proteins, 20S proteosome, GM6 and GRESAG 4.2 corresponding to these clustered miRNAs play essential role in trypanosomiasis. These snippets can act as genetic switches modulating host-parasite interaction and provide useful clue toward treatment of trypanosomiasis.

14763. **Marciano, D., Llorente, C., Maugeri, D. A., de la Fuente, C., Opperdoes, F., Cazzulo, J. J. & Nowicki, C., 2008**. Biochemical characterization of stage-specific isoforms of aspartate aminotransferases from *Trypanosoma cruzi* and *Trypanosoma brucei*. *Molecular and Biochemical Parasitology,* **161** (1): 12-20.

Instituto de Química y Fisicoquímica Biólogica IQUIFIB-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junin 956, C1113AAD, Buenos Aires, Argentina. [cnowicki@criba.edu.ar].

Three genes encoding putative aspartate aminotransferases (ASATs) were identified in the *Trypanosoma cruzi* genome. Two of these ASAT genes, presumably corresponding to a cytosolic and mitochondrial isoform, were cloned and expressed as soluble His-tagged proteins in *Escherichia coli*. The specific activities determined for both *T. cruzi* isozymes were notably higher than the values previously reported for *Trypanosoma brucei* orthologues. To confirm these differences, *T. brucei* mASAT and cASAT were also expressed as His-tagged enzymes. The kinetic analysis showed that the catalytic parameters of the new recombinant *T. brucei* ASATs were very similar to those determined for *T. cruzi* orthologues. The cASATs from both parasites displayed equally broad substrate specificities, while mASATs were highly specific towards aspartate/2-oxoglutarate. The subcellular localization of the mASAT was confirmed by digitonin extraction of intact epimastigotes. At the protein level, cASAT is constitutively expressed in *T. brucei*, whereas mASAT is down-regulated in the bloodstream forms. By contrast in *T. cruzi*, mASAT is expressed along the whole life cycle, whereas cASAT is specifically induced in the mammalian stages. Similarly, the expression of malate dehydrogenases (MDHs) is developmentally regulated in *T. cruzi* while glycosomal MDH is only expressed in epimastigotes and mitochondrial MDH is present in the insect and mammalian stages. Taken together, these findings provide evidence for a metabolically active mitochondrion in the mammalian stages of *T. cruzi*, and suggest that the succinate excreted by amastigotes more likely represents a side product of an at least partially operative Krebs cycle, than an end product of glycosomal catabolism.

14764. **Melchers, J., Diechtierow, M., Feher, K., Sinning, I., Tews, I., Krauth-Siegel, R. L. & Muhle-Goll, C., 2008**. Structural basis for a distinct catalytic mechanism in *Trypanosoma brucei* tryparedoxin peroxidase. *Journal of Biological Chemistry,* **283** (44): 30401-30411.

Department of Structure and Biocomputing, EMBL, 69117 Heidelberg, Germany. [claudia.muhle@kit.edu].

Trypanosoma brucei, the causative agent of African sleeping sickness, encodes three cysteine homologues (Px I-III) of classical selenocysteine-containing glutathione peroxidases. The enzymes obtain their reducing equivalents from the unique trypanothione (bis(glutathionyl)spermidine)/tryparedoxin system. During catalysis, these tryparedoxin peroxidases cycle between an oxidized form with an intramolecular disulphide bond between Cys(47) and Cys(95) and the reduced peroxidase with both residues in the thiol state. Here we report on the three-dimensional structures of oxidized *T. brucei* Px III at 1.4A resolution obtained by x-ray crystallography and of both the oxidized and the reduced protein determined by NMR spectroscopy. Px III is a monomeric protein unlike the homologous poplar thioredoxin peroxidase (TxP). The structures of oxidized and reduced Px III are essentially identical in contrast to what was recently found for TxP. In Px III, Cys(47), Gln(82), and Trp(137) do not form the catalytic triad observed in the selenoenzymes, and related proteins and the latter two residues are unaffected by the redox state of the protein. The mutational analysis of three conserved lysine residues in the vicinity of the catalytic

cysteines revealed that exchange of Lys(107) against glutamate abrogates the reduction of hydrogen peroxide, whereas Lys(97) and Lys(99) play a crucial role in the interaction with tryparedoxin.

14765. **Merritt, E. A., Holmes, M., Buckner, F. S., Van Voorhis, W. C., Quartly, E., Phizicky, E. M., Lauricella, A., Luft, J., DeTitta, G., Neely, H., Zucker, F. & Hol, W. G., 2008**. Structure of a *Trypanosoma brucei* alpha/beta-hydrolase fold protein with unknown function. *Acta Crystallographica Section F Structural Biology and Crystallization Communications,* **64** (6): 474-478.

Structural Genomics of Pathogenic Protozoa (SGPP) Consortium, USA. [merritt@u.washington.edu].

14766. **Militello, K. T., Wang, P., Jayakar, S. K., Pietrasik, R. L., Dupont, C. D., Dodd, K., King, A. M. & Valenti, P. R., 2008**. African trypanosomes contain 5-methylcytosine in nuclear DNA. *Eukaryotic Cell,* **7** (11): 2012-2016.

Department of Biology, State University of New York at Geneseo, Geneseo, NY 14454, USA. [militello@geneseo.edu].

It is currently unclear if there are modified DNA bases in *Trypanosoma brucei* other than J-base. We identify herein a cytosine-5 DNA methyltransferase gene and report the presence and location of 5-methylcytosine in genomic DNA. Our data demonstrate that African trypanosomes contain a functional cytosine DNA methylation pathway.

14767. **Niemann, M., Brecht, M., Schluter, E., Weitzel, K., Zacharias, M. & Goringer, H. U., 2008**. *Tb*MP42 is a structure-sensitive ribonuclease that likely follows a metal ion catalysis mechanism. *Nucleic Acids Research,* **36** (13): 4465-4473.

Genetics, Darmstadt University of Technology, Schnittspahnstrasse 10, 64287 Darmstadt, Germany. [goringer@hrzpub.tu-darmstadt.de].

RNA editing in African trypanosomes is characterized by a uridylate-specific insertion and/or deletion reaction that generates functional mitochondrial transcripts. The process is catalyzed by a multi-enzyme complex, the editosome, which consists of approximately 20 proteins. While for some of the polypeptides a contribution to the editing reaction can be deduced from their domain structure, the involvement of other proteins remains elusive. *Tb*MP42, is a component of the editosome that is characterized by two C(2)H(2)-type zinc-finger domains and a putative oligosaccharide/oligonucleotide-binding fold. Recombinant *Tb*MP42 has been shown to possess endo/exoribonuclease activity *in vitro*; however, the protein lacks canonical nuclease motifs. Using a set of synthetic gRNA/pre-mRNA substrate RNAs, we demonstrate that *Tb*MP42 acts as a topology-dependent ribonuclease that is sensitive to base stacking. We further show that the chelation of Zn^{2+} cations is inhibitory to the enzyme activity and that the chemical modification of amino acids known to coordinate Zn^{2+} inactivates r*Tb*MP42. Together, the data are suggestive of a Zn^{2+}-dependent metal ion catalysis mechanism for the ribonucleolytic activity of r*Tb*MP42.

14768. **O'Brien, T. C., Mackey, Z. B., Fetter, R. D., Choe, Y., O'Donoghue, A. J., Zhou, M., Craik, C. S., Caffrey, C. R. & McKerrow, J. H., 2008.** A parasite cysteine protease is key to host protein degradation and iron acquisition. *Journal of Biological Chemistry*, **283** (43): 28934-28943.

Department of Pathology and Sandler Center for Basic Research in Parasitic Diseases, and the §Department of Biochemistry and Biophysics, California Institute for Quantitative Biomedical Research (QB3), University of California, San Francisco, California 94158, USA. [jmck@cgl.ucsf.edu].

Cysteine proteases of the Clan CA (papain) family are the predominant protease group in primitive invertebrates. Cysteine protease inhibitors arrest infection by the protozoan parasite, *Trypanosoma brucei*. RNA interference studies implicated a cathepsin B-like protease, *Tb*catB, as a key inhibitor target. Utilizing parasites in which one of the two alleles of *Tb*catB has been deleted, the key role of this protease in degradation of endocytosed host proteins is delineated. *Tb*catB deficiency results in a decreased growth rate and dysmorphism of the flagellar pocket and the subjacent endocytic compartment. Western blot and microscopic analysis indicate that deficiency in *Tb*catB results in accumulation of both host and parasite proteins, including the lysosomal marker p67. A critical function for parasitism is the degradation of host transferrin, which is necessary for iron acquisition. Substrate specificity analysis of recombinant *Tb*catB revealed the optimal peptide cleavage sequences for the enzyme and these were confirmed experimentally using FRET-based substrates. Degradation of transferrin was validated by SDS-PAGE and the specific cleavage sites identified by N-terminal sequencing. Because even a modest deficiency in *Tb*catB is lethal for the parasite, *Tb*catB is a logical target for the development of new anti-trypanosomal chemotherapy.

14769. **Ochsenreiter, T., Anderson, S., Wood, Z. A. & Hajduk, S. L., 2008**. Alternative RNA editing produces a novel protein involved in mitochondrial DNA maintenance in trypanosomes. *Molecular and Cellular Biology,* **28** (18): 5595-5604.

Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA. [shajduk@bmb.uga.edu].

The mitochondrial genome of trypanosomes is composed of thousands of topologically interlocked circular DNA molecules that form the kinetoplast DNA (kDNA). Most genes encoded by the kDNA require a posttranscriptional modification process called RNA editing to form functional mRNAs. Here, we show that alternative editing of the mitochondrial cytochrome c oxidase III (COXIII) mRNA in *Trypanosoma brucei* produces a novel DNA binding protein, alternatively edited protein 1 (AEP-1). AEP-1 localizes to the region of the cell between the kDNA and the flagellum and purifies with the tripartite attachment complex, a structure believed to physically link the kDNA and flagellar basal bodies. Expression of the

DNA binding domain of AEP-1 results in aberrant kDNA structure and reduced cell growth, indicating that AEP-1 is involved in the maintenance of the kDNA. Perhaps most important, our studies show a gain of function through an alternatively edited mRNA and, for the first time, provide a link between the unusual structure of the kDNA and RNA editing in trypanosome mitochondria.

14770. **Ortiz, D., Sanchez, M. A., Quecke, P. & Landfear, S. M., 2008**. Two novel nucleobase/pentamidine transporters from *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*. **e - Publication ahead of print 17 October 2008.**

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

African trypanosomes are unable to synthesize purines *de novo* and must salvage preformed purine nucleosides and nucleobases from their hosts. The *Trypanosoma brucei* genome project has identified 12 members of the equilibrative nucleoside transporter family, most of which have been characterized previously as nucleoside and/or nucleobase transporters. Here the 11th member of this family, *Tb*NT11.1, has been functionally expressed in null mutants of *Leishmania* that are deficient in purine nucleoside or nucleobase uptake and identified as a high-affinity purine nucleobase transporter. Expression of *Tb*NT11.1 in *Xenopus* oocytes revealed that it is also a transporter for the diamidine drug pentamidine that is the principal drug employed to treat early stage human African trypanosomiasis and may thus contribute to the uptake of this therapeutically important compound. In addition, characterization of the 12th member of the family, *Tb*NT12.1, reveals that it is an adenine/pentamidine transporter.

14771. **Oyola, S. O., Bringaud, F. & Melville, S. E., 2008**. A kinetoplastid BRCA2 interacts with DNA replication protein CDC45. *International Journal of Parasitology*. **In press, corrected proof.**

Department of Pathology, University of Cambridge, UK. [so515@york.ac.uk].

The gene BRCA2, first identified as a breast cancer susceptibility locus in humans, encodes a protein involved in DNA repair in mammalian cells and mutations in this gene confer increased risk of breast cancer. Here we report a functional characterisation of a *Trypanosoma brucei* BRCA2 (*Tb*BRCA2) orthologue and show that the protein interacts directly with *Tb*RAD51. A further protein-protein interaction screen using *Tb*BRCA2 identified other interacting proteins, including a trypanosome orthologue of CDC45 which is involved in initiation and progression of the replication fork complex during DNA synthesis. Deletion of the *Tb*BRCA2 gene retards cell cycle progression during S-phase as judged by increased incorporation of BrdU and an increased percentage of cells with one nucleus and two kinetoplasts. These results provide insights into the potential role played by BRCA2 in DNA replication and reveal a novel interaction that couples replication and recombination in maintaining integrity of the genome.

14772. **Oza, S. L., Chen, S., Wyllie, S., Coward, J. K. & Fairlamb, A. H., 2008**. ATP-dependent ligases in trypanothione biosynthesis: Kinetics of catalysis and inhibition by phosphinic acid pseudopeptides. *Febs Journal,* **275** (21): 5408-5421.

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

Glutathionylspermidine is an intermediate formed in the biosynthesis of trypanothione, an essential metabolite in defence against chemical and oxidative stress in the Kinetoplastida. The kinetic mechanism for glutathionylspermidine synthetase (EC 6.3.1.8) from *Crithidia fasciculata* (*Cf*GspS) obeys a rapid equilibrium random ter-ter model with kinetic constants K(GSH) = 609 µM, K(Spd) = 157 µM and K(ATP) = 215 µM. Phosphonate and phosphinate analogues of glutathionylspermidine, previously shown to be potent inhibitors of GspS from *Escherichia coli,* are equally potent against *Cf*GspS. The tetrahedral phosphonate acts as a simple ground state analogue of glutathione (GSH) (K(i) approximately 156 µM), whereas the phosphinate behaves as a stable mimic of the postulated unstable tetrahedral intermediate. Kinetic studies showed that the phosphinate behaves as a slow-binding bisubstrate inhibitor [competitive with respect to GSH and spermidine (Spd)] with rate constants k(3) (on rate) = 6.98 x 10(4) M(-1) x s(-1) and k(4) (off rate) = 1.3 x 10(-3) s(-1), providing a dissociation constant K(i) = 18.6 nM. The phosphinate analogue also inhibited recombinant trypanothione synthetase (EC 6.3.1.9) from *C. fasciculata, Leishmania major, Trypanosoma cruzi* and *Trypanosoma brucei* with K(i)(app) values 20-40-fold greater than that of *Cf*GspS. This phosphinate analogue remains the most potent enzyme inhibitor identified to date, and represents a good starting point for drug discovery for trypanosomiasis and leishmaniasis.

14773. **Prohaska, K. & Williams, N., 2008**. Assembly of the *Trypanosoma brucei* 60S ribosomal subunit nuclear export complex requires trypanosome-specific proteins P34 and P37. *Eukaryotic Cell*. **Published online ahead of print 27 August 2008**.

Department of Microbiology and Immunology & Witebsky Center for Microbial Pathogenesis and Immunology, 253 Biomedical Research Building, University at Buffalo, Buffalo, NY 14214, USA. [nw1@acsu.buffalo.edu].

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

14774. **Raftery, J., 2008**. Target selection: triage in the structural genomics battlefield. *Methods in Molecular Biology,* **426**: 37-47.

School of Chemistry, The University of Manchester, Manchester, UK.

A synopsis of some of the approaches to protein target selection is given, with an emphasis on using web resources to converge on well-ordered, readily crystallizable proteins that are maximally different from known structures. This is illustrated with the genomes of the pathogens causing tuberculosis and sleeping sickness.

14775. **Regmi, S., Rothberg, K. G., Hubbard, J. G. & Ruben, L., 2008**. The RACK1 signal anchor protein from *Trypanosoma brucei* associates with eukaryotic elongation factor 1A: a role for translational control in cytokinesis. *Molecular Microbiology,* **70** (3): 724-745.

Department of Biological Sciences, Southern Methodist University, Dallas, TX 75275, USA. [lruben@mail.smu.edu].

RACK1 is a WD-repeat protein that forms signal complexes at appropriate locations in the cell. RACK1 homologues are core components of ribosomes from yeast, plants and mammals. In contrast, a cryo-EM analysis of trypanosome ribosomes failed to detect RACK1, thus eliminating an important translational regulatory mechanism. Here we report that *Tb*RACK1 from *Trypanosoma brucei* associates with eukaryotic translation elongation factor-1A (eEF1A) as determined by tandem MS of TAP-*Tb*RACK1 affinity eluates, co-sedimentation in a sucrose gradient, and co-precipitation assays. Consistent with these observations, sucrose gradient purified 80S monosomes and translating polysomes each contained *Tb*RACK1. When RNAi was used to deplete cells of *Tb*RACK1, a shift in the polysome profile was observed, while the phosphorylation of a ribosomal protein increased. Under these conditions, cell growth became hypersensitive to the translational inhibitor anisomycin. The kinetoplasts and nuclei were misaligned in the postmitotic cells, resulting in partial cleavage furrow ingression during cytokinesis. Overall, these findings identify eEF1A as a novel *Tb*RACK1 binding partner and establish TbRACK1 as a component of the trypanosome translational apparatus. The synergy between anisomycin and *Tb*RACK1 RNAi suggests that continued translation is required for complete ingression of the cleavage furrow.

14776. **Reifur, L. & Koslowsky, D. J., 2008**. *Trypanosoma brucei* ATPase subunit 6 mRNA bound to gA6-14 forms a conserved three-helical structure. *Rna,* **14** (10): 2195-2211.

Comparative Medicine and Integrative Biology, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824, USA. [koslowsk@msu.edu].

T. brucei survival relies on the expression of mitochondrial genes, most of which require RNA editing to become translatable. In trypanosomes, RNA editing involves the insertion and deletion of uridylates, a developmentally regulated process directed by guide RNAs (gRNAs) and catalyzed by the editosome, a complex of proteins. The pathway for mRNA/gRNA complex formation and assembly with the editosome is still unknown. Work

from our laboratory has suggested that distinct mRNA/gRNA complexes anneal to form a conserved core structure that may be important for editosome assembly. The secondary structure for the apocytochrome b (CYb) pair has been previously determined and is consistent with our model of a three-helical structure. Here, we used cross-linking and solution structure probing experiments to determine the structure of the ATPase subunit 6 (A6) mRNA hybridized to its cognate gA6-14 gRNA in different stages of editing. Our results indicate that both unedited and partially edited A6/gA6-14 pairs fold into a three-helical structure similar to the previously characterized CYb/gCYb-558 pair. These results lead us to conclude that at least two mRNA/gRNA pairs with distinct editing sites and distinct primary sequences fold to a three-helical secondary configuration that persists through the first few editing events.

14777. **Sakurai, T., Sugimoto, C. & Inoue, N., 2008**. Identification and molecular characterization of a novel stage-specific surface protein of *Trypanosoma congolense* epimastigotes. *Molecular and Biochemical Parasitology,* **161** (1): 1-11.

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Nishi 2-13, Inada-cho, Obihiro, Hokkaido 080-8555, Japan. [ircpmi@obihiro.ac.jp].

The cattle pathogen *Trypanosoma congolense* expresses life cycle stage-specific surface molecules involved in adaptation to different host and vector environments. Here we report the discovery and molecular characterization of a novel stage-specific GPI-anchored surface glycoprotein that is selectively expressed in the epimastigote (EMF) life cycle stage of *T. congolense*. Culture supernatants of EMF but not of procyclic culture forms (PCFs) promoted adhesion of PCF parasites in an *in vitro* assay. Biosynthetic labelling experiments showed that these EMF culture supernatants contained a 100kDa trypanosome-derived protein that was not present in supernatants from PCF. We named this molecule "congolense epimastigote-specific protein" (CESP). The gene encoding CESP was isolated from an EMF cDNA library after immunoscreening. The multicopy gene had a 2 070-bp open reading frame that encodes a polypeptide of 689 amino acids with a predicted mass of 72.9kDa. The discrepancy between the predicted (72.9kDa) and observed (100kDa) masses may be explained partially by glycosylation of the molecule which has six potential N-glycosylation sites and a predicted GPI anchor. Indeed, metabolic labelling of CESP with [(3)H] ethanolamine revealed that CESP was a GPI-anchored protein. Confocal laser scanning microscopy showed that CESP was expressed only on the surface of the EMF stage of the parasite. The identification of CESP as a unique component of culture supernatants from EMF and that such supernatants can confer plastic-adhesive ability on PCF suggest that CESP is worth further investigation as an adhesion molecule that perhaps allows *T. congolense* EMF to adhere to the tsetse proboscis.

14778. **Sampathkumar, P., Roach, C., Michels, P. A. & Hol, W. G., 2008**. Structural insights into the recognition of peroxisomal targeting signal 1 by *Trypanosoma brucei* peroxin 5. *Journal of Molecular Biology,* **381** (4): 867-880.

Department of Biochemistry, Biomolecular Structure Center, University of Washington, Box 357742, Seattle, WA 98195, USA.
[wghol@u.washington.edu].

14779. **Schwede, A., Ellis, L., Luther, J., Carrington, M., Stoecklin, G. & Clayton, C., 2008**. A role for Caf1 in mRNA deadenylation and decay in trypanosomes and human cells. *Nucleic Acids Research,* **36** (10): 3374-3388.

Zentrum fur Molekulare Biologie der Universität Heidelberg, Im Neuenheimer Feld 282, D-69120 Heidelberg, Germany. [cclayton@zmbh.uni-heidelberg.de].

The eukaryotic Ccr4/Caf1/Not complex is involved in deadenylation of mRNAs. The Caf1 and Ccr4 subunits both potentially have deadenylating enzyme activity. We investigate here the roles of Ccr4 and Caf1 in deadenylation in two organisms that separated early in eukaryotic evolution: humans and trypanosomes. In *Trypanosoma brucei*, we found a complex containing CAF1, NOT1, NOT2 and NOT5, DHH1 and a possible homologue of Caf130; no homologue of Ccr4 was found. Trypanosome CAF1 has deadenylation activity, and is essential for cell survival. Depletion of trypanosome CAF1 delayed deadenylation and degradation of constitutively expressed mRNAs. Human cells have two isozymes of Caf1: simultaneous depletion of both inhibited degradation of an unstable reporter mRNA. In both species, depletion of Caf1 homologues inhibited deadenylation of bulk RNA and resulted in an increase in average poly(A) tail length.

14780. **Sculaccio, S. A., Rodrigues, E. M., Cordeiro, A. T., Magalhães, A., Braga, A. L., Alberto, E. E. & Thiemann, O. H., 2008**. Selenocysteine incorporation in Kinetoplastid: Selenophosphate synthetase (SELD) from *Leishmania major* and *Trypanosoma brucei*. *Molecular and Biochemical Parasitology,* **162** (2): 165-171.

Physics Institute of São Carlos, University of São Paulo (USP), Av. Trabalhador Sãocarlense 400, 13566-590 São Carlos-SP, Brazil; Department of Genetics and Evolution, Federal University of São Carlos, São Carlos, Brazil; Chromatography Group, Chemistry Institute of São Carlos, São Carlos, Brazil. [thiemann@if.sc.usp.br].

Selenophosphate synthetase (EC 2.7.9.3), the product of the selD gene, produces the biologically active selenium donor compound, monoselenophosphate, from ATP and selenide, for the synthesis of selenocysteine. The kinetoplastid *Leishmania majo*r and *Trypanosoma brucei* selD genes were cloned and the SELD protein overexpressed and purified to apparent homogeneity. The selD genes in *L. major* and *T. brucei* are respectively 1197 and 1179bp long encoding proteins of 399 and 393 amino acids with molecular masses of 42.7 and 43kDa. The molecular mass of 100kDa for both (*L. major* and *T. brucei*) SELDs is consistent with dimeric proteins. The kinetoplastid selD complements *Escherichia coli* (WL400) selD deletion confirming it is a functional enzyme and the specific activity of these enzymes was determined. A conserved Cys residue was identified both by multiple sequence alignment as well as by functional complementation and activity assay of the mutant (Cys to Ala) forms of the SELD identifying this residue as essential for the catalytic function.

14781. **Shi, J., Franklin, J. B., Yelinek, J. T., Ebersberger, I., Warren, G. & He, C. Y., 2008**. Centrin4 coordinates cell and nuclear division in *T. brucei*. *Journal of Cell Science,* **121** (18): 3062-3070.

Department of Biological Sciences, National University of Singapore, Singapore 117543. [dbshyc@nus.edu.sg].

Centrins are Ca^{2+}-binding proteins that have been implicated in a number of biological processes, including organelle duplication, mRNA export, DNA repair and signal transduction. In the protozoan parasite *Trypanosoma brucei* we have previously described *Tb*Centrin2, which is present on a bi-lobed structure, and involved in the duplication and segregation of the Golgi complex. Recently, another centrin, *Tb*Centrin4, was also found at the bi-lobe and has been implicated in organelle segregation and cytokinesis. We now show that cytokinesis is not inhibited, but that a dysregulation of nuclear and cell division leads to the production of zoids - daughter siblings that contain all organelles except the nucleus. Our results, therefore, suggest that *Tb*Centrin4 is involved in processes that coordinate karyokinesis and cytokinesis.

14782. **Siegel, T. N., Hekstra, D. R. & Cross, G. A., 2008**. Analysis of the *Trypanosoma brucei* cell cycle by quantitative DAPI imaging. *Molecular and Biochemical Parasitology,* **160** (2): 171-174.

Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA. [george.cross@rockefeller.edu].

Trypanosoma brucei has two DNA compartments: the nucleus and the kinetoplast. DNA replication of these two compartments only partially coincides. Woodward and Gull [**Woodward, R., & Gull K., 1990**, *Journal of Cell Science*; **95**: 49-57] comprehensively studied the relative timing of the replication and segregation of nuclear DNA (nDNA) and kinetoplast DNA (kDNA). Others have since assumed the consistency of morphological indicators of cell-cycle stage among strains and conditions. We report the use of quantitative DAPI imaging to determine the cell-cycle stage of individual procyclic cells. Using this approach, we found that kinetoplast elongation occurs mainly during nuclear S phase and not during G2, as previously assumed. We confirmed this finding by sorting cells by DNA content, followed by fluorescence microscopy. In addition, simultaneous quantitative imaging at two wavelengths can be used to determine the abundance of cell-cycle-regulated proteins during the cell cycle. We demonstrate this technique by co-staining for the non-acetylated state of lysine 4 of histone H4 (H4K4), which is enriched during nuclear S phase.

14783. **Signorell, A., Jelk, J., Rauch, M. & Butikofer, P., 2008**. Phosphatidylethanolamine is the precursor of the ethanolamine phosphoglycerol moiety bound to eukaryotic elongation factor 1A. *Journal of Biological Chemistry,* **283** (29): 20320-20329.

Institute of Biochemistry and Molecular Medicine, University of Bern, Bern, Switzerland. [peter.buetikofer@mci.unibe.ch].

In addition to its conventional role during protein synthesis, eukaryotic elongation factor 1A is involved in other cellular processes. Several regions of interaction between eukaryotic elongation factor 1A and the translational apparatus or the cytoskeleton have been identified, yet the roles of the different post-translational modifications of eukaryotic elongation factor 1A are completely unknown. One amino acid modification, which so far has only been found in eukaryotic elongation factor 1A, consists of ethanolamine-phosphoglycerol attached to two glutamate residues that are conserved between mammals and plants. We now report that ethanolamine-phosphoglycerol is also present in eukaryotic elongation factor 1A of the protozoan parasite *Trypanosoma brucei*, indicating that this unique protein modification is of ancient origin. In addition, using RNA-mediated gene silencing against enzymes of the Kennedy pathway, we demonstrate that phosphatidylethanolamine is a direct precursor of the ethanolamine-phosphoglycerol moiety. Down-regulation of the expression of ethanolamine kinase and ethanolamine-phosphate cytidylyltransferase results in inhibition of phosphatidylethanolamine synthesis in *T. brucei* procyclic forms and, concomitantly, in a block in glycosylphosphatidylinositol attachment to procyclins and ethanolamine-phosphoglycerol modification of eukaryotic elongation factor 1A.

14784. **Signorell, A., Rauch, M., Jelk, J., Ferguson, M. A. & Butikofer, P., 2008**. Phosphatidylethanolamine in *Trypanosoma brucei* is organized in two separate pools and is synthesized exclusively by the Kennedy pathway. *Journal of Biological Chemistry,* **283** (35): 23636-23644.

Institute of Biochemistry and Molecular Medicine, University of Bern, Buhlstrasse 28, Bern, Switzerland. [peter.buetikofer@mci.unibe.ch].

Phosphatidylethanolamine is a major phospholipid class of all eukaryotic cells. It can be synthesized via the CDP-ethanolamine branch of the Kennedy pathway, by decarboxylation of phosphatidylserine, or by base exchange with phosphatidylserine. The contributions of these pathways to total phosphatidylethanolamine synthesis have remained unclear. Although *Trypanosoma brucei*, the causative agent of human and animal trypanosomiasis, has served as a model organism to elucidate the entire reaction sequence for glycosylphosphatidylinositol biosynthesis, the pathways for the synthesis of the major phospholipid classes have received little attention. We now show that disruption of the CDP-ethanolamine branch of the Kennedy pathway using RNA interference results in dramatic changes in phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine. By targeting individual enzymes of the pathway, we demonstrate that de novo phosphatidylethanolamine synthesis in *T. brucei* procyclic forms is strictly dependent on the CDP-ethanolamine route. Interestingly, the last step in the Kennedy pathway can be mediated by two separate activities leading to two distinct pools of phosphatidylethanolamine, consisting of predominantly alk-1-enyl-acyl- or diacyl-type molecular species. In addition, we show that phosphatidylserine in *T. brucei* procyclic forms is synthesized exclusively by base exchange with phosphatidylethanolamine.

14785. **Sturm, N. R., Martinez, L. L. & Thomas, S., 2008**. Kinetoplastid genomics: The thin end of the wedge. *Infection, Genetics and Evolution,* **8** (6): 901-906.

Department of Microbiology, Immunology & Molecular Genetics, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA 90095, USA. [nsturm@ucla.edu].

The completion of the genome sequencing projects for major pathogens *Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania major* has enabled numerous studies that would have been difficult or impossible to perform otherwise. New technologies in sequencing and protein analyses promise further rapid expansion in our capabilities. The keys to successful use of these new tools are recognizing the power and limitations of studies performed thus far, grasping the unrealized potential of new and developing technologies, and creating access to a multidisciplinary set of skills that will facilitate research, particularly in the bioinformatic analysis of the reams of data that will be forthcoming. In this paper, we provide an overview of kinetoplastid genomics studies with emphasis on studies advanced through genomic data, and a preview of what may come in the near future.

14786. **Subramanya, S., Hardin, F. C., Steverding, D. & Mensa-Wilmot, K., 2008**. Glycosylphosphatidylinositol-phospholipase C regulates transferrin endocytosis in the African trypanosome. *Biochemical Journal*. **Published as Immediate Publication 11 September 2008.**

Cellular Biology, University of Georgia, Athens, Georgia 30602, USA. [mensawil@uga.edu].

Glycosylphosphatidylinositol phospholipase C (GPI-PLC) is expressed in bloodstream form *Trypanosoma brucei*, a protozoan that causes human African trypanosomiasis. Loss of GPI-PLC genes reduces virulence of a pleomorphic strain of the parasite, for reasons that are not clear. Herein, we report that GPI-PLC stimulates endocytosis of tranferrin (Tfn) 300 percent-500 percent. Surprisingly, GPI-PLC is not detected at endosomes, suggesting that the enzyme does not interact directly with the endosomal machinery. Therefore, we hypothesized that a diffusible product of GPI-PLC enzyme reaction (possibly diacylglycerol (DAG)) mediated biological effects of the protein. Two sets of data support this assertion. First, a catalytically inactive Gln81Leu mutant of GPI-PLC, expressed in a GPI-PLC null background, had no effect on endocytosis, indicating that enzyme activity is essential for the protein to stimulate endocytosis. Second, exogenous DAGs 1-oleyl 2-acetyl-sn-glycerol (OAG) and dimyristoylglycerol (DMG), independently stimulated endocytosis of Tfn. Further, the DAG mimic phorbol-12-myristate-13-acetate (PMA), a phorbol ester also activated endocytosis in *T. brucei*. DAG-stimulated endocytosis is a novel pathway in the trypanosome. We surmise that (i) GPI-PLC regulates Tfn endocytosis in *T. brucei*, (ii) GPI-PLC is a signalling enzyme, and (iii) DAG is a second messenger for GPI-PLC. We propose that regulation of endocytosis as a physiological function of GPI-PLC in bloodstream *T. brucei*.

14787. **Sutak, R., Lesuisse, E., Tachezy, J. & Richardson, D. R., 2008**. Crusade for iron: iron uptake in unicellular eukaryotes and its significance for virulence. *Trends in Microbiology,* **16** (6): 261-268.

Department of Pathology and Bosch Institute, University of Sydney, Sydney, NSW 2006, Australia. [d.richardson@pathology.usyd.edu.au].

The effective acquisition of iron is a pre-requisite for survival of all organisms, especially parasites that have a high iron requirement. In mammals, iron homeostasis is meticulously regulated; extracellular free iron is essentially unavailable and host iron availability has a crucial role in the host-pathogen relationship. Therefore, pathogens use specialized and effective mechanisms to acquire iron. In this review, we summarize the iron-uptake systems in eukaryotic unicellular organisms with particular focus on the pathogenic species: *Candida albicans, Tritrichomonas foetus, Trypanosoma brucei* and *Leishmania* spp. We describe the diversity of their iron-uptake mechanisms and highlight the importance of the process for virulence.

14788. **Sutterwala, S. S., Hsu, F. F., Sevova, E. S., Schwartz, K. J., Zhang, K., Key, P., Turk, J., Beverley, S. M. & Bangs, J. D., 2008**. Developmentally regulated sphingolipid synthesis in African trypanosomes. *Molecular Microbiology***, 70** (2): 281-296.

Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, 1550 Linden Drive, Madison, WI 53706, USA. [jdbangs@wisc.edu]

Sphingolipids are essential components of eukaryotic membranes, and many unicellular eukaryotes, including kinetoplastid protozoa, are thought to synthesize exclusively inositol phosphorylceramide (IPC). Here we characterize sphingolipids from *Trypanosoma brucei*, and a trypanosome sphingolipid synthase gene family (*Tb*SLS1-4) that is orthologous to *Leishmania* IPC synthase. Procyclic trypanosomes contain IPC, but also sphingomyelin, while surprisingly bloodstream-stage parasites contain sphingomyelin and ethanolamine phosphorylceramide (EPC), but no detectable IPC. *In vivo* fluorescent ceramide labelling confirmed stage-specific biosynthesis of both sphingomyelin and IPC. Expression of *Tb*SLS4 in *Leishmania* resulted in production of sphingomyelin and EPC suggesting that the *Tb*SLS gene family has bi-functional synthase activity. RNAi silencing of *Tb*SLS1-4 in bloodstream trypanosomes led to rapid growth arrest and eventual cell death. Ceramide levels were increased more than three-fold by silencing suggesting a toxic downstream effect mediated by this potent intracellular messenger. Topology predictions support a revised six-transmembrane domain model for the kinetoplastid sphingolipid synthases consistent with the proposed mammalian sphingomyelin synthase structure. This work reveals novel diversity and regulation in sphingolipid metabolism in this important group of human parasites.

14789. **Tran, T., Buscher, P., Vandenbussche, G., Wyns, L., Messens, J. & De Greve, H., 2008**. Heterologous expression, purification and characterisation of the extracellular domain of trypanosome invariant surface glycoprotein ISG75. *Journal of Biotechnology,* **135** (3): 247-254.

Department of Molecular and Cellular Interactions, Vrije Universiteit Brussel, Pleinlaan 2, Brussels, Belgium. [thao.tran@vub.ac.be].

The invariant surface glycoprotein ISG75 is a transmembrane glycoprotein occurring on the surface of the bloodstream-form *Trypanozoon*. This study describes the expression and purification of the N-terminal extracellular domain of ISG75, a novel target for development of diagnostic tests for trypanosomosis. To facilitate disulphide formation in the cytoplasm, a 1 287-bp cDNA fragment encoding ISG75 from *Trypanosoma brucei gambiense* was expressed in a thioredoxin reductase, glutathione oxidoreductase double mutant *Escherichia coli* strain. An accessory plasmid pRIL, providing the argI, ileY, and leuW tRNAs, was necessary for efficient heterologous translation of the ISG75 mRNA. The recombinant double-tagged (streptavidine and histidine) ISG75 was purified by two-step affinity chromatography. Addition of L-glutamic acid and L-arginine in the buffer solutions was crucial to stabilise the protein during purification. The purified soluble protein was characterised by circular dichroism spectroscopy, reverse-phase high pressure liquid chromatography and mass spectrometry. It has an alpha-helical folded conformation, is homogeneous and pure (99 percent). Furthermore, sera of *Trypanosoma brucei*-infected animals specifically recognise this recombinant ISG75; and rabbit antiserum raised against the recombinant ISG75 detects all species of the *Trypanozoon* subgenus in parasite preparations.

14790. **Umeyama, T. & Wang, C. C., 2008**. Polo-like kinase is expressed in S/G2/M phase and associated with the flagellum attachment zone in both procyclic and bloodstream forms of *Trypanosoma brucei*. *Eukaryotic Cell,* **7** (9): 1582-1590.

Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94158-2280, USA. [ccwang@cgl.ucsf.edu].

Trypanosoma brucei, the aetiologic agent of African sleeping sickness, divides into insect (procyclic) and bloodstream forms. These two forms are subject to distinct cell cycle regulations, with cytokinesis controlled primarily by basal body/kinetoplast segregation in the procyclic form but by mitosis in the bloodstream form. Polo-like kinases (PLKs), known to play essential roles in regulating both mitosis and cytokinesis among eukaryotes, have a homologue in *T. brucei*, *Tb*PLK, which regulates only cytokinesis. In our previous study, overexpressed triply haemagglutinin-tagged *Tb*PLK (*Tb*PLK-3HA) in the procyclic form localized to a mid-dorsal point and the anterior tip of the cell along the flagellum attachment zone (FAZ). In our current study, *Tb*PLK-3HA expressed at the endogenous level was identified at the same dorsal location of both procyclic and bloodstream forms, albeit it was no longer detectable at the anterior tip of the cell. Endogenously expressed *Tb*PLK fused with an enhanced yellow fluorescent protein (EYFP) localized to the same dorsal location along the FAZs in living procyclic and bloodstream cells. Fluorescence-activated cell sorter analysis of hydroxyurea-synchronized procyclic cells revealed that *Tb*PLK-EYFP emerges during S phase, persists through G(2)/M phase, and vanishes in G(1) phase. An indicated

*Tb*PLK-EYFP association with the FAZs of G(2)/M cells may thus represent a timely localization to a potential initiation site of cytokinesis, which agrees with the recognized role of *Tb*PLK in cytokinetic initiation.

14791. **Urbaniak, M. D., Crossman, A. & Ferguson, M. A., 2008**. Probing *Trypanosoma brucei* glycosylphosphatidylinositol biosynthesis using novel precursor-analogues. *Chemical Biology and Drug Design,* **72** (2): 127-132.

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

Glycosylphosphatidylinositol precursor-analogues were synthesized in which the natural diacylglycerol lipid was replaced with either of two steroidal moieties. The ability of the steroidal glycosylphosphatidylinositol precursor-analogues to prime the glycosylphosphatidylinositol biosynthetic pathway was assessed in a trypanosomal cell-free system. The N-acetyl-D-glucosaminylphosphatidylinositol de-N-acetylase was only able to act upon the N-acetylglucosamine form of one of the two analogues. However, the glucosamine form of both analogues could be mannosylated, but inositol was neither acylated nor modified with ethanolamine phosphate. The use of alternative groups, such as sterols, in place of the natural diacylglycerol lipid may enable the production of more drug-like, substrate-based inhibitors.

14792. **Urbaniak, M. D., Yashunsky, D. V., Crossman, A., Nikolaev, A. V. & Ferguson, M. A., 2008**. Probing enzymes late in the trypanosomal glycosylphosphatidylinositol biosynthetic pathway with synthetic glycosylphosphatidylinositol analogues. *ACS Chemical Biology,* **3** (10): 625-634.

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

Glycosylphosphatidylinositol (GPI)-anchored proteins are abundant in the protozoan parasite *Trypanosoma brucei*, the causative agent of African sleeping sickness in humans and the related disease nagana in cattle, and disruption of GPI biosynthesis is genetically and chemically validated as a drug target. Here, we examine the ability of enzymes of the trypanosomal GPI biosynthetic pathway to recognize and process a series of synthetic dimannosyl-glucosaminylphosphatidylinositol analogues containing systematic modifications on the mannose residues. The data reveal which portions of the natural substrate are important for recognition, explain why mannosylation occurs prior to inositol acylation in the trypanosomal pathway, and identify the first inhibitor of the third alpha-mannosyltransferase of the GPI biosynthetic pathway.

14793. **Uzureau, P., Daniels, J. P., Walgraffe, D., Wickstead, B., Pays, E., Gull, K. & Vanhamme, L., 2008**. Identification and characterization of two trypanosome TFIIS proteins exhibiting particular domain architectures and differential nuclear localizations. *Molecular Microbiology,* **69** (5): 1121-1136.

Laboratoire de Parasitologie Moleculaire, ULB IBMM, rue des Pr Jeneer et Brachet 12, B-6041 Gosselies, Belgium. [keith.gull@path.ox.ac.uk].

Nuclear transcription of *Trypanosoma brucei* displays unusual features. Most protein-coding genes are organized in large directional gene clusters, which are transcribed polycistronically by RNA polymerase II (pol II) with subsequent processing to generate mature mRNA. Here, we describe the identification and characterization of two trypanosome homologues of transcription elongation factor TFIIS (*Tb*TFIIS1 and *Tb*TFIIS2-1). TFIIS has been shown to aid transcription elongation by relieving arrested pol II. Our phylogenetic analysis demonstrated the existence of four independent TFIIS expansions across eukaryotes. While *Tb*TFIIS1 contains only the canonical domains II and III, the N-terminus of *Tb*TFIIS2-1 contains a PWWP domain and a domain I. *Tb*TFIIS1 and *Tb*TFIIS2-1 are expressed in procyclic and bloodstream form cells and localize to the nucleus in similar, but distinct, punctate patterns throughout the cell cycle. Neither TFIIS protein was enriched in the major pol II sites of spliced-leader RNA transcription. Single RNA interference (RNAi)-mediated knock-down and knockout showed that neither protein is essential. Double knock-down, however, impaired growth. Repetitive failure to generate a double knockout of *Tb*TFIIS1 and *Tb*TFIIS2-1 strongly suggests synthetical lethality and thus an essential function shared by the two proteins in trypanosome growth.

14794. **Vandemeulebroucke, A., De Vos, S., Van Holsbeke, E., Steyaert, J. & Versees, W., 2008**. A flexible loop as a functional element in the catalytic mechanism of nucleoside hydrolase from *Trypanosoma vivax*. *Journal of Biological Chemistry,* **283** (32): 22272-22282.

Department of Ultrastructure, Vrije Universiteit Brussel (VIB), Pleinlaan 2, Brussels 1050, Belgium. [jan.steyaert@vub.ac.be].

The nucleoside hydrolase of *Trypanosoma vivax* hydrolyzes the N-glycosidic bond of purine nucleosides. Structural and kinetic studies on this enzyme have suggested a catalytic role for a flexible loop in the vicinity of the active sites. Here we present the analysis of the role of this flexible loop via the combination of a proline scan of the loop, loop deletion mutagenesis, steady state and pre-steady state analysis, and x-ray crystallography. Our analysis reveals that this loop has an important role in leaving group activation and product release. The catalytic role involves the entire loop and could only be perturbed by deletion of the entire loop and not by single site mutagenesis. We present evidence that the loop closes over the active site during catalysis, thereby ordering a water channel that is involved in leaving group activation. Once chemistry has taken place, the loop dynamics determine the rate of product release.

14795. **Vaughan, S. & Gull, K., 2008**. The structural mechanics of cell division in *Trypanosoma brucei*. *Biochemical Society Transactions,* **36** (3): 421-424.

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK. [keith.gull@path.ox.ac.uk].

Undoubtedly, there are fundamental processes driving the structural mechanics of cell division in eukaryotic organisms that have been conserved throughout evolution and are being revealed by studies on organisms such as yeast and mammalian cells. Precision of structural mechanics of cytokinesis is however probably no better illustrated than in the protozoa. A dramatic example of this is the protozoan parasite *Trypanosoma brucei*, a unicellular flagellated parasite that causes a devastating disease (African sleeping sickness) across sub-Saharan Africa in both man and animals. As trypanosomes migrate between and within a mammalian host and the tsetse vector, there are periods of cell proliferation and cell differentiation involving at least five morphologically distinct cell types. Much of the existing cytoskeleton remains intact during these processes, necessitating a very precise temporal and spatial duplication and segregation of the many single-copy organelles. This structural precision is aiding progress in understanding these processes as we apply the excellent reverse genetics and post-genomic technologies available in this system. Here we outline our current understanding of some of the structural aspects of cell division in this fascinating organism.

14796. **Wakaguri, H., Suzuki, Y., Katayama, T., Kawashima, S., Kibukawa, E., Hiranuka, K., Sasaki, M., Sugano, S. & Watanabe, J., 2008**. Full-Malaria/Parasites and full-Arthropods: databases of full-length cDNAs of parasites and arthropods, update 2009. *Nucleic Acids Research*. **Published online 4 November 2008**.

Department of Medical Genome Sciences, Graduate School of Frontier Sciences, Human Genome Center, Institute of Medical Science, The University of Tokyo. 4-6-1, Shirokanedai, Minatoku, Tokyo 108-8639, Bioinformatics Project, Science & Technology Systems, Inc. 4F 1-20-1, Shibuyaku Shibuya, Tokyo 150-0002 and Department of Parasitology, Institute of Medical Science, The University of Tokyo. 4-6-1, Shirokanedai, Minatoku, Tokyo 108-8639, Japan. [jwatanab@ims.u-tokyo.ac.jp].

Full-Malaria/Parasites is a database for transcriptome studies of Apicomplexa and other parasites, which is based on our original full-length cDNA sequences and physical cDNA clone resources. In this update, the database has been expanded to contain the shotgun sequencing for the entire sequences of 14 818 non-redundant full-length cDNA clones from six apicomplexa parasites and 6.8 million of transcription start sites (TSS), both of which had been produced by novel protocols using the oligo-capping method and the Illumina GA sequencer. The former should be the ultimate data for exact annotation of the expressed genes, while the latter should be useful for ultra-deep expression analysis. Furthermore, we have launched Full-Arthropods, a full-length cDNA database for arthropods of medical importance. Full-Arthropods contains 50 343 one-pass sequences, 10 399 shotgun complete sequences and 22.4 million TSS tags in anopheles mosquitoes that transmit malaria, tsetse flies that transmit trypanosomiasis and dust mites that cause allergic dermatitis and bronchial

asthma. By providing the largest integrated full-length cDNA data resources in the apicomplexa parasites as well as their vectors, Full-Malaria/Parasites and Full-Arthropods should help combat parasitic diseases. Full-Malaria/Parasites and Full-Arthropods are accessible from http://fullmal.hgc.jp/.

14797. **Wendler, A., Irsch, T., Rabbani, N., Thornalley, P. J. & Krauth-Siegel, R. L., 2008**. Glyoxalase II does not support methylglyoxal detoxification but serves as a general trypanothione thioesterase in African trypanosomes. *Molecular and Biochemical Parasitology.* **e – Publication ahead of print 19 September 2008.**

Biochemie-Zentrum der Universit**a**t Heidelberg, Im Neuenheimer Feld 504, 69120 Heidelberg, Germany. [ircpmi@obihiro.ac.jp].

Glyoxalase I and II form a ubiquitous glutathione-dependent pathway for the detoxification of reactive and mutagenic ketoaldehydes. Methylglyoxal produced as spontaneous by-product of glycolysis is probably the main physiological substrate. Consequently, African trypanosomes with their exorbitant glucose turnover were expected to have a most efficient detoxification system. *Trypanosoma brucei* possesses a trypanothione [bis(glutathionyl)spermidine]-dependent glyoxalase II but lacks a glyoxalase I gene. Methylglyoxal reductase as well as dehydrogenase activities are negligible. However, the concentrations of methylglyoxal and advanced glycation end products in the parasites are similar to those in different mammalian cells and the mechanism of methylglyoxal elimination remains elusive. Glyoxalase II is an abundant protein. Overexpression of the gene as well as RNA interference in bloodstream and procyclic cells did not result in a growth phenotype. Deletion of both alleles in procyclic parasites revealed that the enzyme is not essential at least under culture conditions. Recombinant glyoxalase II hydrolyzed the trypanothione-thioesters of methylglyoxal, glyoxal and 4,5-dioxovalerate, substrates of the classical glyoxalase system, with high efficiency. The absence of a glyoxalase I, however, renders these thioesters unlikely as physiological substrates. Here we show that trypanothione-thioesters can be generated from the respective coenzyme A derivative by transesterification. S-acetyl- and S-propionyltrypanothione obtained by this spontaneous reaction proved to be excellent substrates of *T. brucei* glyoxalase II. This offers a function for the parasite glyoxalase II as general trypanothione thioesterase independent of ketoaldehyde detoxification.

14798. **Willert, E. K. & Phillips, M. A., 2008**. Regulated expression of an essential allosteric activator of polyamine biosynthesis in African trypanosomes. *PLoS Pathogens,* **4** (10): e1000183.

Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, USA.
[margaret.phillips@UTSouthwestern.edu].

Trypanosoma brucei is the causative agent of African sleeping sickness. The polyamine biosynthetic pathway has the distinction of being the target of the only clinically proven anti-trypanosomal drug with a known mechanism of action. Polyamines are essential for cell growth, and their metabolism is extensively regulated. However, trypanosomatids appear to lack the regulatory control mechanisms described in other eukaryotic cells. In *T. brucei*, S-

adenosylmethionine decarboxylase (AdoMetDC) and ornithine decarboxylase (ODC) are required for the synthesis of polyamines and also for the unique redox-cofactor trypanothione. Further, trypanosomatid AdoMetDC is activated by heterodimer formation with a catalytically dead homologue termed prozyme, found only in these species. To study polyamine regulation in *T. brucei*, we generated inducible AdoMetDC RNAi and prozyme conditional knockouts in the mammalian blood form stage. Depletion of either protein led to a reduction in spermidine and trypanothione and to parasite death, demonstrating that prozyme activation of AdoMetDC is essential. Under typical growth conditions, prozyme concentration is limiting in comparison to AdoMetDC. However, both prozyme and ODC protein levels were significantly increased relative to stable transcript levels by knockdown of AdoMetDC or its chemical inhibition. Changes in protein stability do not appear to account for the increased steady-state protein levels, as both enzymes are stable in the presence of cycloheximide. These observations suggest that prozyme and ODC are translationally regulated in response to perturbations in the pathway. In conclusion, we describe the first evidence for regulation of polyamine biosynthesis in *T. brucei* and we demonstrate that the unique regulatory subunit of AdoMetDC is a key component of this regulation. The data support ODC and AdoMetDC as the key control points in the pathway and the likely rate-limiting steps in polyamine biosynthesis.

14799. **Wurst, M., Robles, A., Po, J., Luu, V. D., Brems, S., Marentije, M., Stoitsova, S., Quijada, L., Hoheisel, J., Stewart, M., Hartmann, C. & Clayton, C., 2008**. An RNAi screen of the RRM-domain proteins of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*. **Available online 18 September 2008.**

Zentrum fur Molekulare Biologie der Universität Heidelberg (ZMBH), Im Neuenheimer Feld 282, D69120 Heidelberg, Germany. [cclayton@zmbh.uni-heidelberg].

In eukaryotes, proteins containing RNA recognition motifs (RRMs) are involved in many different RNA processing reactions, RNA transport, and mRNA decay. Kinetoplastids rely extensively on post-transcriptional mechanisms to control gene expression, so RRM domain proteins are expected to play a prominent role. We here describe the results of an RNA interference screen targeting 37 of the 72 RRM-domain proteins of *Trypanosoma brucei*. RNAi targeting eight of the genes caused clear growth inhibition in bloodstream trypanosomes, and milder effects were seen for nine more genes. The small, single-RRM protein *Tb*RBP3 specifically associated with ten mRNAs in trypanosome lysates, but RBP3 depletion did not affect the transcriptome.

14800. **Ziková, A., Panigrahi, A. K., Uboldi, A. D., Dalley, R. A., Handman, E. & Stuart, K., 2008**. Structural and functional association of *Trypanosoma brucei* MIX protein with cytochrome c oxidase complex. *Eukaryotic Cell,* **7** (11): 1994-2003.

Seattle Biomedical Research Institute, 307 Westlake Ave. North, Suite 500, Seattle, WA 98109-5219, USA. [ken.stuart@sbri.org].

A mitochondrial inner membrane protein, designated MIX, seems to be essential for cell viability. The deletion of both alleles was not possible, and the deletion of a single allele led to a loss of virulence and aberrant mitochondrial segregation and cell division in

Leishmania major. However, the mechanism by which MIX exerts its effect has not been determined. We show here that MIX is also expressed in the mitochondrion of *Trypanosoma brucei*, and using RNA interference, we found that its loss leads to a phenotype that is similar to that described for *Leishmania*. The loss of MIX also had a major effect on cytochrome c oxidase activity, on the mitochondrial membrane potential, and on the production of mitochondrial ATP by oxidative phosphorylation. Using a tandem affinity purification tag, we found that MIX is associated with a multiprotein complex that contains subunits of the mitochondrial cytochrome c oxidase complex (respiratory complex IV), the composition of which was characterized in detail. The specific function of MIX is unknown, but it appears to be important for the function of complex IV and for mitochondrial segregation and cell division in *T. brucei*.

TC/D/I0811E/1/05.09/330